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SCIENCE IN WEST AFRICA

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THE first meeting in Nigeria of the West African Science Association was held in Ibadan during December 16–21.

The West African Science Association is formed by the Science Associations of Ghana, Nigeria and Sierra Leone, membership being open to any other scientific worker in any other country or territory in West Africa—including the French-speaking territories. Its purpose is to provide a forum for discussion, by scientists from all parts, of their common problems and to emphasize to public and Governments alike the fundamental importance of science and technology in the development of these areas.

This meeting had an additional significance, for it was the first major international scientific conference to be held in independent Nigeria. It was held, fortunately, at a time when much of the initial exuberance of political liberation had died down and it was possible to assess the problems ahead in a relatively sober atmosphere. This is important, for there is a real danger of complacency, a tendency to confuse the terms 'freedom' and 'prosperity'—not surprising, perhaps, when one sees the amazing transformation in Lagos, the modern buildings, the hotels, the new roads.

The generation of a potentially powerful African State with a seat in the United Nations Organization is no small thing. Perhaps under different conditions the nations would be little disturbed by the emergence of an independent Nigeria ; but with a world divided into separate and mutually distrustful camps the result is a great deal of outside interest arising not from any deep-seated and humanitarian concern, nor from the prospects of great financial reward, but for purely political considerations. Capital from outside is available for many purposes ; but it is a capital investment based not on sound economic principles but on political expediency.

These points are made merely to emphasize that Nigeria and Ghana—to mention the two territories with which we are more concerned in this context—are passing through a 'honeymoon' period. It is now, or possibly never, that they must take their chance. Capital must be spent wisely if wise development is to occur. Capital wasted now will, in the future, be merely a monument to an opportunity lost.

It may seem surprising that a meeting of scientists should concern itself with such matters. In fact, the meeting as such did not. But it was obviously the background against which much of the discussion took place, for the West African scientist is acutely concerned with the direction of future development, and a meeting such as this gives the best possible picture of the problems.

To one observer, at least, from all the papers and discussions emerged the impression that of all West Africa's problems none was more urgent than the matter of health and feeding. It is easy, in this apparently happy, cheerful community, to forget the grim realities behind. It is easy to forget that half the children born never live to adult life, that even among the adult population, disease extracts a dreadful toll. This would not be quite so bad if we did not already know enough to reduce these figures drastically. But all major health schemes are expensive, and the fact must be faced that West Africa simply cannot afford to keep its children alive. Even so, merely to keep children alive is not enough. They must be fed properly. Nigeria, though it is one of the most densely populated parts of the whole continent, is still a territory with vast, under-populated, undeveloped tracts of land. It is theoretically capable of maintaining a very much higher population. The other territories of West Africa are in a similar state—a great potential of food given over at present to the tsetse fly and incredibly inefficient farming methods.

The development problems implied in all this are, of course, many-sided. There are economic and social problems as well as technological problems—and, of course, educational problems are a vital part of the whole picture. But this meeting was concerned primarily with the technological—including the medical—problems involved. One gained an impression of advancing knowledge on all fronts. On the medical side it was made abundantly clear what modern knowledge properly applied could achieve. On the agricultural side there was an impression of slow advance. The tsetse fly is still unconquered, but it is clearly in retreat. For the rest, it is a matter now, not so much of more research, as of funds to apply what is already known, and to pay the technologists and scientists who will be needed.

Development is admittedly a total thing. For a nation really to emerge it needs far more than freedom. It needs schools, universities, cities—but above all it needs a reasonable expectation of life for its inhabitants and food to feed them. There is a most urgent need for the limited capital available to be well spent. Office blocks and handsome government buildings are no solution ; they have their value, but one suspects that many of them are merely testimony to the validity of Parkinson's law—and these territories cannot afford Parkinson's law.

The suggestion was made—and endorsed enthusiastically—that a Scientific and Economic Advisory Council be set up as a matter of urgency, the task of which would be to advise the Government on the problems of development and how best to use the amount of money available. This would be a start—but even more necessary is a radical reappraisal of values; the vital role of science and technology is still not appreciated. The economic and social status of the scientist still lags far behind that of many professions—and the lot of the science teacher, that most critical member of any modern community, is pitiful. (These remarks probably apply equally to Europe, even to America, but these countries are grown up and are at liberty to commit technological suicide if they wish.) To Africa, technological advance is not just a matter of keeping ahead of, or level with, or catching up with other countries—it is a matter of survival, not in some hypothetical future war but now.

The meeting which prompted these musings made it abundantly clear that scientific workers in West Africa are fully aware of all that has been said. They feel deeply their responsibilities to the future development of these territories. But they are also acutely aware of the difficulties. Scientists, technologists, science-teachers are pitifully few in number and, relative to other professions, underpaid. There is far too little money forthcoming for the vitally important land development schemes which have been mooted, for the damming of rivers, for the intensive campaigns against the tsetse fly, for all the other necessary adjuncts to land development. There are the competing claims of industrialization. But this apart, a great deal of what is available would seem to be diverted at present into non-essential projects.

In Nigeria, at least, the Science Association has begun to face up to its wider responsibilities. It is not just a forum for the holding of yet another conference. It has a vital task in the education of the general public and of the Government. It is probably the only body in Nigeria with the machinery to drive home the basic fact that economic progress depends on technological progress. It has begun its task with vigour, but whether or not it will succeed in instilling the necessary sense of urgency remains to be seen.

UTILIZATION OF NITRATE BY MICRO-ORGANISMS

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INTENSIVE work on various physiological and biochemical aspects of the assimilation and dissimilation of nitrate by micro-organisms and higher plants has been undertaken in many laboratories. We now present a synthesis of the results in an attempt to clarify the present position in regard to the mechanism of the utilization of nitrate by micro-organisms.

Many micro-organisms and higher plants utilize nitrate-nitrogen, and the overall process whereby nitrate is reduced to ammonia with the subsequent formation of amino-acids, protein and other nitrogenous cell constituents is known as 'nitrate assimilation'. Under certain conditions, however, several micro-organisms can use nitrate or some of its reduction products as terminal hydrogen acceptors in place of oxygen. This process has been called 'nitrate respiration' by Sato[1] or 'dissimilatory nitrate reduction' by Verhoeven[2]. A similar process has been described in higher plants[3].

Many classifications have been proposed for the various types of nitrate reduction, but none has been entirely satisfactory. Jensen[4] suggested five categories according to the products of the reaction, whereas Verhoeven[2,5] differentiated between three types of nitrate reduction as follows: (1) *assimilation*, in which nitrate is reduced only for the building up of cell materials; (2) *incidental dissimilation*, in which nitrate acts as a non-essential hydrogen acceptor; (3) *true dissimilation*, in which nitrate acts, at least under certain conditions, as the essential hydrogen acceptor which enables the organism to grow. The separation of incidental and true nitrate dissimilation does not seem to be of great value and has only a teleological significance. Organisms which can carry out incidental dissimilation are those that have a fermentative respiration when both oxygen and nitrate are limiting factors. Thus, Verhoeven[5] showed that his strain of *Escherichia coli* produced large amounts of lactic and succinic acids under anaerobic conditions, but in the presence of either nitrate or oxygen complete oxidation to carbon dioxide occurred. Japanese workers[6,7] have classified nitrate-reducing organisms on the basis of the behaviour of their cytochromes towards nitrate. This is not now satisfactory since in *Neurospora crassa* and *Escherichia coli* an iron system appears to be required for the dissimilatory nitrate reductase but not for the assimilatory enzyme. We propose that nitrate reduction may be best considered under two headings: (1) *nitrate assimilation*, in which the end products are nitrogenous cell constituents; (2) *nitrate respiration* or *nitrate dissimilation*, in which nitrate is used instead of oxygen, usually under anaerobic conditions, as the terminal hydrogen acceptor. Denitrification may be regarded as a special case of nitrate respiration and is defined as the production of nitrogen gas or the oxides of nitrogen by the reduction of nitrate, nitrite or any other suitable intermediate.

Suggested Intermediates

Some of the compounds which are of interest in the intermediary metabolism of nitrate are shown in Table 1. Gayon and Dupetit[8] presented equations for the overall process of denitrification, but recognized only nitrite as an intermediate. Franzen and

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Table 1. SUGGESTED INTERMEDIATES IN NITRATE REDUCTION

Oxidation-reduction state of nitrogen atom	Formula	Compound
+5	NO_3^-	Nitrate
+4	NO_2	Nitrogen dioxide
+3	NO_2^-	Nitrite
+2	NO $N_2O_3^=$	Nitric oxide Nitrohydroxylamate
+1	(NOH) N_2O $N_2O_2^=$ $NO_2:NH_2$ $NH(OH)_2$	Nitroxyl Nitrous oxide Hyponitrite Nitramide or imido nitric acid Dihydroxyammonia
0	N_2 $OH.NH.NH.OH$	Nitrogen Dihydroxyl hydrazine
−1	NH_2OH	Hydroxylamine
−2	$H_2N.NH_2$	Hydrazine
−3	NH_4^+	Ammonia

Löhmann[9] and Beijerinck and Minkmann[10] compiled comprehensive lists of possible products of nitrate reduction, but Kluyver and Donker[11] were the first to draw up a scheme for the stepwise reduction of nitrate to nitrous oxide and nitrogen gas. Since then, many pathways have been suggested, based on the assumption that reduction proceeds via a number of two-electron steps. The Delft school[2,5,12] have been particularly active in this field and have presented schemes involving complex molecular re-orientation at the hyponitrite-level of oxidation. Nicholas[13] suggested that nitrate assimilation proceeds according to the following pathway:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O_2^= \longrightarrow NH_2OH \longrightarrow NH_4^+$$

Nitrate. Gayon and Dupetit[8] demonstrated that nitrite is the first product of nitrate reduction. Quastel, Stephenson and Whetham[14] found that *Bacterium coli* produced nitrite when grown anaerobically; nitrite has since been identified as a product of nitrate reduction in bacteria, fungi and higher plants[5,15]. The agronomic importance of nitrate reductase activity in maize plants has been demonstrated by Hageman and Flesher[16].

The sequence of electron transfer during nitrate reductase activity by the purified enzyme from *Neurospora crassa* has been shown to be[13]:

$$\begin{tikzcd}
TPNH \arrow[r] & FAD \arrow[r] \arrow[d] & Mo \arrow[r] & NO_3^- \\
& \text{2,3}',\text{6-trichlorophenol indophenol} \arrow[ru] & &
\end{tikzcd}$$

A similar molybdo-flavoprotein system has been described in the yeast *Hansenula anomala*[17] and in higher plants[18].

Both molybdenum and cytochrome have been shown to participate in nitrate reduction by denitrifying bacteria[19]. The sequence of electron transfer to the alternative hydrogen acceptors oxygen and nitrate in whole cells of *Pseudomonas aeruginosa* is:

$$\begin{tikzcd}
DPNH \arrow[r] & FAD \arrow[r] & \text{cytochrome } c \arrow[r] \arrow[d] & \text{nitrate reductase (Mo)} \arrow[r] & NO_3^- \\
& & \text{cytochrome oxidase} \arrow[d] & & \\
& & O_2 & &
\end{tikzcd}$$

Sadana and McElroy[20] proposed a similar scheme for nitrate reduction in *Achromobacter fischeri* (≡ *Photobacterium fischeri*), but claimed that molybdenum was not involved. Although they grow the bacteria in peptone medium without the addition of molybdenum, it is unlikely that their cultures were in fact deficient in the micronutrient since no purification methods were used. Thus, further work is required to determine whether *Phytobacterium fischeri* requires molybdenum for the reduction of nitrate.

Many schemes have been postulated for nitrate reduction in *Escherichia coli*, and Taniguchi and Itagaki[21] have recently obtained a homogeneous molybdo-protein which reduces nitrate with cytochrome b_1 as the electron donor. It is probable that the different pathways suggested by various workers using this organism can be explained by the different hydrogen donors in the nitrate reductase assay and by the varied conditions under which the organism was grown. Thus the preparations have probably all contained the same terminal nitrate reductase (that is, the molybdo-protein) with either succinic dehydrogenase[22], lactic dehydrogenase[23], diaphorase[23] or quinone reductase[24] as the penultimate hydrogen-donating system. Table 2 illustrates that differences also occur between strains. Thus *E. coli* (*Pf*) can dissimilate nitrate under anaerobic conditions, and both iron and molybdenum deficiencies reduce the nitrate reductase activity. Nitrite accumulates, and the glucose in the medium is almost entirely oxidized to carbon dioxide. Strain *BLA*, however, can utilize nitrate as the sole nitrogen source for growth[25], but not as a hydrogen acceptor during anaerobic respiration. In this case, iron deficiency has no effect on nitrate reductase activity, indicating that a cytochrome system is not involved. The metabolism of this strain under anaerobic conditions is fermentative, and organic acids accumulate in the medium.

Table 2. GROWTH OF *Escherichia coli* UNDER ANAEROBIC CONDITIONS

The cells were grown in a medium containing both NH_4^+ and NO_3^- ions (ref. 23) under anaerobic conditions for 24 hr. at 37° C.

Strain	*B.L.A.**	*Pf*†
Nitrate reductase activity of iron-deficient cells as a percentage of the activity of normal cells	103	42
Nitrate reductase activity of molybdenum-deficient cells as a percentage of the activity of normal cells	51	48
Carbon dioxide production during growth	+	++++
Nitrite ion in the culture medium	±	++++
Lactic acid in the culture medium	+++	++
Succinic acid in the culture medium	++++	−

* Strain *B.L.A.* was derived from strain *B*, kindly supplied by Dr. R. Herriott, Biochemistry Department, Johns Hopkins University School of Hygiene and Public Health, Baltimore, by sub-culturing it on nitrate medium.

† Strain *Pf* was a gift from Dr. J. Erkama, Biochemistry Department, University of Helsinki.

It has been suggested that cytochromes participate in nitrate reduction only in the dissimilatory process[19,26]. This is confirmed by the results in Table 3, which show that iron deficiency depressed nitrate reductase activity only in organisms dissimilating nitrate under anaerobic conditions. In contrast to this, molybdenum deficiency invariably decreased nitrate reductase activity under either aerobic or anaerobic conditions. It is noteworthy that a deficiency of either iron or molybdenum reduced the enzyme activity of *Rhizobium japonicum* extracted from soy-bean nodules[27].

Table 3. EFFECTS OF IRON DEFICIENCY AND AERATION ON THE NITRITE REDUCTASE ACTIVITY OF MICRO-ORGANISMS

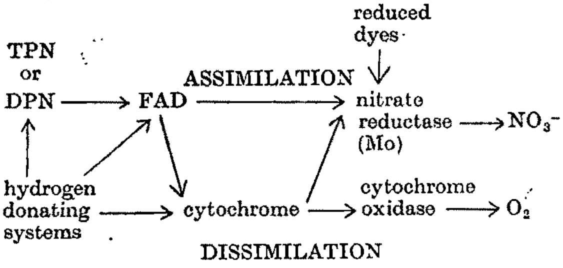
The enzyme activities of homogenates prepared from iron-deficient cells are given as percentages of those of homogenates prepared from normal cells grown under the same conditions of aeration

Organism	Nitrogen source in culture medium	Condition of growth	
		Anaerobic	Aerobic
Clostridium pasteurianum (*W–S*)	Nitrate	122	*
Escherichia coli (*B.L.A.*)	Nitrate + ammonium	103	108
Escherichia coli (*Pf*)	,, ,,	12	106
Micrococcus denitrificans (N.C.I.B. 8944)	,, ,,	28	†
Neurospora crassa (*Em* 5297*a*)	Nitrate	45	130
Pseudomonas aeruginosa (N.C.I.B. 8704)	Nitrate + ammonium	40	†

* Obligate anaerobe which can use neither oxygen nor nitrate as a terminal electron acceptor.

† The denitrifying organisms have negligible nitrate reductase activity under aerobic conditions.

These results suggest that a generalized scheme for electron transport during microbial nitrate reduction can be constructed as shown in scheme 1.



Scheme 1. Alternative pathways of electron transfer to nitrate and oxygen in micro-organisms

This emphasizes the homology of nitrate dissimilation and aerobic respiration, first suggested by Gayon and Dupetit[8]. Takahashi, Taniguchi and Egami[28] have suggested that dissimilatory nitrate reduction is coupled to phosphorylation, and this seems to be probable in view of the similarities in the electron-transfer sequence during respiration with either oxygen or nitrate as the terminal acceptor, and the large change in free energy ($-\Delta F$ approximately 40 kcal./mole) during the reduction of nitrate by reduced diphosphopyridine nucleotide. It is feasible that the coupling reaction might provide a control mechanism whereby a cytochrome system is involved in nitrate dissimilation and not in nitrate assimilation.

Similar alternative hydrogen-donating systems probably occur in higher plants. Thus, Mendel and Visser[29] suggested that the reducing power for nitrate reduction was of respiratory origin in the dark and of photosynthetic origin in illuminated leaves. Stoy[30] concluded that nitrate reduction in the light and dark "are identical in essential parts and that the only difference lies in the various ways in which the hydrogen donors, necessary for the nitrate reduction, can be generated".

Nitrite. Yamagata[31] found a nitrite reductase enzyme in cell-free extracts of *Bacillus pyocyaneus* and Taniguchi *et al.*[32] showed that extracts of *B. pumilis* reduced nitrite when reduced methylene blue was the electron donor. Nitrite reduction has also been demonstrated in species of *Azotobacter*, *Debaryomyces*, *Desulfovibrio*, *Escherichia* and algae (see refs. 5, 13, 25).

Nitric oxide has occasionally been observed as a product of denitrification[33,34], and it has now been identified as the immediate product of nitrite reduction during denitrification by *P. stutzeri*[35], *P. aeruginosa*[25,36] and *M. denitrificans*[25]. The product of assimilatory nitrite reduction has not previously been determined, although the enzyme has been studied in considerable detail[37]. Medina and Nicholas[38] suggested that hyponitrite was the first product of the reduction, but Nicholas and Jones (unpublished results), using a chromatographic technique[39], could not detect it during nitrite reduction by the purified enzyme from *Neurospora*. It is doubtful, however, whether this technique was sufficiently sensitive to detect the small amounts of product expected. The results obtained by Walker[40] in this laboratory suggest that nitric oxide is formed during nitrite reduction by the purified *Neurospora* enzyme.

The assimilatory nitrite reductase purified from *N. crassa*[37] and the dissimilatory enzyme from *P. aeruginosa*[36] appear to be very similar, since they are both flavoproteins which require copper, iron and sulphydryl groups for maximum activity. Walker and Nicholas[36] and Yamanaka *et al.*[41] have shown that the *Pseudomonas* enzyme has cytochrome oxidase activity, but this does not appear to be its primary function, since nitrite is a much more efficient electron acceptor than is oxygen.

Iwasaki and Mori[42] claimed that nitrite and hydroxylamine could react enzymatically to give nitrous oxide and nitrogen, but this could not be confirmed in this laboratory[25] or by Asano[43].

Recent work[44] makes it probable that the disappearance of nitrite noted in soy-bean leaves by Nason *et al.*[45] may not have been due to a nitrite reductase. There is little doubt, however, that a nitritase enzyme exists in plant tissue, since nitrate can be utilized as the sole nitrogen source and nitrite is the only known product of the nitrate reductase enzyme. Butt and Beevers[46] suggested that nitrite is reduced by reduced triphosphopyridine nucleotide generated in the pentose phosphate pathway.

Nitric oxide. We have recently produced evidence that nitric oxide, or a compound with which it equilibrates, is an intermediate in both assimilatory and dissimilatory nitrate reduction[47]. There are two main lines of evidence: (1) nitric oxide is the immediate product of nitrite reduction in *Neurospora* and in several denitrifying bacteria; (2) nitric oxide reductase activity is found in micro-organisms and higher plants which are actively reducing nitrate, but the enzyme is absent or much reduced in organisms grown on ammonium-nitrogen only.

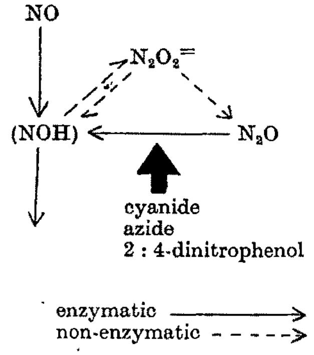
Inhibition of hydrogenase activity by nitric oxide *in vitro* has been noted in species of *Azotobacter*, *Clostridium*, *Escherichia* and *Hydrogenomonas*[47,48], but this is unlikely to have very great significance *in vivo* since nitric oxide probably has only a transient existence in association with the enzyme; indeed, it is formed as the free gas rarely even by denitrifying organisms[47]. This is confirmed by our observations that nitric oxide can act as the acceptor during hydrogenase activity in extracts of *M. denitrificans* and only at relatively high concentrations (greater than 0·05 atmosphere) is it inhibitory. We have also confirmed the observations of Keilin and Hartree[49] than nitric oxide can form a complex with cytochrome *c*, but it is doubtful whether this is of physiological significance.

Hyponitrite, nitroxyl and nitrous oxide. Many workers[2,11,13,50] have suggested that nitrate reduction involves an intermediate at this level of oxidation. Nitroxyl, hyponitrite, nitrous oxide, nitramide and dihydroxyammonia have all been postulated, but experimental evidence for any of these is scanty.

Assimilation of hyponitrite has been reported in *E. coli*[51] and in *N. crassa*[38]. The instability of hyponitrite[52,53] makes experimental work and the interpretation of results difficult. We have confirmed that hyponitrite decomposes rapidly at physiological *p*H, and the presence of whole cells or cell-homogenates of *P. aeruginosa* did not change the rate of non-enzymatic production of gas[25]. This finding is in agreement with the results of Allen and van Niel[52] with *P. stutzeri* and of Kluyver and Verhoeven[54] with *P. aeruginosa* and *M. denitrificans*. These results, however, do not rule out hyponitrite as an intermediate, since it could have a transient existence in association with enzyme surfaces.

Several workers have reported that nitrous oxide can be reduced to nitrogen gas or ammonia by bacteria[2,51,52,54,55]. It is unlikely, however, that nitrous oxide is a usual intermediate in denitrification, since nitrogen production from nitrous oxide can be inhibited by levels of cyanide, azide or 2 : 4-dinitrophenol which have no effect on the rate of production of nitrogen gas from nitrite[52,55]. Experiments on simultaneous adaptation have produced conflicting results; but Sacks and Barker[55] using *P. denitrificans*, and McNall and Atkinson[51] using *E. coli*, found that cells adapted to nitrate utilized nitrous oxide only after a lag period. It is likely that nitrous oxide is utilized *per se* since it is the formal anhydride of hyponitrous acid and dissolves in water to give a true solution[56].

These results can be integrated by assuming that a compound like nitroxyl (NOH), containing one nitrogen atom, is the intermediate in nitrate reduction, and that this is in equilibrium with hyponitrite which can decompose to give nitrous oxide. A further adaptive enzyme is necessary to utilize nitrous oxide. This is illustrated in scheme 2, and is similar in outline to that presented by Lees[57] for the nitrification process.



Scheme 2. Some intermediates during the assimilation and dissimilation of nitrate

The scheme is supported by the following lines of evidence: (1) Nitrous oxide has frequently been reported as a product of denitrification[8,58]. Recent work by Delwiche[59] makes it probable that nitrous oxide is formed non-enzymatically when a compound at the nitroxyl level of oxidation accumulates. (2) The occasional reports of simultaneous adaptation to nitrate and nitrous oxide[52,54] could be caused by induction of the enzyme utilizing nitrous oxide by small amounts of the gas formed during growth. (3) The existence in solution of hyponitrite or nitrous oxide explains the nitrogen 'losses' observed by several workers[60], who measured only nitrate, nitrite, nitrogenous gases, ammonia and cell nitrogen during denitrification. (4) This scheme explains the utilization of hyponitrite, since it would exist in equilibrium with nitroxyl and would also produce nitrous oxide which could be utilized. (5) The true intermediate is likely to contain only one nitrogen atom, as do the other intermediates. A compound such as hyponitrite would involve the consecutive formation and breakage of an N–N bond and this, although possible, is, from a consideration of the energy changes involved, unlikely.

Hydroxylamine. Verhoeven[2] detected hydroxylamine in the culture medium during dissimilatory nitrate reduction by *Denitrobacillus licheniformis* and the reduction of hydroxylamine to ammonia has been demonstrated in a number of micro-organisms[51,61].

Hydroxylamine reductases from *N. crassa*[13] and from *P. aeruginosa*[62] have been shown to be flavoproteins requiring manganese for activity. Kono and Taniguchi[63] have also implicated a cytochrome in reduction of hydroxylamine by a denitrifying *Micrococcus*. The Japanese workers concluded that "hydroxylamine reductase may have physiological meaning other than the mere reduction of hydroxylamine"[64]. This seems unlikely, since the product of the reduction has been shown to be ammonia[40,62], and we have demonstrated[25] that *P. aeruginosa* assimilates small amounts of nitrate-nitrogen (15 per cent of the total cell nitrogen) even while denitrifying actively in a peptone-nitrate medium.

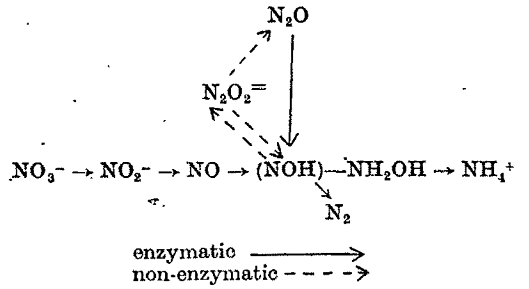
Extracts of higher plants have been shown to oxidize hydroxylamine by means of a peroxidase system stimulated by manganese[44,65]. There is no evidence that this has physiological significance *in vivo*, although there have been reports that nitrate occasionally accumulates in detached leaves kept in the dark[30]. Cresswell and Hewitt[65] suggested that this system "may well provide a basis for the nitrification of hydroxylamine". This may be true for heterotrophic nitrification, for example, by *Aspergillus flavus*[66] (although even here it is of doubtful physiological significance), but is unlikely to apply to autotrophic nitrification by *Nitrosomonas spp.*, where the process is linked to a system yielding energy.

The oxime pathway for utilization of hydroxylamine appears to have little significance except as a detoxication mechanism (see the review by Nicholas[13]).

Aromatic compounds. It has been suggested that alternative or simultaneous mechanisms for reducing nitrate could involve aromatic nitro compounds. There is, however, little evidence for this[13,67]. Enzymes capable of reducing a range of aromatic nitro-compounds have been demonstrated[67,68], but there is no evidence for enzymes responsible for the nitration of the benzene nucleus and this is an obligate step in any such hypothesis.

Common Pathways in Assimilation and Denitrification

The final products of nitrate assimilation are, by definition, nitrogenous cell components. During nitrate dissimilation, however, the following products can be formed: nitrite, nitric oxide, nitrous oxide, nitrogen, hydroxylamine or ammonia. The results already discussed show that the processes involved in both assimilatory and dissimilatory nitrate reduction are very similar. Thus, some of the intermediates have been shown to be the same, and the properties of the enzymes are alike. The assimilatory route has been described in detail previously[13], but it appears that it should now include nitric oxide as a likely intermediate. Schemes for nitrate dissimilation, and for denitrification in particular, have usually been complex[2], but we have shown that the sequence of intermediates may be similar to that in the assimilatory route, and both processes are presented in scheme 3.


N_2O
$N_2O_2^=$
$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow (NOH)—NH_2OH \rightarrow NH_4^+$
N_2
enzymatic
non-enzymatic

Scheme 3. A pathway for the reduction of nitrate in micro-organisms

It is clear that in dissimilation of nitrate the rate of reduction of nitrate will be much greater than that in assimilation of nitrate, since in dissimilation the nitrate has a respiratory function. Some intermediates, which probably normally exist only as enzyme-bound complexes, may therefore accumulate. On this basis, it is clear that the products of nitrate dissimilation depend on the relative activities of the enzymes of the reductive sequence present in the organism. Thus, those organisms which produce large amounts of nitrite (for example, *E. coli*, *N. crassa*) have a very active nitrate reductase and little or no nitrite reductase activity. On the other hand, some organisms (for example, *B. licheniformis*[2]) produce ammonia during dissimilation, and here all the enzymes in the reductive sequence must be very active. Denitrifying organisms occupy an intermediate position; in a few cases nitric oxide is produced[34], and here the nitric oxide reductase is presumably less active than the nitrite reductase. Similarly, we have suggested how nitrous oxide is produced when the intermediate at the nitroxyl-level accumulates. Denitrification is an efficient form of dissimilation of nitrate in that the products are gaseous and toxic compounds such as nitrite do not accumulate in the medium. This may well have ecological significance. We have demonstrated that *P. aeruginosa* can assimilate nitrate-nitrogen even while actively denitrifying[25], but some denitrifying organisms require ammonium-nitrogen for growth[34,69]. In these cases it is likely that at least one of the later enzymes, probably hydroxylamine reductase, is absent. This is an analogous situation to the *Neurospora* mutants, which can reduce nitrate but not nitrite[70].

The homology of aerobic respiration and dissimilatory nitrate reduction has been pointed out already. That the aerobic process is more efficient is suggested by the following findings: (1) Proliferating bacteria denitrify only under anaerobic conditions, and aeration prevents the induction of the nitrate-reducing enzymes[19,25]. (2) Cell yield is usually lower in bacteria-dissimilating nitrate than in those grown aerobically[25,71]. This might be caused by the formation of a smaller number of high-energy phosphate bonds during the oxidation of carbohydrate substrates by nitrate than by oxygen, that is, a less efficient use of the energy sources. Confirmation of this idea can only come from a study of the extent to which phosphorylation is coupled to nitrate dissimilation. (3) Even cells possessing the nitrate-reducing enzymes use oxygen rather than nitrate as the terminal hydrogen acceptor[19,25,72]. Oxygen has no effect on the enzymes *per se*, but the cytochrome oxidase appears to be more efficient than the nitrate reductase in oxidizing the reduced cytochrome which is involved in the dissimilatory system.

Conclusions

The hypothesis presented here, which serves to link the assimilation and dissimilation of nitrate, is consistent with the data available at the present time. It also agrees with the concept of the "unity of biochemistry"[11,12], which suggests that if similar chemical reactions are carried out by different organisms, then the mechanisms involved are also likely to be similar. Thus, several unrelated organisms can dissimilate nitrate, and it is more likely that a process such as denitrification represents a fairly simple adaptation of a widespread existing system (that is, nitrate assimilation) rather than a *de novo* formation of an entirely different pathway. Similarly, it is probable that a closely related system for nitrate reduction exists in higher plants and, furthermore, the reverse process of nitrification may well involve the same intermediates.

It seems likely, therefore, that the process of nitrate assimilation follows the inorganic sequence to ammonia. Nitrate dissimilation appears to be merely an adaptation of the assimilatory pathway, partly by the intervention of cytochromes in the penultimate electron transfer sequence. Denitrification is a special case of nitrate dissimilation in which the main end-products are nitrogenous gases.

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Coggins identified the organic acids in the culture medium.

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THE EVOLUTION OF OVERDOMINANCE: NATURAL SELECTION AND HETEROZYGOTE ADVANTAGE

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HETEROSIS or hybrid vigour, inbreeding depression and the maintenance of balanced polymorphisms in natural populations have all been associated in one form or another with overdominance. The occurrence of overdominance may be viewed either as an intrinsic phenomenon or as the outcome of an evolutionary process, and in this article we shall argue for the latter possibility.

The term 'overdominance' is applied either when the heterozygote for two alleles at a locus, or more generally when the heterozygote for two genetically different but homologous sections of a chromosome, is superior in mean fitness to either homozygote. Heterosis, or hybrid vigour, on the other hand, is not defined in terms of such specific genetic situations. It describes the observation of a higher mean, with respect to a quantitatively varying character, for the hybrid between two more or less pure lines than for either pure line. When the quantitative character considered is not fitness, Dobzhansky[1] has suggested the term 'luxuriance' should be used instead of heterosis, so that heterosis may be taken to refer specifically to an observed excess fitness of the hybrid. Overdominance may be a cause of heterosis; but the occurrence of heterosis does not imply the existence of overdominance.

Overdominance may also be a cause of the complementary phenomenon of inbreeding depression. The natural causes of heterosis and inbreeding depression have been the subject of much discussion. Some writers, notably Haldane[2] and Lerner[3], have pinpointed overdominance as the basic underlying mechanism, and so have been led to assume an intrinsic advantage for heterozygous genotypes. On the other hand, Fisher[4] and especially Mather[5] have attached overriding importance to non-allelic gene interactions as a cause for heterosis and inbreeding depression. There is on this view no longer a need to attach any intrinsic importance to the phenomenon of overdominance. We shall first of all review the evidence for the occurrence of overdominance and then show how it may evolve.

Causes of Inbreeding Depression

One of the simplest explanations for inbreeding depression is the accumulation of deleterious recessives. However, the loss in vigour from this cause has been shown to be no more than 1–5 per cent[4,6], whereas heterosis of the order of 20 per cent is quite common. On the other hand, the calculation by Fisher[7] of the loss of reproductive value on inbreeding a random-mating population polymorphic for an overdominant pair of alleles, and similar calculations by Haldane[8] and Crow[6], show that overdominance at a comparatively small number of loci might be sufficient to explain observed levels of heterosis and inbreeding depression. There is, however, little direct evidence that supports the simple view of intrinsic overdominance, or 'heterosis *per se*' as it is frequently called, as the basic underlying mechanism. As Mather[5] points out, the occurrence of overdominance in relation to hybrid vigour has scarcely, if ever, been proved, for it is difficult to distinguish from non-allelic gene interaction. Thus, Schuler and Sprague[9], for example, found no evidence in maize for overdominance with respect to specific genetic markers. Further, Jinks[10], using di-allele crossing techniques, found overdominance always in association with non-allelic gene interactions. It has also been shown by Williams[11] that heterosis for a complex character may arise through multiplicative interactions between its various components.

Overdominance, Balanced Polymorphisms and Linkage

Overdominance was shown by Fisher[12] to be a theoretically necessary condition for a balanced polymorphism in a random mating population, when the selective values remain constant. Although, when selective values vary, it is possible to construct models which retain two alleles in a random mating population without overdominance[13,14], it is probable that most of the known cases of balanced polymorphisms are, in fact, associated with the occurrence of overdominance. The association between polymorphism and close linkage was first pointed out by Fisher[15]. One of the first examples of a probable complex of closely linked genes was discovered by Ernst[16] in the distylic polymorphism of some species of *Primula*. Further, Fisher[7] showed how interaction between two or more factors could lead to the tightening of linkage between them. It seems likely, therefore, that we can explain the evolution of closely linked complexes of genes, such as occur in *Primula*, by the accumulation of favourable modifiers linked to a segregating polymorphic locus. The importance of linkage and non-allelic interaction in the building up of complex polymorphic loci has been stressed by Sheppard[17] and Mather[5]. More recently, Clarke and Sheppard[18–20], in a series of papers on mimicry in various African species of *Papilio*, have provided convincing evidence that the mimetic forms are most probably controlled by the various alleles of a 'super-gene' which has evolved in this way. There seems to be little doubt that such polymorphisms are clear examples of an association between overdominance and linkage between interacting genes.

The concept of the balance of polygenic combinations was first developed in a classic paper by Mather[21], although it was, perhaps, foreshadowed by Fisher[7] when writing on "simple metrical characters". On the assumption that natural selection favours intermediate phenotypes, gametic combinations which are well 'balanced' with respect to high and low factors will be continually selected at the expense of poorly balanced combinations which give rise to extreme values. The poorly balanced gametes will be continually produced from well-balanced gametes by recombination so that natural selection will favour closer linkage between factors occurring in a well-balanced combination, or combinations of factors which are already more closely linked. In outbreeding species, selection may thus be expected to cause the accumulation of chromosomes heterozygous for linked balanced polygenic combinations which, although phenotypically uniform, release genetic variability by recombination.

In the simple case of two segregating loci A–a, B–b with A, B acting in one direction, and a, b acting in the other, natural selection will favour the balanced repulsion heterozygote Ab/aB. Evidence for the importance of recombination in releasing significant genetic variability comes from the artificial selection experiments of Mather and Harrison[22], and more recently from the striking phenomenon of repeated response to selection described by Thoday and Boam[23]. More specific evidence for the occurrence of linked balanced complexes in a disruptive selection experiment is given by Gibson and Thoday[24]. The importance of linked interacting gene complexes is also emphasized by experiments with *Drosophila* obtained from natural populations, such as those of Dobzhansky and Levene[25] and of Spiess[26]. There is, perhaps, an analogy between the balanced polygenic combination and the balanced lethal heterozygote and, as pointed out by Mather[5], the former as much as the latter are clearly examples of overdominant situations in the more general form as defined at the beginning of this article.

This brief review of the evidence for the occurrence of overdominance shows that it is most unlikely to be a phenomenon commonly associated with just a single pair of alleles differing at only one genetic site. Even in its simplest form, in a balanced polymorphism, overdominance is closely associated with linkage between interacting genes, and more generally is dependent to an appreciable extent on the genetic background. The dependence on the genetic background is clearly illustrated by the work of Levene, Pavlovsky and Dobzhansky[27], and Dobzhansky[28] with various chromosome rearrangements in *Drosophila pseudo-obscura*, which are frequently polymorphic in natural populations.

Evolution of Dominance

The dependence of overdominance on the genetic background bears a striking resemblance to the similar situation with respect to dominance in such cases as the crinkled dwarf mutant in cotton, which was the first example used by Fisher[29] as evidence for his theory of the evolution of dominance. This theory relates to the common occurrence of the dominance of a wild-type gene over rare deleterious alleles. Since the deleterious allele is rare, it will occur much more frequently in a heterozygous than in a homozygous state, if it is assumed that the heterozygote is less fit than the wild-type homozygote. Fisher argued that modifiers affecting the deleterious mutant would therefore be selected almost entirely for their effect on the heterozygote, and that selection of such modifiers would gradually lead to the resemblance of the mutant heterozygote and the wild-type homozygote. The selection for modifiers will depend on the frequency of the heterozygote, and so increase as its frequency increases, but selection will always be slow so long as the mutant is rare. This will be the case so long as the mutant homozygote is at an appreciable disadvantage. As pointed out by Fisher[30], selection for modification of the mutant homozygote will be slow until the heterozygote is fairly frequent "except in so far as some such an effect has already been produced by the selective modification of the heterozygote". Fisher's theory of the evolution of dominance was criticized by Wright[31] and Haldane[32] soon after its first publication, principally on account of the very slow rate at which modifiers of the mutant heterozygote would be selected. However, subsequent experimental evidence for the breakdown of dominance on outcrossing such as in the work on cotton[33] or in Ford's[34] work with *Triphœna comes* (*Hb*), and more recently in the work of Clarke and Sheppard[18-20] in the mimicry alleles in *Papilio* species, leaves little doubt that, at least in a fair proportion of cases, dominance is an evolutionary phenomenon and its expression depends on the genetic background.

It was pointed out by Fisher[15] that if, for any reason, the heterozygote occurs in appreciable proportions, such as is the case in a balanced polymorphism, evolution of dominance may occur much more rapidly than in the modification of the dominance of an ordinary deleterious mutant. This will, however, apply only to such genetic factors as affect external appearance, where it may be an advantage for the heterozygote to resemble one or other homozygote, but the basic polymorphism is determined by some constitutional effect of the factor other than that on external appearance. It can be shown (Bodmer, unpublished) that modifiers of the three genotypes, formed by two alleles at a locus, occurring in a balanced polymorphism, will be selected according to the prevailing genotype frequencies. Since in such a situation the 'mutant' homozygote is no longer rare, there is not the same differential selection for modifiers of the heterozygote as in the situation to which Fisher originally applied his theory. However, during the initial stages of progress of an advantageous gene, the gene will occur principally in heterozygotes and there may then be opportunities for differential selection of modifiers of the advantageous heterozygote[35]. This is a situation not apparently envisaged by Fisher in his original development of the theory of the evolution of dominance, but one which is exemplified in the classic work of Kettlewell[36] on the industrial melanic forms of the moth *Biston betularia*.

Increase of Newly Occurring Genes

The conditions under which a newly occurring gene will become established have been investigated by Bodmer and Parsons[35] for three specific genetic systems. Their general conclusion was that the newly formed heterozygote must be at an advantage in outbreeding species, and that the measure of the advantage needed to establish the gene decreases as the amount of inbreeding increases. Thus, consider the simple case of a new allele A being introduced into a population which is predominantly aa. Let the frequency of the allele A be q, and of a be p where $p + q = 1$, and suppose the amount of inbreeding to be measured by a constant inbreeding coefficient F. Then if the viabilities of the genotypes AA, Aa, and aa are respectively a, h and b, the frequencies of the three genotypes after selection will be:

$$\begin{array}{ll} AA & a(q^2 + pqF)/T \\ Aa & 2hpq(1 - F)/T \\ aa & b(p^2 + pqF)/T \end{array}$$

where T is such that the frequencies after selection add up to unity. If, initially, A is rare, then q is small and neglecting terms in q^2 the condition that the frequency of A should increase is:

$$aF + h(1 - F) > b$$

and this is the condition for the gene A to become established in the population. When $F = 0$ and there is random mating this gives $h > b$, which is simply the condition that the new heterozygote should be fitter than the prevailing homozygote. When $F = 1$ and there is complete selfing we have $a > b$, and this means that the new homozygote must be fitter than the old homozygote. As F goes from 0 to 1 for increasing amounts of inbreeding, the relative importance of the heterozygote viability h, in determining the initial increase of the gene A, diminishes. Thus the importance of initial heterozygote advantage is entirely a function of the breeding system.

Evolution of Overdominance

Now Haldane[37], in a paper on the theory of selection for melanism in moths, has given an elegant calculation for the total number of heterozygotes produced in the course of the substitution of a new advantageous gene. He argues that if this total number of heterozygotes is H times the average population size in any one generation, then selection for modifiers of the heterozygote will be equivalent to the effects of H generations of selection on a population consisting entirely of heterozygotes. He later qualifies this statement, however, by saying that when the viabilities of the heterozygote and the new homozygote are equal (and, of course, greater than that of the old homozygote) "there is no selective pressure tending to select modifiers of heterozygotes".

This statement is followed up in his paper on "The Cost of Natural Selection"[38], where he considers the number of "selective deaths" as an effective measure of the selection for modifiers of any particular genotype. On this premise, when there are no differences in fitness between genotypes, there can be no selection of modifiers which improve the fitness of any one genotype. It is clear that such a model excludes the possibility of evolving overdominance, for once the viability of the heterozygote has been selected up to that of the new advantageous homozygote, Haldane would argue that there could be no further differential selection of modifiers improving the heterozygote viability. This situation arises from his assumption that on the whole a species "can only maintain its numbers by utilizing its reproductive capacity to the full", and so taking as his basic model a situation in which there is a deterioration in the environment causing a lowering of the mean fitness. Selection of a new gene in this environment is then acting only to return the mean species fitness to its former level. This does not allow the possibility of selection for a gene increasing the mean fitness without any prior deterioration of the environment. It is surely necessary to have a theoretical model which can allow for a positive evolutionary change of this sort. As Thoday (personal communication) has suggested to us, Haldane's basic assumptions do not take into account "the cost of living".

In his original calculations on the theory of dominance, Fisher[30] expressed the selection for modifiers of the heterozygote in terms of the fraction of the ancestry of future generations ascribable to heterozygotes or, as Haldane[39] called it, their "ultimate reproductive value". The calculation assumes a steady state with respect to the rare mutant, the frequency of heterozygotes being maintained by recurrent mutation. In this case the ultimate reproductive value of a heterozygote remains the same, at least during an interval of time when the heterozygote viability has been improved only a little. There is no clear parallel to this concept when we consider the progress of an advantageous gene in an outbreeding population. As we have shown here, such a gene must start off by having an advantage in the heterozygote over the prevailing wild-type homozygote. Even if the new homozygote is initially fitter than the heterozygote, when the new gene is rare and so occurs principally in heterozygotes, the contribution of heterozygotes to future ancestry is still much more than that of homozygotes. However, as the gene frequency increases, and so the proportion of homozygotes relative to heterozygotes increases, this situation will change and homozygotes will end up by contributing much more, in total, to future ancestry.

Following Haldane[37], it is possible to calculate the total number of heterozygotes Aa and homozygotes AA produced as a new advantageous gene increases in a population consisting initially mostly of the genotype aa. By weighting the frequency of a genotype in any generation with its contribution, in terms of reproductive value, to the subsequent generation it is also possible to calculate the total contribution of reproductive value made by the genotypes Aa, AA during the evolutionary progress of the new gene A. Assuming viabilities $1+S_1$, $1+S_1-S$ and 1 for the genotypes AA, Aa and aa respectively, we have calculated the total number, or total reproductive value, contributed by the genotypes AA, Aa as the gene frequency of A increases from q_0 to q_1 for a series of different values of S_1 and S. If we assume that q_0, the initial frequency of A, is small, then these contributions depend largely on $1/S_1$ (where S_1 is the excess viability of AA over aa) for any given value of q_1. Table 1 shows the number of heterozygotes Aa produced by the time A has reached gene frequencies ranging from 0·1 to 0·9 for two different sets of viabilities. In the first case $S_1 = 1$ per cent and $S = 0$, so that A is fully dominant, and in the second case $S_1 = 5$ per cent and $S = 2{\cdot}5$ per cent, so that A is now an 'additive' gene with respect to fitness. The actual numbers in Table 1 represent multiples of the population size (assumed constant) in any generation. The ratio of the number of Aa as compared with the number of AA is given in the last column of Table 1 to the nearest whole number. This ratio is more or less independent of the viabilities S_1 and S, and appears to be a characteristic of the pattern of increase of a new gene in a random-mating diploid population.

Table 1

q_1	No. of heterozygotes Aa $S_1=1$ per cent $S=0$	 $S_1=5$ per cent $S=2{\cdot}5$ per cent	Ratio of heterozygotes Aa to homozygotes AA
0·1	21·3	8·2	37
0·2	45·1	16·4	17
0·3	72·0	24·6	10
0·4	103·2	32·8	7
0·5	140·0	41·0	5
0·6	185·1	49·2	4
0·7	243·2	57·4	3
0·8	325·1	65·6	2
0·9	465·1	73·8	1

where q_1 is the frequency of the 'new' gene A and the viabilities
of AA Aa aa
are $1+S_1$ $1+S_1-S$ 1 respectively.

It is clear that when the frequency of A is small far more heterozygotes than homozygotes have been produced and this excess is still appreciable when q_1 is 40 per cent or even 50 per cent. Thus, if modifiers can have been selected at all in the time taken to reach this gene frequency, they will have been selected much more for their effect on the heterozygote Aa than on the homozygote AA. It is the total number of heterozygotes produced, not the number which have died selectively, which gives an approximate measure of the number of equivalent generations of selection available for a population consisting entirely of heterozygotes.

It can be shown that the difference between the total number produced and between the total contribution of reproductive value is generally only of the order of magnitude of the gene frequency. The details of these calculations will be published elsewhere. The results provide a theoretical basis for the statements by Mather[5,40] that outbreeding species are exposed to natural selection in a predominantly heterozygous condition. It is, of course, clear that as soon as modifiers have increased the fitness of the heterozygote over that of both homozygotes, a balanced polymorphic situation will be achieved in which the heterozygote is permanently maintained in the population.

In a further calculation, Haldane[37] has obtained an expression for the increase in frequency of an independent dominant modifier of the heterozygote, throughout the course of the increase of the advantageous gene. In our notation, the modifier would increase in frequency by a factor :

$$F = \left(\frac{S_1 - S}{S}\right)^{2\mu/(S_1 - 2S)}$$

where μ is the advantage conferred on the heterozygote by the modifier. Haldane considers only the case $\mu = S$ when the modifier brings the viability of the heterozygote Aa up to the level of the homozygote AA. When $\mu = S$, $F \rightarrow 1$ as $S \rightarrow 0$ and the A gene tends to complete dominance. F is large and tends to infinity only as $S/S_1 \rightarrow 1$, in which case the heterozygote Aa has the same viability as the prevailing wild-type homozygote aa and only heterozygotes carrying the modifier will be selected. Only allowing the case $\mu = S$, Haldane again excludes the possibility of evolving overdominance. If $\mu \neq S$, and is a constant, it is easily seen that $F \rightarrow \infty$ as $S \rightarrow 0$, for then the heterozygotes carrying the modifier are effectively overdominant and only such heterozygotes will be permanently maintained in the population. If, for example, $S_1 = 10$ per cent, $S = 1$ per cent and $\mu = 10$ per cent, then $F = 100$, whereas Haldane considers values of F greater than 20 most improbable. Although lack of dominance of the modifier will diminish its relative rate of increase, linkage with the advantageous gene will certainly enormously increase its advantage, more especially if the modifier is initially rare. This will also be true of modifiers tending to increase the viability of Aa when S/S_1 is nearly 1.

It seems clear, from these calculations, that an advantageous gene may evolve overdominance during the course of its evolutionary progress. Experimental evidence for this lies in the work of Levene, Pavlovsky and Dobzhansky[27]. It is also, of course, well known that if a gene is initially overdominant in the heterozygote, a balanced polymorphism will result. As pointed out by Fisher[7], this means that although overdominance as an intrinsic phenomenon may be rare, cases of overdominant genes so maintained in a population will be particularly brought to our notice. However, the evidence, as mentioned earlier in the paper, for the widespread occurrence of linkage between interacting genetic factors in association with overdominance, argues against the simplest form of overdominance associated with a genetic difference at a single site (a muton or recon in Benzer's[41] terminology). As Lewis[42] mentions, even the simplest biochemical models of heterosis in *Neurospora* and *Aspergillus* heterocaryons are the result of the complementary effect of two dominant genes.

Evidence from Micro-organisms

Even in micro-organisms with probably only limited facilities for outcrossing there is considerable evidence for the existence of linked blocks of genes controlling closely related biochemical reactions, which must have an evolutionary origin. Thus, Demerec *et al.*[43] have shown in *Salmonella* that four loci controlling sequential steps in the synthesis of tryptophan occur in a closely linked complex. Similar results have been found for loci concerned with the synthesis of histidine and proline in *Salmonella*, and also for tryptophan loci in *E. coli*[44]. That such blocks are most likely to have an evolutionary origin is emphasized by the fact that the same four steps in tryptophan synthesis, which are controlled by a linked complex in *Salmonella* and *E. coli*, are controlled by four unlinked loci in *Neurospora*[45]. Furthermore, such linked complexes are not a peculiarity of the enteric bacteria because recent work of Gross and Fein[46] has shown the existence of a linked complex in *Neurospora* concerned with the synthesis of aromatic compounds. The work of Pardee *et al.*[47] in *Escherichia coli* on the loci involved in the control of the synthesis of β-galactosidase further shows the existence of linkage between genes which are interacting at an extremely basic level. This is a situation which surely must have an evolutionary origin.

If linkage and interaction are excluded as the basic ingredients for heterosis, and as we have defined it overdominance, it is necessary to search for some intrinsic advantage of overdominance. It has been suggested by Haldane[2] that a heterozygote may be advantageous because it may give rise to more biochemical diversity than either homozygote, as occurs, for example, with the hæmoglobins. However, the occurrence of interaction products from a heterozygote[48,49] belies this simple interpretation. Moreover, the postulated mechanism of Crick and Orgel (personal communication) for heterocaryon complementation certainly suggests the possibility that some heterozygotes are likely to be intrinsically disadvantageous. Rather it seems, from the sort of survey of heterozygote viabilities carried out by Stern and Novitski[50], that a fairly wide spectrum of heterozygote viabilities is likely to occur and that there is little reason to favour any particular combination.

Conclusion

In conclusion, we would argue that overdominance as it is commonly found in outbreeding species is, at least in many cases if not all, an evolutionary phenomenon which results from the nature of an outbreeding diploid species in exposing new geno combinations in a predominantly heterozygous condition. Even in cases where overdominance is found in ostensibly inbreeding species, these almost exclusively have a previous history of outbreeding[51] during which the overdominance may have evolved in the way we have suggested. Our mechanism is just another example of the way in which the breeding system determines the genetic structure of a species, in the same way that outbreeding leads to the balanced genotype of Mather or the integrated gene pool of Dobzhansky. The root of all this organization lies in the advantage of the outbreeding system which, as Darlington[52], Mather[21], Thoday[53], and probably Darwin before them have emphasized, is its flexibility in producing new variation upon which natural selection may act. The evolutionary process is the accumulation of successively interacting genetic factors which together build up the highly adapted and 'integrated' genotype which we now study.

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NEWS and VIEWS

Metallurgy and Fuel Technology at Cardiff: Prof. W. R. D. Jones

PROF. W. R. D. JONES will retire from the chair of metallurgy and fuel technology at the University College of South Wales and Monmouthshire, Cardiff, at the end of the present academic session. He graduated from that College in 1919, and in the same year commenced teaching duties. Coming from a family well known in mining circles, and forming a close association with the iron and steel industry in South Wales, his research work has nevertheless largely been directed towards light alloys containing magnesium. His determination of the copper–magnesium phase-diagram brought him his D.Sc. in 1932, and in the following year he was appointed head of the Department in succession to Prof. A. A. Read. Prof. Jones has always taken a very full part in academic work, being at various times deputy principal, dean of the Faculty of Science and a member of the Academic Board of the University of Wales. Combined with this he promoted a vigorous research school, the publications of which in the *Journal of the Institute of Metals* are notable contributions to our knowledge of magnesium alloys. Other work has been concerned with strain-ageing of steels and the viscosity of molten metals and alloys. His genial personality has made him popular with staff and students and he is a well-known figure in public and social life in Cardiff. He became actively interested in the South Wales Institute of Engineers and was elected president in 1953.

Dr. H. K. M. Lloyd

DR. H. K. M. LLOYD, who has been appointed to succeed Prof. W. R. D. Jones, is senior lecturer in metallurgy in the University of Nottingham. He graduated with honours in metallurgy at the University College of Swansea in 1942, and then gained industrial experience as a metallurgist in a steelworks, and afterwards became experimental officer in the Aeronautical Inspection Directorate. As a result of research on hydrogen in steel in the University of Sheffield he was awarded a Ph.D. in 1948 and the Brunton Medal, and continued for a year on the teaching staff. Since 1949 Dr. Lloyd has lectured at the University of Nottingham, first in the Mechanical Engineering Department, and from 1954 in the new Department of Metallurgy, which he largely established and equipped. He has maintained close contacts with the iron and steel industry and various engineering firms, which have sponsored some of his research work. This has chiefly been concerned with engineering metallurgy, and his work has been published in a variety of journals. In 1950 Dr. Lloyd was invited to examine metallurgical problems in an oil installation in the Persian Gulf, and during 1951 was awarded a Nuffield travelling fellowship and visited plants in the United States and Canada. His interests have extended to technical institutions and he is a governor of Corby Technical College, while as a founder member of the East Midlands Metallurgical Society he became president in 1953. In returning to Wales, Dr. Lloyd will feel quite at home in the metallurgical work which awaits him at Cardiff.

Mechanical Engineering at Sydney: Prof. P. T. Fink

AT the beginning of the current academic year, Peter Thomas Fink was appointed to the chair of mechanical engineering in the University of Sydney. Prof. Fink was educated in Birmingham, England, and the University of Sydney, where he graduated in aeronautical engineering in 1944. Continuing at Sydney as a research student and teaching fellow under Prof. A. V. Stephens, he worked on problems in aircraft stability in conjunction with the Commonwealth Scientific and Industrial Research Organization (Melbourne) until his appointment as assistant lecturer under Prof. A. A. (now Sir Arnold) Hall at the Department of Aeronautics, Imperial College of Science and Technology, London, in 1947. Prof. Fink spent the next ten years at the Imperial College as assistant lecturer, lecturer, and (from 1956) reader in aeronautics, playing a prominent part in the expansion of teaching and research activities under Prof. Hall and later Prof. H. B. Squire. During this period he published papers on aircraft stability and aerodynamics, including some important contributions to the understanding of the flow past slender delta wings. Prof. Fink also collaborated with Mr. Donald Campbell in the design of his record-breaking hydroplane. In 1957 he returned to Sydney to take up an appointment there as reader in aeronautical engineering.

The Iron and Steel Institute : Awards

THE Council of the Iron and Steel Institute has made the following awards : *Bessemer Gold Medal for 1961* : Mr. William Barr, director and chief metallurgist, Colvilles, Ltd., president during 1959–60 of the Institute ; *Sir Robert Hadfield Medal for 1961* : Mr. Emrys Davies, managing director, Brymbo Steel Works ; *Williams Prizes for 1960 (£50 each)* : Mr. T. McHugh and Mr. F. A. Kirk (Low Moor Fine Steels, Ltd.) for the paper "Some Aspect of Steel Extrusion" (*J. Iron and Steel Inst.*, 194, 423 ; 1960) ; their co-author, Mr. R. Cox, was not eligible for an award ; and Mr. J. Chapman (Steel, Peech and Tozer) and Mr. W. Montgomery (Round Oak Steel Works, Ltd.) for the paper "The Effect of Flow Distribution on Air Preheat in the Open-hearth Furnace" (*J. Iron and Steel Inst.*, 196, 15 ; 1960).

A New Department for Technical Co-operation

IN the House of Commons on March 21, the Prime Minister announced new procedures for dealing with technical assistance overseas. Hitherto, requests for such assistance have been dealt with through different administrative channels, depending on whether they came from a dependent territory, an independent country of the Commonwealth or a foreign country. Such arrangements were adequate in the past, but with the growth of size and importance of plans for helping overseas countries with trained men and women, special equipment, and so on, closer co-ordination has become necessary; the Government, therefore, proposes to set up a new Department of Technical Co-operation. The new Department, Mr. Macmillan said, would be in the charge of a Minister of equivalent rank to that of a Minister of State, and while the Foreign Secretary, the Commonwealth Secretary and the Colonial Secretary would continue to be responsible for matters of general policy, within these limits the new Department would take over the present responsibility of these three overseas Departments for the provision of technical assistance other than capital projects. It would be concerned with much of the technical assistance provided with the help of the United Kingdom through international organizations as well as with that given direct to the recipient countries. As one of its duties the new Department would administer the scheme set out in the White Paper of last October for the continued employment of overseas offices; but the transfer, promotion and discipline of the Overseas Service in dependent territories would remain the responsibility of the Colonial Secretary.

The statement was warmly welcomed by Mr. H. Gaitskell for the Opposition, and in reply to questions Mr. Macmillan added that the Vote would be separate and the Minister would be a member of the House of Commons. Capital aid would remain with the three overseas Departments, and the existing Colonial organization would continue. Educational assistance through the United Nations Educational, Scientific and Cultural Organization would still be a matter for the Ministry of Education, and the work of the British Council in providing teachers of English would continue, though Mr. Macmillan agreed that all this would need co-ordination, and that the new Department would take over many requests for educational assistance. It might also be preferable to continue to operate in inter-university matters through existing non-governmental bodies such as the Inter-University Council for Higher Education Overseas, or the Council for Overseas Colleges of Arts, Science and Technology and the Association of Universities of the British Commonwealth, although he thought the new Department might be able to help here, too. In reply to a suggestion that the new Department might have specific responsibility for the Inter-University Council, Mr. Macmillan said he would not like to give a precise answer without consulting the Chancellors of the Universities. Mr. Macmillan said, reasonably enough, that it would take some time to work out all the details. The new Minister would be concerned with providing training facilities in Britain for men and women from these territories, particularly from within the Commonwealth, as well as with finding and sending abroad technically trained people of British nationality. Some would belong to the Overseas Services, and others would be serving on contract for a limited period. A similar statement, made simultaneously by Lord Hailsham in the House of Lords, added that the proposals would mean bringing together work done in overseas Departments, and that in general the staff would come from these Departments. He also made it clear that the new Department would not be responsible for the Colonial Development Corporation, relations with which would continue to be handled through the Colonial Office and the Commonwealth Relations Office. The necessary Bill was read for the first time in the House of Commons on March 23.

The American Institute of Biological Sciences

SUPPORTED by a grant from the National Science Foundation, the American Institute of Biological Sciences has undertaken a Biological Sciences Communication Project to seek ways of improving the overall effectiveness of the control and dissemination of biological information. An examination of the methods of acquiring, indexing, storing and retrieving printed scientific literature will be followed by an investigation into the needs for and use of information by biologists, and of the characteristics of the dissemination activities of existing agencies. The effectiveness of visiting biologist's programmes, conferences and symposia will also be appraised and the project will consider developing a practical method for obtaining and disseminating in the

United States valuable research information obtained by United States scientists in visiting other countries. The project will also take account of the problem of scientific communication as influenced by the training of scientists and of the needs of teachers, administrators and science writers, which may be quite different from those of the research worker. An advisory committee has been formed to assist the Institute in providing liaison among allied biological groups.

Funds for Industrial Research in the United States

A PRELIMINARY report issued by the National Science Foundation indicates that funds for research and development by industry in the United States amounted in 1959 to 9,400 million dollars, an increase of 15 per cent on 1958, and the total is expected to reach 10,000 million dollars in 1960 (*Reviews of Data on Research and Development*, No. 24. N.S.F. 60–61. Washington 25, D.C., December 1960). Of the total, 5,400 million dollars came from federal funds, an increase of 17 per cent, whereas that financed by industry itself increased by 12 per cent. Expenditure on fundamental research was 344 million dollars, an increase of 17 per cent on 1958. Three-quarters of the Federal contribution went to two industries heavily engaged in research and development projects concerned with missiles, rockets and satellites, the aircraft industry alone receiving 2,500 million dollars, and the other industry, electrical equipment and communication, 1,600 million dollars.

Computing Chemical Plant Automation

THE first British-produced digital computing system to be used to control a chemical process without human intervention has been ordered by Imperial Chemical Industries, Ltd., for use in their chemical plant at Fleetwood, Lancs. The machine concerned is the Ferranti *Argus* computer, which, because it uses only solid-state electronic elements, is deemed reliable enough to operate in relatively 'docile' chemical processes without a constant human monitor. It is interesting to note that the computer is of the pluggable sequence type ; the 'plugs' in this case being small ferrite rods which alter the coupling between mesh points of a set of mutually orthogonal conductors. In line with recent thought, the *Argus* has facilities for time sharing, the acceptance and emission of analogue signals, and the capacity of accepting additional drum storage of up to 50,000 words. The Imperial Chemical Industries, Ltd., installation has the basic capacity of 1,024 orders and core storage for 1,024 words. More than 300 analogue signals of six different types can be read in and outputs to control 100 pneumatic valves are provided. The arithmetical speeds are such that 50,000 additions or 10,000 multiplications can be performed each second.

Coal Research in Australia

THE Coal Research Division of the Commonwealth Scientific and Industrial Research Organization has during the past four years published an attractive series of 16-page pamphlets (*Coal Research in C.S.I.R.O.*, Nos. 1 (May 1957)–11 (August 1960). Melbourne : Commonwealth Scientific and Industrial Research Organization) at the rate of three a year, describing facets of its work. Presentation and style are excellent ; the articles are brief, popularly written and broadly informative, yet technically precise. Directed towards industrial producers and users of coal, the series would appear to serve four purposes and these may be illustrated with reference to recent articles. The 'educative' function is represented at present by a series on macerals, petrological constituents of coals roughly analogous to the minerals in rocks. Their nature and appearance and their influence on properties of coal are described with the aid of typical photomicrographs. Descriptions of 'techniques' and of 'recent research work' are closely related to one another, though the emphasis differs greatly between general application and specific results according to the topic under discussion. Examples of the former are "Fluidization Technique", "Porosity Measurements", and fundamental methods of investigation such as "Molecular Still for Tar Distillation" and "High Sensitivity Gas Chromatography". Among the latter may be mentioned "Coal Structural Studies", "Coal Macerals in Solution", "Char Blending makes Better Coke" and "Air-Flow in Cupolas" ; the aim, methods used, type of information obtained, and likely applications or significance are all touched on. Finally, some articles might be regarded first and foremost as 'surveys of a field', as "Coke Production and Cupola Practice", "Utilization of High-Ash and Small Coals" and "Pitch for Making Electrode Carbon". The last-mentioned article, for example, gives information on methods of preparation and properties required. Each issue concludes with abstracts of papers published by staff of the Coal Research Division. While the breadth of treatment assists understanding and is to be preferred to a parochial approach, the impression is sometimes created (since acknowledgment of work elsewhere is not made) that the techniques, methods of interpretation, and detailed findings alike originated in the Research Organization's laboratories. Sometimes this is so ; but often the work described forms part of the world-wide advance, and the article is partly concerned with this broader advance. These pamphlets deserve attention and success for their competent attempt to 'sell' research in industry.

Technological Development in Poland

A RECENT issue of the *Review of the Polish Academy of Sciences* (No. 2, 5, April–June 1960) contains a survey by I. Malecki of fifteen years of technology in Poland and one by T. Nowicki of the six-years work of the Polish Academy of Sciences' Institute of Basic Technical Problems founded in 1953. Dealing first with the starting up of technology in Poland after the War, and the reconstruction and development of its technical basis, Dr. Malecki describes the development of particular industries, including transport and communications. Power production increased by 310 per cent between 1946 and 1958, production of cast iron and steel from 1,200,000 tons and 1,900,000 tons, respectively, in 1948, to 3,900,000 tons and 5,600,000 tons in 1958. There were major advances in the engineering industry which represented 19 per cent of industrial production in 1958 compared with 13 per cent in 1938, and the output of the chemical industry rose nine-fold compared with 1938. The industry now provides 70 per cent of pharmaceuticals compared with 12·5 per cent in 1949. The Institute of Basic Technical Problems comprises eight scientific centres covering three main fields : electrotechnics, mechanics and technical physics, and includes departments for the theory of telecommunication, electrical engineering, electronics, mechanics of continuous media, fluid mechanics, vibrations research and analogues, and a laboratory. for research on isotopes.

Radioisotopes and Radiation in Entomology

THE International Atomic Energy Agency in Vienna recently convened a symposium under the above title with the object of discussing and developing plans for the wider use of radioisotopes and radiation in the control of insect pests. It was held in Bombay during December 5–9, 1960, when twenty-five papers were read and discussed by delegates from the host country, India, Federal Republic of Germany, France, Italy, Pakistan, Philippines, United Kingdom, United States and U.S.S.R. The papers covered a pertinent range of topics, such as the use of irradiation as a direct means of disinfesting food products, as an indirect means by inducing sterility in male insects, and so interrupting reproduction in field populations, and the use of radioactive tracers in entomological and insect biochemical research. There was lively but constructive discussion, for example, in the respective merits of static radioactive materials and electrical machines as radiation sources. Through the kindness of the Indian Government the delegates visited the Atomic Energy Establishment and the new reactor at Trombay, where impressive facilities have been created for the peaceful applications of atomic energy. There are major agricultural and insect pest problems in the less-developed regions of the world in which irradiation and radioisotope techniques can undoubtedly play a valuable part. Excellent facilities for the application of these techniques now exist in India, so that the symposium was indeed timely and effective. The balanced and up-to-date nature of the programme will render the collected proceedings a valuable document provided that publication is not long delayed.

Sabouraudia

THE aptly named *Sabouraudia*, the new journal of the recently formed International Society for Human and Animal Mycology and the first number of which was published during January, can truly be regarded as a milestone marking the rapid extension of the science of mycology into the sphere of human and animal mycoses (Vol. 1, Part 1. Edited by J. C. Gentles. Pp. 72 + 12 plates. (Edinburgh and London: E. and S. Livingstone, Ltd., 1961). Subscription price per volume (four issues) 80*s*. or 12 dollars. Single parts 20*s*. or 3 dollars). Indeed, as pointed out in an excellent review of the subject by the president, Dr. Juan Makinnon, order is steadily being brought out of chaos by the insistence of a few discerning enthusiasts on the observance of the rules of the international codes of nomenclature for bacteriology and botany. It is, perhaps, the recent trend towards accuracy in species diagnosis and nomenclature of the causal fungi, in contrast to the over-emphasis on clinical aspects characteristic of older work, which has been responsible for the enhanced status of medical and veterinary mycology. This is well exemplified by two papers describing some of the techniques being evolved for obtaining the sexual, or 'perfect', states of parasites content to be known previously only by their conidial states. The remaining six papers are of an agreeably high standard, and mycology will be well served if the journal continues to maintain this standard. It is printed clearly on good quality, quarto-sized paper with excellently produced illustrations, and includes book and film reviews and news of scientific societies in a total of 71 pages.

Protection of Cleaned Iron Objects

THE protection of iron objects after they have been cleaned has for a long time been a major problem in the conservation of museum objects. In the February issue of the *Museums Journal*, Mr. K. J. Barton details some experiments and conclusions on this subject. The most interesting result is that the phosphate coated object (using one of the commercial phosphoric acid cleaners) shows no evidence of corrosion under the experiments. The one disadvantage of this coating is that the resultant colour is poor and always patchy. This can be overcome by dyeing the phosphate coating black with 'Hematine' crystals, thus giving a matt surface which can be polished with a stiff brush. Other tests covered vapour-phase inhibitors using cyclohexylamine carbonate. Shell Nos. 220 and 260 were found to be the most effective and their use is limited to objects made of steel, iron, tinplate, zinc and aluminium. They must not be used in association with lead or copper since they are liable to attack.

Atmosphere Temperature Fluctuations

THE subject of climatic change is one which has for long interested climatologists and meteorologists, but especially so during recent years. Direct temperature observations, the recession of glaciers, the movement of fish and insects northwards in the northern hemisphere, as well as a similar movement of the tree-line have all indicated an appreciable rise in atmospheric temperature in many parts of the world during the past few decades. A recent contribution to the subject has been made by G. S. Callendar, of the Ministry of Aviation, in a paper which appears in the *Quarterly Journal of the Royal Meteorological Society* (87, No. 371; Jan. 1961). From his analysis he concludes that the rising trend is significant from the Arctic to about 45° S. lat., but is small and indeed locally non-apparent in regions below 35° N. as yet. A number of theories to account for the rise have been advanced, and there has been much speculation as to whether or for how long it will continue. One widely held theory is to the effect that the rising trend is a result of increased incoming solar radiation, due either to an increased output of solar energy or to an increased atmospheric transparency resulting from a decrease in volcanic activity. Callendar considers that such a hypothesis is not inconsistent with the observed zonal short-period fluctuations, but that it fails to explain the pattern of the overall climatic trend. The latter is considered to be not incompatible with the increased carbon dioxide theory, which is to the effect that the trend could be due to back radiation from the additional carbon-dioxide produced by fossil-fuel combustion. The points at issue seem to be: Does Nature control the whole process, or has human activity caused the trend, on which Nature has merely imposed the fluctuations ?

Corrosion Prevention

A SPECIAL exhibition on metallic corrosion and its prevention is being organized at the Science Museum, South Kensington, during March 27–April 22. This collection of exhibits has been assembled by the Corrosion Group of the Society of Chemical Industry to mark the first International Congress on Metallic Corrosion, to be held in London during April 10–14. It has been estimated that the yearly cost of corrosion to Great Britain alone is at least £200 million (£5 a head per annum); the subject is, therefore, of great national economic importance and of direct interest to the layman. For this reason although the exhibition will be of particular interest to the delegates to the Congress, it has been designed so as to be intelligible and interesting to the non-specialists who form the majority of visitors to the Museum. It sets out to answer in simple terms the questions: What is Corrosion? How and when does it take place? What can be done to prevent it?

University Entrance

A SECOND edition of *University Entrance—The Basic Facts* has appeared. It will enable one to find out (*a*) which universities provide first-degree courses and in what subjects; (*b*) the minimum to be attained in matriculation for entering individual universities; (*c*) faculty and departmental requirements; (*d*) the approximate cost of study based on amounts paid at present by the Ministry of Education to scholars who need maximum help; (*e*) what the present policy is regarding grants to students; the approximate scale of awards and parental contribution. It is suggested to readers that they watch the national or educational Press for announcements about the Government's future policy on financial help to students. The pamphlet, published by the National Union of Teachers, can be obtained from Hamilton House, Mabledon Place, London, W.C.1 (price 4*s*. 6*d*. post free). It should be of great help to head-masters, teachers, parents and students.

Friedrich Vieweg and Son Anniversary Competition

ON April 1, 1786, Friedrich Vieweg established at Brunswick a publishing house that was to become associated with such great names as Goethe, Herder, Alexander and Wilhelm von Humboldt, Justus von Liebig, Gottfried Keller, Wilhelm Raabe, Hermann von Helmholtz, Wilhelm von Bode, Madame Curie, Arnold Sommerfeld, Ludwig Prandtl, Albert Einstein, Max von Laue and Erwin Schrödinger. During the past years the house has been mainly concerned with the publication of books and periodicals in the fields of mathematics, natural sciences and engineering. On the occasion of its one hundred and seventy-fifth anniversary the house of Friedrich Vieweg is offering a number of prizes for papers in the field of mathematics, physics and chemistry for publication in book form, to be judged by a panel of distinguished scientists. Further information can be obtained from Friedrich Vieweg und Sohn, Postfach 185, Burgplatz 1, Braunschweig, Germany.

Announcements

DR. J. T. DELACOUR, formerly director of the Los Angeles County Museum and president emeritus of the International Council for Bird Preservation, has been awarded the Addison Emery Verrill Medal of the Peabody Museum, Yale University.

DR. A. R. BATTERSBY, lecturer in chemistry in the University of Bristol, has been awarded the Corday-Morgan Medal and Prize of the Chemical Society, in consideration of his work on the stereochemistry of emetine and its congeners, and also on the chemistry of curare alkaloids and the biogenesis of papaverine. The award consists of a silver medal and a monetary prize.

THE British Iron and Steel Research Association will hold open days at its Sheffield Laboratories on June 15 and 16. Further information is available from the Association, 11 Park Lane, London, W.1.

THE spring meeting of the Parasitology Group of the Institute of Biology is to be held at Wills Hall, Bristol, during April 11–13. Included in the programme will be an invited address by Prof. C. P. Read on "The Host-Parasite Interface: Molecular Basis of Parasitism". Further information can be obtained from Dr. L. P. Joyner, Central Veterinary Laboratory, Ministry of Agriculture, Fisheries and Food, New Haw, Weybridge, Surrey.

A POSTGRADUATE course in nuclear technology (chemical) is being administered by the Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London. The course is to be full-time and of one year's duration. Applications for admission should be made by June 1 to the Registrar, Imperial College of Science and Technology, London, S.W.7, from whom further information and application forms can be obtained.

Two lectures on "Recent Advances in the Radiation Chemistry of Aqueous Systems" will be given by Prof. Gabriel Stein of the Department of Physical Chemistry, Hebrew University of Jerusalem, at the Imperial College of Science and Technology, London, during May 3–10. The first lecture will be on basic processes in aqueous solutions and the second on some systems of biochemical interest. Further information can be obtained from Dr. A. J. Swallow, Department of Chemical Engineering and Chemical Technology, Prince Consort Road, London, S.W.7.

THE European Association for Research on Plant Breeding is holding a conference on "Environmental Factors in the Distribution of Fruit Varieties" at Het Huis de Provincie, Arnhem, during April 27–28. The programme will include two sessions, namely: (1) surveying work on local growth conditions and plant responses; (2) analysis of causal relations. Further information can be obtained from the Institute of Horticultural Plant Breeding, P.O. Box 16, Wageningen, The Netherlands.

THE British Leather Manufacturers' Research Association is holding open days at its Laboratories, Milton Park, Egham, Surrey, during May 2–4. Included in the programme are two lectures, one by Mr. H. Bradley, director of research, British Boot, Shoe and Allied Trades Research Association, on "Requirements of the Shoe Industry from the Leather Industry", and the other by Dr. K. W. Pepper, director of the British Leather Manufacturers' Research Association, on "Adhesion in the Manufacture of Leather and Leather Goods". Further information can be obtained from the director at the Laboratories.

ERRATUM. In *Nature* of March 18, p. 865, column 2, line 16, for 'Melville' read 'Metcalfe'.

THE PRESENT STATE OF ERGODIC THEORY*

By Dr. I. E. FARQUHAR

Queen Mary College, London

THE purpose of ergodic theory is to establish the necessary and sufficient conditions for a system to approach equilibrium from a non-equilibrium situation. It does not define the manner in which a system approaches equilibrium; that is the province of transport theory and master equations. Ergodic theory has, in fact, the characteristics of a mathematical existence theorem, and it possesses, accordingly, both the strength and the weakness of such a theorem. Its strength lies in its generality—it deals with arbitrary physical systems in arbitrary situations. Its weakness appears whenever attempts are made to apply it to any specific physical situation.

The procedure is to derive conditions for the equality of time and ensemble averages, in classical statistics, of phase functions, and in quantum statistics of expectation values of operators. Here there is illustrated one example of the lack of specific application of ergodic theory, for the time averages considered are always evaluated over an infinite period. To interpret these time averages physically, that is, as values observable physically during the time intervals occupied by measurements on the system, it is necessary to assume that the appropriate relaxation time of the system is much shorter than the duration of the measurement. Yet ergodic theory in itself provides no estimate of this relaxation time, so that, to apply ergodic theory in any specific case, recourse must be had either to some other theory, or to experiment, in order to obtain this relaxation time. Further, just as it says nothing about values of relaxation times, so ergodic theory yields no information regarding values of transport coefficients, although it is perfectly consistent with the occurrence of transport processes.

In classical statistics, Birkhoff's theorem[1] remains basic. It asserts the equality of time and ensemble averages almost everywhere on the surface of constant energy in phase space corresponding to the energy of an isolated system, provided this energy surface is metrically indecomposable. This is equivalent to the requirement that there are no invariant integrals of the motion independent of the energy. It has, in fact, been shown[2] that time and ensemble averages are equal, almost everywhere on the reduced manifold in phase space which is defined by the set of *all* invariants of motion of the system.

Although metrical indecomposability is thus a criterion for ergodicity, the trouble is that it is not known how many invariants of the motion an arbitrary physical system possesses. Hence, efforts have been made to circumvent the question of metrical indecomposability by restricting the scope of the theorems.

One method of doing so is to give full weight to the fact that statistical mechanics deals with macroscopic systems. Birkhoff's theorem is a result of general dynamics, independent of the number of degrees of freedom of the system. But it may be inquired whether time and ensemble averages are equal asymptotically in the limit as the number of degrees of freedom of the system becomes infinite. An asymptotic ergodic theorem of this form, without any requirement of metrical indecomposability, has been given by Khinchin[3] by restricting the class of phase functions, the ergodic behaviour of which is required. Khinchin's restriction is to the class of phase functions the phase dispersion of which is small, although it is only for phase functions which are sums of corresponding functions for the individual molecules of a system with separable Hamiltonian that Khinchin has shown the phase dispersion to be small. In certain cases where Birkhoff's theorem asserts that a system is non-ergodic, Khinchin's approach gives the positive result that the departure from ergodic behaviour is, nevertheless, vanishingly small in the limit of an infinite number of degrees of freedom.

In all these cases, the theorems are valid 'almost everywhere', and so some form of 'coarse-graining', by introducing phase cells, is required to avoid effects associated with exceptional phase points from showing up in practice.

In quantum statistics the basic treatment has been that of von Neumann[4], who showed how phase cells and 'coarse-grained' operators may be defined, which are consistent with the quantum-mechanical commutation rules. It is in terms of such coarse-grained operators and corresponding coarse-grained distributions that thermodynamical properties are described. By making use of the macroscopic nature of the system, here reflected in the high density of quantum energy-levels, von Neumann established an ergodic theorem, but only at the expense of introducing an averaging over all possible coarse distributions; these are treated as equally probable. Without such an averaging, von Neumann could not eliminate reference to the particular coarse-grained distribution used. Von Neumann's proof depended on the Hamiltonian of the system being non-degenerate and this requirement of non-degeneracy came to be regarded as the quantum-mechanical ergodicity condition, analogous to the classical criterion of metrical indecomposability.

However, it was shown recently[5] that von Neumann's argument does not in fact yield any criterion for ergodicity, since his proof may be carried through even if the Hamiltonian is degenerate. It is the assumption in von Neumann's approach, of equal *a priori* probabilities for coarse distributions, that leads to the result that *all* systems are ergodic. This overstrong assumption removes not only the possibility of obtaining ergodicity conditions[5], but also any requirement on the time evolution of the system other than that the norm of the state vector be constant. In particular, the time evolution operator need not be unitary[6]. The assumption of equi-probability of coarse distributions thus leads to results which are too general to be of physical significance. This does not, of course, invalidate von Neumann's general method of averaging over coarse distributions, but only the special case in which the *a priori* distribution of these coarse distributions is assumed equi-probable.

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* Substance of paper at a conference on "Statistical Mechanics and Irreversibility" arranged by the Institute of Physics and the Physical Society and held at Queen Mary College, London, during December 19–20.

An alternative proposal put forward in a series of recent papers by an Italian school[7] is to average, not over all coarse distributions for a fixed initial state, but over all initial states for a fixed coarse distribution. It is assumed that all initial states are *a priori* equally probable and hence the averaging methods developed by von Neumann may be used. Once again, this treatment is too general to yield any ergodicity criterion; but, unlike von Neumann's method, it does require that the time evolution operator of the system be unitary.

To obtain ergodicity conditions it would be necessary, in both von Neumann's and the Italians' treatments, to make a less strong assumption regarding the *a priori* distribution, whether of coarse distributions or of initial states. The Italians themselves[8] have gone some way towards relaxing this strong assumption by replacing the averaging over all initial states of the entire energy shell by separate averaging within each phase cell; the initial states within any phase cell again are assumed equally probable. This procedure does yield ergodicity conditions, but the conditions are somewhat formal and, although they can be linked with the Hamiltonian of the system, the connexion has not been worked out fully. Furthermore, as with the classical criterion of metrical indecomposability, it would appear difficult to determine whether actual physical systems satisfy the conditions. Other ergodicity conditions, which again have a rather tenuous connexion with the Hamiltonian, have been obtained by Landsberg[9], who considers a class of averaging processes which includes, as special cases, averaging over coarse distributions and over initial states. The class is defined in such a way that averages are independent of particular phase cells and quantum states, and, as with Khinchin's theorem, a condition on the averaging procedure is required.

Ergodic theory has been attacked[10] on the grounds that it is not of physical significance to consider, as it does, an isolated system. Two points may be made here: first, in the attempt to relate dynamical and thermodynamical properties of a system, ergodic theory provides only a first step; once ergodic behaviour has been established, the system of interest, hitherto considered isolated, must be coupled to a large heat-bath, and only the combined system is then regarded as isolated. The interaction between system and surroundings is vital. Secondly, those who favour introducing irreversibility[10] by way of external influences at the boundaries of a non-isolated system do not appear to be justified in assuming arbitrarily that these external influences are random. Although an arbitrary assumption regarding *a priori* probabilities appears both in von Neumann's and in the Italians' treatments, this assumption refers to methods of handling the system rather than to the system itself. The assumptions therefore are not 'assumptions of disorder', in the sense in which the term was used in early work on kinetic theory.

The work of van Hove[11] on master equations does not fall within the province of ergodic theory, since it deals with particular systems and seeks to study in detail their time-development. It seems, however, that a generalization of this approach may well prove to be a most fruitful method of studying the approach to equilibrium of arbitrary systems.

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TERPENE CHEMISTRY

A SYMPOSIUM on terpene chemistry, organized by the Chemical Society, was held in the Chemistry Department of the Imperial College of Science and Technology on February 23, and attracted an audience of more than two hundred. The programme consisted of six main lectures.

The first session was opened by the president, Sir Alexander Todd, who gave a warm welcome to the speakers and visitors from abroad. Prof. E. R. H. Jones was chairman of the opening session, in which the first paper on "Terpene Compounds with Ten-membered Rings" was given by Prof. F. Šorm, of Prague. After a brief historical introduction, the chemistry of germacrome, a typical ten-membered cyclic terpene, was reviewed in detail, special reference being made to the trans-annular reactions which are so characteristic of this series. A theoretical treatment of the non-classical interaction of double bonds in this series was also provided. Further examples of structural determinations of this class of terpenes were provided by costunolide, balchanolide and *iso*balchanolide, cnicin, scabiolide and parthenolide.

Later, Prof. B. C. L. Weedon (Queen Mary College) reviewed his recent work on structure determination and synthesis of carotenoids and their biological precursors. Particularly striking results have been achieved with the aid of nuclear magnetic measurements carried out and interpreted by Dr. L. M. Jackman at the Imperial College. Examples included spirilloxanthin, spheroidene and the paprika ketones capsorubin, capsanthin and capsanthone. Physical methods have also been applied successfully in determining the structures of a variety of hydrolycopenes, for example, phytoene, ζ-carotene and neurosporene, believed to be biogenetic intermediates of carotenoids. New methods for building up the carotenoid ring systems were also described as part of the syntheses of canthaxanthin and astacene.

After lunch, Prof. D. H. R. Barton introduced Prof. O. Jeger (Zurich), who discussed the structures of a series of oxidation products of the diterpenes manool and sclareol. Many of these had potential values in the perfumery industry, and evidence for the structures suggested was based on numerous physical measurements as well as chemical degradations and correlations with other diterpene degradation products; a notable feature of this symposium was the extensive use which chemists are making of nuclear magnetic resonance measurements in structural problems. Prof. H. C. H. Erdtman (Stockholm) then correlated, so far as was possible, the distribution of terpenes among the various orders of the Coniferae and stressed the taxonomic importance of this group of organic compounds. This extensive survey will have far-reaching effects on biogenetic studies of the terpenes; in the course of the work several new terpenes were discovered and the elucidation of these structures was also described.

In the final session, the two papers presented related to the determination of the structure of the complex terpenoid bitter principle, clerodin. Prof. Johnson, as chairman, introduced Prof. Monteath Robertson, who described the potentialities of the X-ray diffraction method in the elucidation of structures of molecules containing up to one hundred atoms (other than hydrogen).

Prof. Robertson's colleague, Dr. G. A. Sim, then illustrated the general points by discussing the case of clerodin (as a simple derivative) in detail. This elegant work was only one of several similar topics solved by this method in the Glasgow laboratories. Prof. Barton, in his usual lucid style, then discussed the underlying chemistry of this substance and showed how the structure had been derived on the basis of chemical degradations. His work was also complementary to the nuclear magnetic resonance measurements carried out by Dr. Jackman, who described this aspect of the problem. In the ensuing discussion, Dr. K. H. Overton (Glasgow) outlined his elucidation of the stereochemistry of the bitter principle, columbin.

A. W. JOHNSON

AUSTRALIAN ARID ZONE TECHNICAL CONFERENCE

AN Arid Zone Technical Conference was held at Warburton, Victoria, during December 5–9. It was attended by more than seventy delegates representing a very wide range of organizations and sciences. Several overseas visitors were present as observers, including Dr. P. C. Raheja and Dr. Y. Satyanarayan (Central Arid Zone Research Institute, India), Dr. M. H. Aslan (Desert Range Research Station, Egypt) and Mr. M. U. Khan (Meteorological Service, Pakistan).

The programme was organized into nine major sessions. In Session 1, the physical characteristics of the Australian arid zone were outlined in terms of geology (L. C. Noakes, Bureau of Mineral Resources, Canberra), geomorphology (J. A. Mabbutt, Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O.), Canberra), soils (C. G. Stephens, C.S.I.R.O., Adelaide), vegetation (N. C. W. Beadle, University of New England, Armidale), and climate (H. L. Ashton, Bureau of Meteorology, Melbourne). This material was primarily descriptive in nature and provided a continent-wide background for those scientists not directly active in arid zone problems, or concerned with only one geographical locality in the arid zone.

Sessions 2 and 3 were devoted to the principles and problems of plant and animal production and husbandry, and formed the central basis of the conference. Review papers were presented on plant production (R. O. Slatyer, C.S.I.R.O., Canberra), and utilization (R. M. Moore, C.S.I.R.O., Canberra), and on animal production (W. V. Macfarlane, Australian National University, Canberra), and animal husbandry (G. R. Moule, C.S.I.R.O., Prospect and J. H. Whittem, Animal Industry Branch, Alice Springs) and an additional thirty-four contributed papers covered a wide range of specific subjects within these fields of research. In Session 2 data were presented indicating that the existing communities are relatively inefficient in the conversion of rainfall to plant material, and that this phenomenon is due in part to extreme soil mineral deficiency and the physiological and ecological characteristics of the individual species, as well as to the low and intermittent rainfall regime. Experimental data were also presented on the possibilities of introducing new plant species and the problems associated with their establishment, persistence and natural dissemination. This information, together with a recognition of the desirability of concentrating water run-off for more effective use, led to the general concept of concentrating effort for increased plant production on the most favoured parts of the landscape, rather than attempting a blanket improvement of the whole countryside.

The management of the pasture communities under grazing is of special importance under arid conditions, partly because of the need to maintain reserves of perennial evergreen species for drought reserve and partly because of the delicate plant-climate balance and the ease with which communities can be damaged by overgrazing. Also, efficient utilization is influenced by property development, particularly by spacing of watering points and fencing. It was considered that the most advantageous developments in this field can only be achieved if the requirements and responses of the animal are also considered. In consequence, integrated studies of both plants and animals, with the development of range condition standards and management procedures, was recommended.

The main form of commercial production in arid areas in Australia appears likely to remain some form of animal production and this will be restricted mainly to sheep and cattle. In Session 3, it was emphasized that there is need for more information about both species, but particularly cattle, and with special reference to adaptation, nutrition and reproduction in the more tropical parts of the zone, where there is at present active development of the cattle industry.

As any widespread change in the level of nutrition in the more arid areas will probably be a slow process, emphasis was placed on the need to breed better adapted and better producing animals, either by selection within the genetic material already in the country or by introducing new genes. Evidence was given of advances in the first field, and it was also indicated that the determination of selection criteria could be assisted by more basic physiological study, one particular example being potassium-levels in the blood.

To a large extent, the cattle population of the arid area and to a lesser extent the sheep population, exist as a series of wild herds and flocks. While property management aims at bringing them under better control, management practices are introduced against a background of almost complete lack of quantitative information on the ecology of the herds and flocks, their drinking and grazing habits, reproductive patterns, the nature and time of mortalities, and their individual, group and herd behaviour. It was recommended that a detailed study of these, using ecological and behavioural approaches developed by wild-life ecologists, should be encouraged.

Sessions 4–7 dealt with aspects of water and included a review paper (R. Powles, Soil Conservation Service, Condobolin) and contributed papers on soil and water conservation, underground water, irrigation and special aspects such as the suppression of evaporation from water storages, the sealing of earth dams, demineralization, and the significance of advection phenomena in arid zone irrigation areas. The importance of evaluating quantitatively the distribution and nature of occurrence of the limited water resources was emphasized. For maximum effectiveness it was suggested that such an inventory should reveal the dynamic characteristics of individual aquifers and catchments, and since it is impossible, at present, to envisage the appraisal of all water resources in this manner, it was proposed that a systematic approach should be adopted in which selected type situations within defined landscape patterns (land systems) could be studied and the results extrapolated as widely as possible.

Session 8 dealt with human settlement in arid regions and included contributions on demography (W. D. Borrie, Australian National University, Canberra), human function (W. V. Macfarlane, Australian National University, Canberra) and human organization (R. K. Macpherson, University of Sydney) in the Australian arid zone. It was apparent that there is a tendency in Australia to take arid zone environments for granted with all their physical disabilities and other stresses on human beings. The conference stressed that much could be done to make living conditions more acceptable.

The conference was impressed by the advantages following from the combined discussion of arid problems by the representatives of many different disciplines and stressed the need to define, in biological terms, the problems of plant and animal production specific to the arid zone ecosystem. Perhaps the main impression gained was a general confidence among delegates that research would achieve more efficient production from arid and semi-arid areas. There was much evidence of research having entered a phase in which quantitative data were becoming available to replace subjective estimates. This particularly applied in such fields as mineral resources, hydrology, microclimatology, geomorphology, plant and animal physiology, animal breeding, economics and human physiology.

C. S. CHRISTIAN
R. O. SLATYER

FUTURE OF NATURAL FAUNAS

THE twenty-sixth annual meeting of the Association of British Zoologists was held at the Zoological Society of London on January 7 under the presidency of Prof. C. M. Yonge. The theme of "Looking Ahead—The Future of Natural Faunas" was introduced by Sir Julian Huxley, who described and illustrated, by means of colour slides, what he had seen on his recent visit to East Africa. The central problem there, he said, was essentially one of human ecology. The fauna was a relic of the pre-human ecological climax and still presented a unique array of types in which some twenty-five or more species appeared to be collaborating in exploiting a number of exceptionally brittle habitats. The advent of pastoralism had brought many problems in its train and the situation was aggravated by the African, who thought of cattle as a symbol of wealth and prestige rather than as a source of protein. Recent studies by a number of investigators, including Fulbright Scholars, had shown that the land could be induced to yield more protein per acre from game than from cattle. In South Africa, a depleted fauna was being given some encouragement in game 'farms' from which there was a large demand for animals for re-stocking purposes. Sir Julian emphasized the importance of the tourist trade as an economic factor of conservation and he pointed out that parks were already being recognized as a source of tribal pride. The future of the fauna of East Africa was certainly in the balance, but the situation was not nearly as bad as he thought it would be when he went out there. Sir Julian concluded by directing attention to the conference at Arusha in Tanganyika in September, which was being organized by the International Union for the Conservation of Nature and other agencies with the object of influencing African public opinion towards conservation and the wise use of natural resources, including wild life.

Mr. Max Nicholson (director-general, Nature Conservancy) said that apart from the virtually unpredictable effects of human population pressure during the next forty years, the future of the British fauna depended to a great extent on climatic changes. These might include some brought about by the agency of man such as a barrage across the Bering Strait, the break-up of Arctic pack-ice by atomic means, the reversal of flow of some of the big Russian rivers and the flooding of depressions in the Sahara. The consequent rise in ocean-levels and the effects on the course of the Gulf Stream might well induce a Mediterranean type of climate in Britain. Birds could be regarded as sensitive indicators of climatic change, and it was significant that the rook and green woodpecker were steadily moving north. Although it was possible that no species would be lost in the next forty years, there was a tendency towards the replacement of a broad range of specialized species by a narrow range of adaptable species. Among the outstanding hazards to which the fauna was subject were the exploitation of waterways, marshland and estuaries, pollution by oil and toxic chemicals, mortality on fast highways, the burning of heathland and the sprawling out of cities. Isolation was becoming increasingly difficult to find and the time might well be coming when it would have to be administratively regulated. Referring to the work of conservancy in managing and studying what he hoped would eventually be some 150 reserves in Britain, a number representing the full spectrum of the country's natural habitats, Mr. Nicholson said that people were getting away from the sentimental idea that reserves were places where nothing was killed. The cropping of vertebrate resources might prove to be the best form of land management.

Dr. L. Harrison Matthews (scientific director of the Zoological Society of London) said that of the three orders of marine mammals, the Cetacea, the Pinnipedia and the Sirenia, the future of the whales was unquestionably precarious, but as the future of whaling was precarious too, the two facts might cancel each other out. There had been a virtual break-down in the international control of the situation; populations of the blue whale might be at a minimum for recovery and the overall length of the fin-whale was getting smaller. However, with the advent of pelagic factory-ships, whaling as an economic proposition might stop long before the whales were killed off, although the Japanese, who hunted for meat, might well go on. He thought that if carefully managed the future of the seals was reasonably secure; the elephant seal was now re-colonizing its former haunts in the Antarctic and the walrus seemed to be holding its own. Less, however, could be said about the future of the sirenids, the dugongs and manatees. The latter were now less common than they were in the Carribean, but were reasonably plentiful in northern South America. Recent trials had shown that the manatee had an unusual appetite for grasses and aquatic herbs, and if it could be successfully introduced into weed-choked waterways it might become a useful and important animal in the ecology of the tropics.

Mr. P. H. Greenwood (British Museum (Natural History)) said that the freshwater fauna of tropical Africa, particularly the fishes, posed numerous evolutionary, zoogeographical and ecological problems. Each lake was characterized by a high degree of endemism, especially among the Cichlidae, which had undergone extensive adaptive radiation in the past ten million years. Likewise, each river system was characterized by its own assemblage of species co-existing with others of a more general distribution. The danger, he thought, was in the widespread introduction of species from one system or lake to another where it was not endemic. These actions were partly the consequence of steps taken to counter-balance the threat or the fact of the over-fishing of the endemic species. The results were unpredictable because so little was known about the overall ecology of the waters, but they might well upset the bionomy of the lakes. Mr. Greenwood pointed out that some species were known to run up rivers and temporary flood streams to spawn in areas inundated during the biannual rains. Such species were particularly vulnerable to the effects of flood control and irrigation projects. As the future of the two British freshwater stations in Uganda and Rhodesia was by no means assured, he hoped that every effort would be made to ensure their continued existence.

Dr. Peter Crowcroft (British Museum, (Natural History)) reviewed what little information was available about changes in the status of Australian mammals, particularly the small marsupials and native rodents which had been replaced over immense areas by the introduced house mouse and ship rat. The introduction of the European fox constituted one of the greatest threats to many terrestial forms, but the available evidence suggested that the arboreal marsupials were in no danger. It was noteworthy, too, that both the Monotremes, the spiny anteater (*Tachyglossus*) and the duck-billed platypus (*Ornithorhyncus*) were widespread and abundant in the eastern States. Although Tasmania possessed less species, it appeared to have a better chance of preserving them, particularly as the fox had not been introduced. The fate of the thylacine or Tasmanian 'tiger' remained in doubt, but the Tasmanian devil (*Sarcophilus*) was abundant. An important new factor in conservation was the recently formed Australian Mammal Society, which was stimulating research and co-ordinating survey work. This should enable further changes to be assessed with far greater accuracy. The paucity of information about the distribution and numbers of Australian mammals in the last century made it difficult to distinguish between those changes caused by settlement and those which were already proceeding naturally, perhaps as a consequence of climatic changes.

JOHN HILLABY

COLONIAL RESEARCH, 1959-60

*COLONIAL RESEARCH, 1959–60**, follows the pattern of recent reports. It is slightly shorter, due in part to the disappearance of a report from the disbanded Colonial Products Council, which is replaced by a brief report from the Tropical Products Institute of the Department of Scientific and Industrial Research on work done on behalf of Colonial territories. It also includes the fifteenth annual reports of the Committee for Colonial Agricultural, Animal Health and Forestry Research and the Colonial Medical Research Council, the sixteenth annual report of the Colonial Social Science Research Council, the thirteenth annual reports of the Colonial Economic Research Committee and the Colonial Pesticides Research Committee, the fifth annual report of the Colonial Road Research Committee, the annual report on fisheries research of the Colonial Fisheries Advisory Committee and reports from the Tsetse Fly and Trypanosomiasis Committee and the Director of the Anti-Locust Research Centre. Besides the annual report on Colonial research, there is a section dealing with research matters not covered by the reports of the specialist advisory bodies, and this refers to building and housing research, industrial and engineering research, meteorology, and geological surveys, as well as to the work of the Falkland

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* Colonial Office. *Colonial Research, 1959–60*: Pp. 381. (Cmnd. 1215.) (London: H.M. Stationery Office, 1960.) 17s. net.

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Islands Dependencies Survey and of the Man-power Research Unit, Jamaica.

The annual report on Colonial research notes the dissolution of the Colonial Research Council, and afterwards of the Colonial Road Research Committee, in June 1960, and of the Colonial Medical Research Committee, in July 1960. The functions of the former have been absorbed by the new Committee on Overseas Road Research, which will advise the Road Research Board of the Department of Scientific and Industrial Research, and also the Colonial Secretary, on Colonial road research. Those of the latter have been absorbed by the new Tropical Medicine Research Board set up by the Medical Research Council, and the new Board will advise the Colonial Secretary, through the Medical Research Council, on all medical research in and for the Colonies financed from Colonial Development and Welfare Funds. To the work of the Overseas Research Council there is only the slightest reference: the Council is said to be considering the retention and recruitment of scientific staff for work overseas, and it is stated that the Council will be able to call for advice on existing research organizations, including the Colonial Research Advisory Committee; but it is apparent that, while these bodies are to keep the Overseas Research Council informed of their activities, they are to report direct to the Colonial Secretary.

There is, in fact, nothing in *Colonial Research, 1959–60,* to provide the conspectus of overseas research which the establishment of the new Overseas Research Council might be expected to provide, or to indicate that the new Council is likely to be in a position to provide such a conspectus in the near future. Nor can any justification be found in its pages for the change of name, and the dropping of a honoured term to which the Colonial Secretary paid a timely and handsome tribute in the House of Commons in December. It would appear that activities will continue much as before, and there is no hint of the vision and imagination that are required to seize the opportunities that overseas research and technical assistance generally now present.

These opportunities are, in fact, stressed by the substantial increase in Colonial Development and Welfare Research Schemes made during the year. The 134 new schemes and 82 supplementary schemes, involving grants totalling £2,370,261, compare with 71 new schemes and 63 supplementary schemes, totalling £798,974 in the previous year, and brought the total allocation to research schemes since 1940 to £21,864,193. About 42·6 per cent of the £2·37 million granted in 1959–60 was for agricultural, animal health and forestry schemes, 15·8 per cent for medical research, 2·6 per cent for fisheries research, 1·9 per cent for tsetse and trypanosomiasis research, 2·1 per cent for social science economic research, 23·7 per cent for pesticides research, 2·4 per cent for products research, and 7·1 per cent for anti-locust research. About 33·5 per cent of the gross allocation was for schemes to benefit the East African territories, 7 per cent for the West African group, 15·5 per cent for South-East Asian territories and Hong Kong, 12·5 per cent for the West Indian Colonies, British Guinea and British Honduras, 1 per cent for the Central African territories and 30·5 per cent for other territories or for schemes of general interest.

Twenty-four new appointments were made during the year to the Research Branch of the Overseas Civil Service; but the total complement in March 1960 was 199 compared with 207 in March 1959. Two Research Fellows, one studying human trypanosomiasis at the West African Institute for Trypanosomiasis Research, and the other investigating arterial hypertension in Jamaica at the University College of the West Indies, completed their investigations during the year; a third Research Fellow, studying the effect of diet on the levels of various co-factors and enzymes concerned with carbohydrate and fat metabolism at the University College of the West Indies was released from his fellowship for other research work. Two further research fellowships and six research studentships were awarded during the year.

Although, as already stated, *Colonial Research* does not provide the conspectus of overseas research that might be expected and that indeed is essential, the reports of the several specialist advisory committees, besides including a list of publications during the year, contain much information on research activities outside the direct responsibility of the particular committees. That of the Committee for Colonial Agricultural, Animal Health and Forestry Research —much the longest—includes accounts of the work of the several regional research organizations in that field in East and West Africa, and the West Indies and of the Imperial College of Tropical Agriculture, and also accounts of research carried out by the Colonial Departments of Agriculture, Forestry and Animal Health. Similarly, the Colonial Fisheries Advisory Committee reports on the work of the Sierra Leone Fisheries Research and Development Unit, the East African Fisheries Research Organization, Jinga, the East African Marine Fisheries Organization, Zanzibar, the Northern Rhodesian–Nyasaland Joint Fisheries Research Organization, the Tropical Fish Culture Research Institute, Malacca, and the Fisheries Research Unit of the University of Hong Kong. The Colonial Medical Research Committee likewise reports on the work of the regional organizations for medical research, and a research undertaken and financed by the Medical Departments of Colonial Territories, while that of the Colonial Pesticides Research Committee, besides reviewing the work done for the Committee by its research units at Porton and in East Africa, by the Agricultural Research Council's Unit of Experimental Agronomy, Oxford, at Long Ashton Research Station, at Rothamsted Experimental Station, at the Imperial College Field Station, Silwood Park, and by the Herbicide Unit of the Regional Research Centre, Trinidad, gives more than half its space to research not under the ægis of the Committee. The report of the Colonial Social Science Research Council has appended to it the annual reports of the East African Institute of Social Research, the Nigerian Institute of Social and Economic Research, the Institute of Social and Economic Research, the University College of the West Indies and of the Rhodes–Livingstone Institute. The report of the Tsetse Fly and Trypanosomiasis Committee is essentially an account of the work of the regional research organizations in East and West Africa, supplemented by a review of activities of territorial departments, and that of the Director of the Anti-Locust Research Centre on Locust Research and Control is no less essentially an account of regional and co-operative effort.

The material is thus largely available for some authoritative conspectus of research effort overseas and also much evidence of the extent to which that effort is interlocked with research effort in Britain generally. What is lacking appears to be the authority or the will. If the Minister for Science is indisposed to accept responsibility which appears to arise directly out of his responsibility for the Overseas Research Council and the other Research Councils, the inquiry already set on foot by the Colonial Secretary into the organization of technical assistance can scarcely fail to shatter the present complacency. It may stimulate something of the reappraisal and oversight of the whole of this research effort and its adequacy in relation to existing and future needs and to available resources of man-power as well as of finance that is overdue.

THE PORTUGUESE MAN-OF-WAR

IN a well-produced report on the Portuguese man-of-war*, Mr. A. K. Totton and Dr. G. O. Mackie have written complementary accounts, natural history and morphology by the former, and behaviour and histology by the latter. The two sections are based, partly on material from the *Discovery* collections, and partly on the results of a three-months trip to the Canaries.

Mr. Totton provides a non-technical description of *Physalia* for the layman and goes on to describe its habits, somersaulting behaviour, flotation and motion in relation to wind and water. From experiments at Arrecife he finds that the animal floats with its long axis at an angle of about 45° from the down-wind direction. He does not accept Woodcock's hypothesis that specimens from the southern hemisphere are nearly always mirror images of those from north of the equator, and the following explanation advanced by Mr. Totton appears to be much more satisfactory: "I think that left or right-handedness in a particular individual must be established on the first windy day that the larva keeps to the surface. The larval tentacle would cause a drag on the windward side, so that the float would be blown (so to speak) to leeward. As new tentacles grow. . . this drag would be increased and the part of the float from which they are budded would become bowed out to windward as a bulge, resulting fortuitously in a left- or right-handed individual".

The main part of Mr. Totton's report, however, is a detailed re-investigation of the complex budding pattern in the cormidial groups of appendages, in which he has been able to substantiate and amplify the basic work of Huxley, Lens and van Riemsdijk, Steche and Okada. These structures are described in great detail, and there are many excellent photographs; but this morphological section would have been easier to understand if more stylized drawings (like text-figure 26), as well as stereo-diagrams, had been used to clarify the complicated pattern of budding.

On origins, the author accepts the well-known idea that the ancestral form was a kind of actinula with an apical float and that this became colonial, as implied by Delage and Hérouard (1901, pl. 28). It is further suggested that "the evolution of *Physalia* and the other siphonophores seems to be linked with that of certain corymorphine, myriotheline and margelopsine hydroids; with *Pelagohydra* and with the so-called Disconantha (*Velella, Porpema, Porpita*)". This is a curious assemblage of forms because the tubularoids (including, *inter alia*, corymorphines and margelopsines), the myriothelines and the so-called Disconantha (Chondrophores) are very distinct hydroid groups. In particular, the Chondrophores have been conclusively shown to be 'pteronemid' hydroids (that is, allied to *Zanclea* rather than *Tubularia*) by Picard, but the author seems to have overlooked this and much other recent work bearing on his problem. Nor does he discuss the possibility of siphonophore evolution from a group other than capitate hydroids, and, in this connexion, the Actinulida have a strong claim for consideration.

There is some confusion in terminology, there being, for example, no distinction drawn between a hydromedusan gonozooid (medusa or medusa bud) and a disconanth gonozooid (reproductive hydranth bearing medusa buds). It is also surprising to find that the author equates "an asexual replicated gonozooid of *Velella*" with a blastostyle of *Climacocodon*. Dr. Mackie (p. 372) also promotes this same view that the blastostyles of *Corymorpha* and the reproductive polyps of *Velella* "are alike and homologous in every respect", but this interpretation is unlikely to be acceptable to hydroid specialists.

On the association of the fish *Nomeus* with *Physalia*, Mr. Totton does not discuss a new interpretation of the immunity of some fish to cœlenterate stings put forward by Davenport and Norris. These authors, working on *Amphiprion* and the sea anemone *Stoichactis*, demonstrated that while the thick mucus covering of the fish remains intact, the nematocysts are not stimulated, but once this is removed (as with contact with a net) the fish is vulnerable. Some such mechanism may protect *Nomeus*.

Dr. Mackie's plan to investigate the behaviour and reactions of *Physalia* was broadened to include also histology in order to establish, in particular, the extent of the nervous co-ordination between the different parts of a siphonophore colony. He was able to examine the muscular and nervous systems, the mesoglœa, the gas gland, the nematocysts and the histology of the digestive regions, the whole being a valuable contribution to siphonophore biology.

In specimens of *Physalia* exposed to the wind, the crest or sail is erected, and, although the way in which the wind acts has not been determined, any stimulus causing an overall tightening of the float musculature will normally result in crest erection. Dr. Mackie aptly describes the mechanism as reminiscent of a pneumatic tyre with an inflatable rubber tube—the tube corresponding, of course, to the pneumosaccus inside the float.

Space does not allow full consideration of these exceptionally well-illustrated reports, but the fate of the gonophores, the great puzzle in *Physalia*, remains unsolved; it is suggested that the eggs undergo development in the depths.

W. J. REES

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* *Discovery Reports*: Vol. 30, Pp. 301–408, Plates 7–28, August 1960: Studies on *Physalia physalis* (L.). Part 1: Natural History and Morphology. Part 2: Behaviour and Histology. By A. K. Totton and G. O. Mackie. (Cambridge: At the University Press, 1960.) 70s. net.

BIRTH OF DELINQUENTS

ALTHOUGH a connexion between economic depressions and other social catastrophes—such as war—with the origin of delinquency in children has long been suspected, little statistical evidence to support the hypothesis has been available. The evidence is now brought out in a report published by the Home Office Research Unit, entitled *Delinquent Generations**, which shows that the peak of the effect is shown to occur not for those children born during the 1939–45 War, but for those born during 1935–42. These are children a large proportion of whom passed through their fourth to fifth year during the War, suggesting that the social disturbances which occur during the fourth and fifth year of life may have a greater effect than at other ages.

The analysis is based on criminal statistics for the period 1946–57 for each year of birth from 1925–26 to 1948–49. The argument is developed from simpler analyses to a complex process of standardization. In the first place the crime-rate for eight-year-old male offenders is followed from 1946 to 1957, and this shows that the crime-rate was least (for this age) when the number of children of that age was greatest; the statistical work also shows that, for children born after the War, there is a positive correlation between the number of children of a given age at risk and the crime-rate for that age, whereas for pre-War births the tendency is towards a negative correlation.

In 1948, with one of the smallest populations of eight-year-old boys, the crime-rate for this age was the worst for any year of the period studied. There was a continuous and marked improvement until 1956, with 1955 showing the lowest crime-rate (together with the peak of the population bulge). Not only did eight-year-old boys have the worst crime-rate in 1948 but also the highest crime-rate for seventeen-year-old boys occurred in 1957, when this same generation was seventeen years old.

Analyses of this kind are then generalized for all ages from eight to twenty years of age, for both males and females separately in England and Wales, and for males in Scotland. It is interesting to find that the same patterns of crime-proneness apply to both sexes and to both Scotland and England, notwithstanding that the rates at different ages are not at all similar.

Another finding is that youths between seventeen and twenty-one in 1955 and onwards, who would, according to the general findings of this research, have been expected to be exceptionally delinquent because of the effects of the War, have in fact been even more delinquent than could have been predicted. Either some new and different factor has operated during recent years, or the earlier influences have an additional effect at adolescence.

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* Home Office. *Delinquent Generations*: a Home Office Research Unit Report. (Studies in the Causes of Delinquency and the Treatment of Offenders, No. 3.) By Leslie T. Wilkins. Pp. iv+19. (London: H.M. Stationery Office, 1960.) 1*s*. 6*d*. net.

BILHARZIASIS

WHILE economic development usually reduces general morbidity, it has been found in a number of countries that improvement of irrigation networks has resulted in the spread of bilharziasis. Ways of dealing with this growing menace were studied at the second African conference on "Bilharziasis", convened jointly by the World Health Organization and the Commission for Technical Co-operation in Africa South of the Sahara, and held during March 30–April 8, 1960, in Lourenço Marques. The report, which has just been published, constitutes a general assessment of the present bilharziasis control situation in Africa where "the pressing problems of control are related rather to the deployment of skills, materials and work than to the uncertainty of methods"*.

Assessment of morbidity is complicated by the chronic nature of the disease which may be recognized only if it entails a period of incapacitation for work. Of the three phases of the disease described in the report, the conference considered the last—that of irreversible pathological effects and *sequelae*—to be by far the most significant from both the public health and the socio-economic point of view. The report directs attention to the pulmonary effects of *S. haematobium* infection, and to the fact that eggs may sometimes be recognized in the sputum of patients. Methods of assessing morbidity are reviewed, and the advantages of certain methods brought out.

The merits of the various types of classic molluscicide (copper sulphate, pentachloriphenate) and also of the more recent products (Bayer 73, I.C.I. 24223, and 'Aqualin') are examined, together with ways in which these products are used in African territories. Water management, land use and agricultural practices were examined at length in relation to the transmission and spread of bilharziasis. Some countries in the African region have adopted legislation relating to the control of communicable diseases, and the report cites an example of one law which might be modified and adapted to local health and economic conditions elsewhere. Methods of irrigation and agriculture and of bilharziasis control aspects of engineering techniques applied to watercourses, ponds and reservoirs are studied, and the frequently neglected possibility of using rainwater is also touched on.

In addition to the steps taken to improve irrigation plans, it would be desirable to construct bridges and culverts, to install safe washing and bathing places and at the same time to develop an effective health education programme so that people may be made aware of the danger of natural waters.

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* World Health Organization. Technical Report Series No. 204: Second African Conference on Bilharziasis (WHO/CCTA), Lourenço Marques, Mozambique, 30 March–8 April, 1960—Report. Pp. 37. (Geneva: World Health Organization: London: H.M. Stationery Office, 1960.) 1 Swiss franc; 1*s*. 9*d*.; 0.30 dollars.

VELOCITY OF LIGHT IN A MAGNETIC FIELD

By Prof. THOMAS ERBER

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RECENT advances in several areas of technology, in particular the production of very high steady ($\sim 0\cdot25$ megagauss) and pulse-imploded (~ 30 megagauss) magnetic fields, have brought closer the prospect of the experimental observation of a number of vacuum polarization effects. It seems appropriate at this time to make a preliminary survey of some of these possibilities and to provide some evaluation of their technical feasibility.

I begin by recalling that non-linear electromagnetic effects enter quantum electrodynamics essentially through the diagram presented in Fig. 1. This depicts schematically the dissociation of a photon into an electron-positron pair, usually virtual, which is afterwards annihilated to re-create a photon. The arrow symbolizes an external electromagnetic field (it may be another photon) which exchanges energy and momentum with the pair in the intermediate state. In this sense quantum electrodynamics gives rise to light-light and light-field interactions.

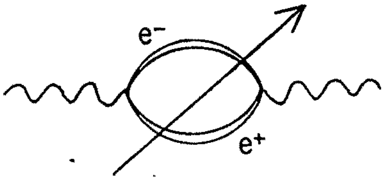

Fig. 1

Suppose the original photon is propagated in the z-direction and that the arrow represents a constant magnetic field the lines of flux of which are in the y-direction. One then finds that the real part of the index of refraction of the vacuum stressed by the external field is given by[1-3]:

$$n = 1 + \frac{\alpha}{4\pi}\left[\frac{B}{B_{cr}}\right]^2 N(\chi) \qquad (1)$$

where:

$$\alpha = \frac{e^2}{\hbar c} \sim \frac{1}{137};$$

$$B_{cr} = \frac{m^2c^3}{e\hbar} = \alpha\frac{e}{r^2} \sim 4\cdot5 \times 10^{13} \text{ gauss};$$

$$\chi = \frac{h\nu}{mc^2}\cdot\frac{B}{B_{cr}};$$

ν is photon frequency; m, electron mass; B, external field (gauss); r, classical electron radius. $N(\chi)$ is a very weakly varying function of χ in almost the entire range of practical interest. For photons polarized in the direction of B, that is, the y-axis, one may take $N(\chi) \sim 0\cdot2$ in the range $0 < \chi < 0\cdot1$; for photons polarized perpendicularly to B a similar approximation gives $N(\chi) \sim 0\cdot3$. Further details are contained in Toll's thesis[3].

I now consider a number of applications:

(1) *Cotton-Mouton effect:* Equation (1) essentially says that there is a selective slowing down of light in a transverse magnetic field. By using polarized light and exploiting $N_{\parallel} \neq N_{\perp}$ one can achieve a rotation of the plane of polarization. This is in complete analogy to the transverse double refraction of optically active media (even to the B^2 dependence) and therefore deserves to be called a 'Cotton–Mouton' effect. From these remarks it follows readily that θ, the angular rotation of the plane of polarization (radians) is given by:

$$\theta \simeq \frac{\alpha}{15}\,\frac{l}{\lambda}\left(\frac{B}{B_{cr}}\right)^2 \qquad (2)$$

where l is the path-length and λ is the wave-length. The key point of this approach is the enormous help of the factor l/λ. Assuming a field of 3×10^7 gauss[4] and a path-length of about 10^2 cm. one finds:

$$\theta \simeq \frac{1}{2\lambda}\,10^{-13} \text{ radians} \qquad (3)$$

At this point it is clear that it would be advantageous to choose a λ well into the γ-ray region. Unfortunately the experimental means for measuring θ are very poor at these frequencies. Since the pulse-imploded fields cannot be maintained for periods longer than about 1μ sec., the accurate γ-ray polarimetry possible with the Mössbauer effect cannot be used.

In the optical range one sacrifices λ but has some compensating advantages: (*a*) The short working times permitted by the pulsed fields are no longer prohibitive since rapid shuttering (Kerr cells) and sufficient intensities are available. (*b*) Path folding techniques may be used for maximum exploitation of the available magnetic volume. (*c*) Very precise measurements of θ, probably down to at least $\sim 5 \times 10^{-7}$ radians, are possible[5]. It seems likely that a moderate extension of present techniques would make the experiment represented by (3) feasible in this region.

(2) *Refraction of light:* Experiments of this type have the unique advantage of not requiring large field volumes; the non-linearities arise from the mere presence of an interface between a field and field-free region of space. This is particularly suited to the pulse-imploded fields since their strong point is the creation of a magnetic 'shock' travelling ahead of the detonation wave. By suitable arrangement of the explosive charges it should be possible to focus these magnetic waves and enhance the non-linearities through field superposition. The observation of refraction effects then becomes the analogue of ordinary schlieren photography.

An extraordinarily sensitive refraction experiment of this kind has been performed by Jones[6]: Using a static magnetic field of 8000 gauss shaped in the form of a prism with apex angle $\sim 100°$, he looked for a deviation with a 'light lever' capable of sensing an angular deflexion $\sim 5 \times 10^{-13}$ radian. In this case:

$$\Delta n \equiv n - 1 \simeq \tfrac{1}{2}\delta > 10^{-13} \tag{4}$$

where δ is the angle of deflexion. No effect was observed. For such feeble fields this follows readily from (1): We have directly that:

$$\Delta n \simeq B^2 \times 10^{-13} \sim 6 \cdot 4 \times 10^{-24} \tag{5}$$

which is many orders of magnitude below the limit set by (4). Even for the largest static field configurations at present contemplated[7] $\Delta n \sim 6 \times 10^{-21}$, which is still far too small. For the pulse-imploded fields, however, $\Delta n \sim 10^{-16}$, and this begins to come into the realm of practicability. It remains to be seen whether the development of intense pulsed optical maser (laser) sources will facilitate the construction of sensitive 'fast' levers.

(3) *Interferometric experiments:* These are among the most straightforward means of detecting a variation in Δn. With conventional interferometer arrangements:

$$\Delta n \equiv \beta\, \lambda / l \tag{6}$$

where β is the fringe shift and l denotes the effective interferometer length. Assuming values of $l \sim 10^2$ cm., $\lambda \sim 5 \times 10^{-5}$ cm., and an optimal β of about 10^{-3}, one estimates[8]:

$$\Delta n \sim 5 \times 10^{-10}$$

Comparing with (5) it becomes evident that this is at least six orders of magnitude too large. This experiment has already been tried by Farr and Banwell[9] using a static transverse field of about 20,000 gauss; essentially no detectable shift in Δn could be established. It is interesting to note that Jones's gain of 10^3 in absolute accuracy is partially off-set by a factor of 1/6 in relative accuracy due to the lower field intensity. This emphasizes again the crucial importance of high field strengths in the detection of non-linear effects.

Interferometric techniques will undoubtedly reap great benefit from the introduction of coherent sources of optical radiation. Unfortunately it does not seem likely that this will make the velocity-shift experiments any easier. Laser sources will simply relax the present limitations on l; this still leaves the very considerable problem of providing expensive and elaborate equipment for manufacturing large magnetic volumes. A likelier avenue of improvement lies along an increase in the accuracy of measuring phase shifts. Coherent light could in principle be heterodyned to produce signals in a convenient radio-frequency range; rapid β-determinations down to about 10^{-4} would then seem to be feasible[10].

(4) *Dispersion effects:* The abrupt shift in the accepted value of the velocity of light (a change of about 5 parts in 10^5) brought about by the introduction of radar techniques during the Second World War has given rise to a persistent undercurrent of speculation about a possible dispersion in the velocity of light. (See for example the discussion in Ditchburn[11].) Recently Softky and Squire[12] have proposed a test of this hypothesis using a nuclear explosion in outer space. The blast would produce a sharply defined pulse of wide-band radiation. It is estimated that by clocking the difference in arrival time of radio-frequency- and γ-rays over a baseline of 2 light-seconds, a dispersive effect of less than one-twentieth of the 'post war' shift (across a frequency spread of 10^{11}) could be reliably detected.

Theoretically speaking such an effect is already contained in the weak dependence on frequency of $N(\chi)$ of (1). (It is important to note that an external field is always required to produce dispersive effects in the Euler–Kockel Lagrangian[1].) Unfortunately even with a generous estimate of 10^{-4} gauss for the effective fields encountered in traversing the interplanetary baseline, the incremental arrival times would be only about $2\Delta n \sim 10^{-36}$ sec., that is, far beyond present instrumental resolution. Any measurement of $\Delta n(RF)/\Delta n(\gamma)$ along these lines would seem to be out of the question in the foreseeable future.

Conversion to frequency shift: Present experimental technique excels in the precise determination of frequency. It would seem most attractive therefore to measure minute velocity shifts in terms of a frequency shift. If indeed $\Delta\nu/\nu \sim \Delta n$ then according to (5) one would merely require an accuracy of $\Delta\nu/\nu \sim 10^{-16}$, which is not at all unreasonable in the light of what has already been achieved in connexion with the Mössbauer effect. Unfortunately, specific schemes for velocity to frequency conversion, quite apart from all instrumental difficulties, seem to involve unfavourable proportionality factors which increase the sensitivity requirements on $\Delta\nu/\nu$ still further. I consider briefly two possibilities, the Doppler effect and γ emission: (i) In a typical Doppler shift experiment, for example the reflexion of light from a moving mirror, one obtains a relative frequency shift given by:

$$\frac{\Delta\nu}{\nu} = 2\,\frac{v}{c}$$

where v is the mirror velocity. Suppose that the experiment were also carried out in a very strong ambient field (H) the sole effect of which is to decrease the velocity of light. In this case one should find a slightly different shift since it is the ratio of the mirror velocity to the actual light velocity that enters. The net change is:

$$\frac{\Delta\nu}{\nu}(H) - \frac{\Delta\nu}{\nu}(0) = 2\,\frac{v}{c}\,\Delta n \tag{7}$$

which shows clearly that in this type of conversion experiment the minute effects of Δn are diminished even further by the presence of the factor v/c. (ii) Another method of converting the velocity shift to a frequency shift hinges on the relationship between the energy and momentum of a photon; $cp = h\nu$. To first order it may be assumed that it is only the photon momentum and not the photon energy which is affected by the presence of an external field. In order to obtain a frequency shift it is necessary to consider a decay process so that the final photon frequency is fixed by both the energy and momentum balance. In calculating this there is some ambiguity involved in carrying through the relativistic kinematics in an anisotropic space but the net frequency shift fortunately is insensitive to the details of the assumptions. I find:

$$E_r(H) - E_r(0) = \frac{E_1}{E_0}\Delta n \qquad (8)$$

where E_1 is the energy available in the γ transition; E_0 is the rest energy of the residual nucleus; and $E_r = (E_1 - h\nu)/E_1$ is the fractional recoil shift of the emitted γ-ray. In this case it is also evident that any effects due to Δn will be minimized by the presence of the unfavourable conversion factor E_1/E_0.

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ENUMERATION OF COMPONENTS IN COMPLEX SYSTEMS BY FLUORESCENCE SPECTROPHOTOMETRY

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THE constancy of the spectral distribution of the fluorescence for a given substance excited with light of different wave-lengths permits the development of a simple quantitative criterion for the determination of the number of independent fluorescent components in a mixture and also results in an interesting extension of the concept of resolution as commonly applied in spectroscopy.

In fluorescence spectrophotometry a number of fluorescence intensity measurements are made, following excitation with different wave-lengths. Both fluorescence observation and excitation cover discrete wave-bands. Continuously recorded fluorescence and fluorescence-excitation spectra are to be considered here as finite series of measurements with effective band-widths determined by the conditions used[1]. In either case the results may be set down as an $m \times n$ matrix, where the m columns are determined by the wave-lengths of excitation and the n rows by the wave-lengths of observation. The matrix elements, F_{ij}, are numbers proportional to the response of the detector, that is, photocurrents in the ordinary case. The i, j element of this *EF* matrix, as I propose to call it, is the fluorescence emitted at wave-length j on excitation with light of wave-lengths i. When several components are present in a mixture the *EF* matrix may be written as:

$$|EF|_{mn} = \begin{vmatrix} \sum\limits_k F_{11}^k & \sum\limits_k F_{12}^k \ldots\ldots & \sum\limits_k F_{1m}^k \\ \sum\limits_k F_{21}^k & \sum\limits_k F_{22}^k \ldots\ldots & \sum\limits_k F_{2m}^k \\ \cdots\cdots & \cdots\cdots & \cdots\cdots \\ \sum\limits_k F_{n1}^k & \sum\limits_k F_{n2}^k \ldots\ldots & \sum\limits_k F_{nm}^k \end{vmatrix} \qquad (1)$$

The superscript k refers to the different components. Of the quanta absorbed in the solution from the exciting beam at wave-length i, a fraction f_i^k will correspond to the k'th component, while the fluorescence emission by the same component at wave-length j is denoted by the absolute quantum yield at this wave-length band q_{ij}^k, where the subscript i is also present to describe the possible, though rare[1,2], eventuality of change of quantum yield with exciting wave-length. Consequently:

$$F_{ij}^k = A_i f_i^k B_j q_{ij}^k \qquad (2)$$

A_i is a row multiplier that stands for the absolute number of exciting quanta absorbed at wave-length i, and B_j a column multiplier proportional to the detector response to a quantum of radiation of j wave-length. A and B can therefore be eliminated from the matrix without affecting the properties in which we are interested, and the *EF* matrix may be written as:

$$|EF|_{mn} = \begin{vmatrix} \sum\limits_k f_1^k q_{11}^k & \sum\limits_k f_1^k q_{12}^k \ldots\ldots & \sum\limits_k f_1^k q_{1m}^k \\ \cdots\cdots & \cdots\cdots & \cdots\cdots \\ \sum\limits_k f_n^k\, q_m^k & \sum\limits_k f_n^k q_{n2} \ldots\ldots & \sum\limits_k f_n^k q_{nm} \end{vmatrix} \qquad (3)$$

An arbitrary 2×2 minor of the matrix is:

$$|EF|_{2,2} = \begin{vmatrix} \sum\limits_k f_i^k q_{ij}^k & \sum\limits_k f_i^k q_{il}^k \\ \sum\limits_k f_n^k q_{nj}^k & \sum\limits_k f_n^k q_{nl}^k \end{vmatrix} \qquad (4)$$

If the spectral distribution of the fluorescence is independent of the exciting wave-lengths as appears to be in all known cases of substances in solution,

$$\frac{q_{ij}^k}{q_{il}^k} = \frac{q_{nj}^k}{q_{nl}^k} = R_{jl}^k \qquad (5)$$

and the value of the last determinant becomes:

$$\Delta = \Big(\sum_k f_i^k q_{il}^k R_{jl}^k\Big)\Big(\sum_k f_n^k q_{nl}^k\Big) - \Big(\sum_k f_i^k q_{il}^k\Big)\Big(\sum_k f_n^k q_{nl}^k R_{jl}^k\Big) \qquad (6)$$

Thus the important rule follows that all the 2×2 determinants of the *EF* matrix are zero when: (1) There is a single fluorescent component in the solution, whether the absorption is due to one or more components ($q_{ij}^k = 0$, for $k > 1$). (2) There is more than one fluorescent species in the solution, but they all possess the same absorption spectrun ($f^1 = 1, f^k = 0$, for $k > 1$). This obviously provides for the possibility of demonstrating the appearance of a new fluorescent species during the life-time of the excited state.

Because of the fixed ratios of the q-values for each component independently of the exciting wave-length (equation 5), it follows that when there are two independent components in the solution the second-order determinants of the *EF* matrix will not all vanish, but the 3 × 3 determinants will vanish identically, and in general, the rank of the *EF* matrix gives the number of components in the system having distinct absorption and fluorescence spectra. Conversely, the 'purity' of a fluorescent solution can be tested and the presence of fluorescent impurities detected by observing whether the 2 × 2 minors of the *EF* matrix vanish. Moreover, the non-vanishing determinants cover the region over which both fluorescence and absorption spectra differ and can be used to single out such a region.

In practice the determinants will not exactly vanish, but approach zero within the experimental error of the method. If the average photocurrent involved in a 2 × 2 determinant is $\bar{F} \pm \delta F$, then a zero determinant will appear to have the value :

$$\Delta = 0 \pm 2\bar{F}\delta F ; \quad \frac{\Delta}{P} \simeq \frac{\delta F}{\bar{F}} \qquad (7)$$

where P is the permanent or value resulting from adding all the determinant products, so that for the 2 × 2 determinant, $P \simeq 2\bar{F}^2$. The last equation permits one to evaluate significant departures from nullity. A ratio of determinant to permanent of 3 times the coefficient of variation of the measured photocurrents can be considered significant. Moreover, the appearance of the non-zero values over compact regions of the spectrum as well as the constancy of sign can be used to refine the criteria for nullity. The value of the determinant will depend on both f and q, and by the use of equation 6 it is possible to see that in favourable cases it will be possible to detect an impurity present as a molar fraction of the total much smaller than the ratio Δ/P.

In agreement with the usual spectroscopic criterial a fluorescence spectrum may be called partially resolved into components, if there are spectral regions which can be demonstrated to belong to each component separately. In a spectrum of two components (2 × 2 minors non-zero, 3 × 3 minors = 0) the spectrum will be partially resolved, if there is at least one region where all 2 × 2 determinants are zero, when both the fluorescence wave-bands are selected from this region, and the exciting wave-lengths chosen arbitrarily, but some 2 × 2 determinants are non-zero when only one component is selected from within the region.

In the experimental use of the *EF* matrix two possible causes of error must be given consideration : the fluorescence spectrum may appear to change with exciting wave-length if the absorption of the exciting radiation becomes weaker than or even comparable to the absorption of any of the observed fluorescence wave-bands by the solution. Also, scattering of the exciting radiation may have a similar effect by adding a short-wave component to the fluorescence, when the wave-bands of excitation and observation become too close to each other. With good monochromators, it is possible to bring these two band-widths very near the theoretical limit. In one of the experiments quoted below, the wave-lengths of observation and excitation were brought within 13 mμ while the sum of the band-widths was 6 mμ, without impairing the results.

The detector response must be linear within the range of photocurrents measured, otherwise the spectrum will appear to change, depending on the signal strength, or in other words B_j will not be a true column multiplier but a function of q. In practice, the detector response must be kept constant through the series of measurements of either rows or columns of the *EF* matrix. In the former case, a series of excitation spectra of defined fluorescence bands is obtained; in the second, a series of fluorescence spectra excited by definite wave-bands is obtained.

Two examples are given below of the usefulness of the matrix treatment of the data of fluorescence spectrophotometry. The first example refers to the fluorescence of human serum albumin. This protein contains seventeen tyrosine and one tryptophan residue, all potentially capable of fluorescence[3], although in previous work no contribution from the tyrosine was detected[4], and the whole emission was attributed to the single tryptophan. The *EF* matrix obtained with a solution of human serum albumin at neutral *p*H is given below. The wave-bands for excitation and observation were both 3·0 mμ.

		Excitation wave-lengths (mμ)		
		275	285	295
Observation wave-lengths (mμ)	308	66	89	65
	325	100	141	205
	350	108	153	290

The value of the 3 × 3 determinant/3 × 3 permanent is 0·002 or zero within error. Thus, there are less than three distinct components detectable. The 2 × 2 minors are analysed in the following table which gives the ratios of Δ/P for each pair of excitation and observation wave-lengths :

		Excitation pairs	
		275–285	285–295
Observation pairs	308–325	+ 0·022	+ 0·35
	325–350	+ 0·003	+ 0·135

The presence of two distinct components in the absorption and fluorescence is clear, since the experimental error in the determination of the photocurrents was about 1 per cent. From the signs of the two by two determinants it can be concluded

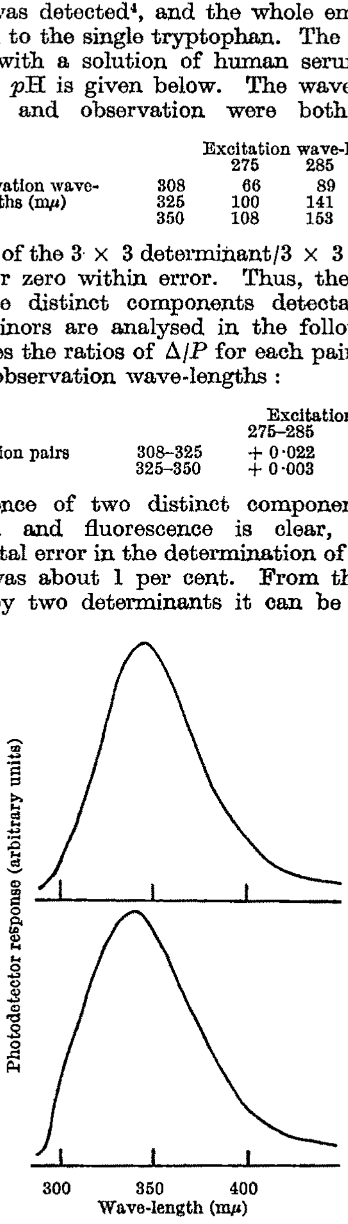

Fig. 1. Fluorescence spectra of 0·5 per cent solution of human serum albumin in water. Excitation and fluorescence band-width of 2 mμ. Upper figure excited at 292 mμ ; lower excited at 278 mμ. All spectra shown are direct recordings uncorrected for grating transmission or photodetector (*EMI* 6255) response

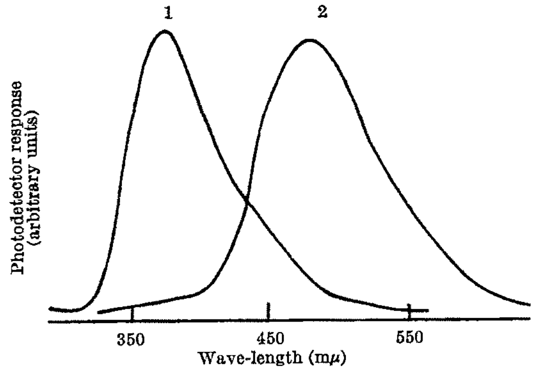

Fig. 2. Fluorescence spectra of 10^{-3} *M* sodium naphthionate in water. Excitation at 340 mμ. Band-widths = 2 mμ. (1) Neutral solution ; (2) 3 *N*, sodium hydroxide solution

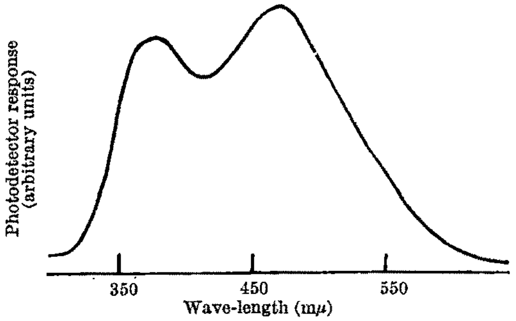

Fig. 3 Fluorescence spectrum of weakly alkaline solution of sodium naphthionate

that the component increasing its absorption fraction over the 285–295 mμ region is the one emitting at the longer wave-lengths as expected from tryptophan. The minors for 275–285 excitation are virtually zero, showing that there is no change in the fractional absorption of the two components over this interval. Following the indications given by the matrix treatment, fluorescence spectrum recordings using narrow bands of excitation at 278 and 292 mμ were undertaken and are shown in Fig. 1, where the contribution from tyrosine appears clearly as a shoulder on the short-wave side of the spectrum, excited at 278 mμ and is virtually absent in the spectrum excited with 292 mμ. The absolute quantum yield of the tyrosine is estimated as between 0·015 and 0·025.

The matrix treatment has also been applied to the fluorescence data from alkaline solutions of sodium naphthionate. The fluorescence spectrum of this compound in neutral solution has a maximum at 395 mμ, while in 2*N* sodium hydroxide the maximum is found at 502 mμ, both spectra being as shown in Fig. 2. Boaz and Rollefson[5] and Förster[6] have concluded from their work on this and similar compounds that the fluorescence shift is due to abstraction of a proton from the amino-group during the lifetime of the excited state so that the long-wave fluorescence is due to the NH^- ion, a species virtually non-existent in the ground-state. The *EF* matrix given below was obtained with a moderately alkaline solution in which both fluorescence bands were present to about the same extent (Fig. 3):

		Excitation wave-lengths (mμ)			
		280	300	340	360
Observation wave-lengths (mμ)	400	81	91	68	98
	450	78	88	66	95
	500	74	83	63	91
	550	43	48	37	53

All nine independent 2 × 2 minors are zero within experimental error, confirming that there is a single species in the ground-state in spite of the complex fluorescence spectrum.

The examples examined show one of the potentialities of fluorescence spectrophotometry as compared with absorption spectrophotometry. In the latter, the determination of the number of components cannot be done without reference to the absorption spectrum of the pure species while in fluorescence spectrophotometry this determination is always possible in principle and may be expected to be practically possible in most cases.

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ABSENCE OF PHOSPHATASE REPRESSION BY INORGANIC PHOSPHATE IN SOME MICRO-ORGANISMS

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THE formation of alkaline phosphomonoesterase in strains of *Escherichia coli*[1,2] and *Bacillus subtilis*[3] has been observed to be repressed in a medium containing inorganic phosphate. It was suggested that phosphate, which is a product of phosphatase action, controlled the level of enzyme synthesis via a negative feed-back system[2]. Such a phosphate–phosphatase regulatory system conceivably could play an important part in the phosphorus economy of the cell. In order to determine if this mechanism applied as well in other organisms and to the acid phosphatases, the effect of inorganic phosphate on the formation of both acid and alkaline phosphatase was studied in various strains of *E. coli* and *Staphylococcus aureus*, and in a fungus, *Neurospora crassa*.

Various strains of *E. coli* were grown in a medium containing per litre : 0·1 gm. crystalline magnesium sulphate, 1·0 gm. ammonium sulphate, 0·5 gm. sodium citrate, 10·0 gm. glucose, 12·2 gm. *tris*-(hydroxymethyl)-aminomethane and thiamine or

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amino-acids, as necessary, to the auxotrophic strains; the *p*H was adjusted to 7·4. The growth medium for coagulase-negative staphylococci contained per litre : 0·04 gm. crystalline magnesium sulphate, 0·001 gm. sodium chloride, 0·001 gm. crystalline ferrous sulphate, 0·02 gm. crystalline manganese sulphate, 1·0 gm. glucose, 1·0 gm. yeast extract (Difco), 12·2 gm. *tris*-(hydroxymethyl)-amino-methane, and *p*H was adjusted to 7·4. The coagulase-positive staphylococci were grown in a medium containing glucose, casein hydrolysate, cystine, salts, thiamine and nicotinic acid in the amounts recommended by Evans[4] except that 12·2 gm. of *tris*-(hydroxymethyl) - aminomethane / litre replaced the inorganic phosphate. All three media also contained 10 μgm. phosphorus/ml. as potassium dihydrogen phosphate. The bacteria were subcultured once before inoculating into the growth media, and incubated at 37° C. on a rotary shaker. Alkaline phosphatase was assayed, after an autolysis with toluene, by the method described by Horiuchi *et al.*[2], with minor modification. The reaction mixture was incubated for 30 min. at 30° C., and *p*-nitrophenol liberated was determined by the procedure of Bessey *et al.*[5]. Acid phosphatase was assayed in the same manner in *p*H 5·6 acetate buffer. The optical densities of cell populations were measured using filter 50 in a Klett–Summerson photometer while inorganic phosphate in the medium was determined by the procedure of Fiske and SubbaRow[6].

Neurospora crassa strain 5297*a* was grown for 48 hr. in a synthetic medium[7] on a rotary shaker at 30° C. The mycelium was then blended aseptically for 30 sec. in a Waring blender and aliquots of the suspension were used to inoculate groups of duplicate flasks containing medium in which the level of potassium dihydrogen phosphate was reduced to 43 μgm. phosphorus/ml. After incubation on the shaker at 30° C., replicate flasks were removed at appropriate intervals of time and the mycelium was harvested by filtration, pressed dry and weighed. Aliquots of the filtered medium were also assayed for inorganic phosphate and phosphatase activity. The weighed mycelia were triturated in a cold mortar with 8 parts (w/w) of washed fine glass beads, and the enzyme was solubilized in distilled water. The supernatant fluid obtained after centrifugation was used for assay of both acid and alkaline phosphatase activities using sodium β-glycerophosphate as the substrate[8].

The effect of inorganic phosphate on alkaline phosphatase formation is shown along with the growth curves of two typical *K*12 strains of *E. coli* in Fig. 1. In strain *K*40, alkaline phosphatase formation took place only in the absence of extracellular inorganic phosphate whereas strain 180 formed the enzyme early in its growth while inorganic phosphate was still present in the medium. Addition of phosphate to a culture of strain *K*40 actively synthesizing alkaline phosphatase resulted in the complete repression of alkaline phosphatase, but not general protein synthesis, whereas in strain 180 there was a stimulation of phosphatase synthesis. These findings in strain *K*40 are consistent with what has been reported by previous workers in *E. coli* strains *K*12, *ML* and *W*[1] and strain *B*[2]. On the other hand, the absence of a phosphate-repressible alkaline phosphatase, as found in strain 180, has previously not been reported. The results of similar experiments with various *E. coli* strains are summarized in Table 1. Only three of ten strains tested were found to have a phosphate-repressible alkaline phosphatase, and at least one strain of each mating type, *Hfr*, *F*+, and *F*−, was not repressible, suggesting there is no direct relationship between phosphate repressibility and mating type. None of the strains had a phosphate-repressible acid phosphatase, in agreement with the findings of Torriani[1]. Strain differences are known to exist in other repressible systems. Thus, Gorini[9] reported that in only two of four strains of *E. coli* tested was arginine a repressor for ornithine transcarbamylase.

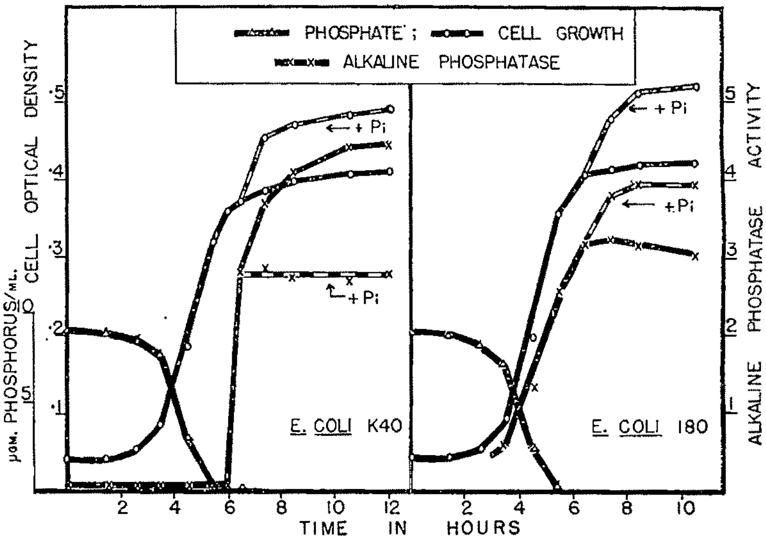

Fig. 1. Relation between the content of inorganic phosphate and the formation of alkaline phosphomonoesterase in two *K*12 strains of *E. coli*. Cells were grown as described in the text. At zero time, 2 ml. of a 12-hr. culture of *E. coli* was inoculated into flasks containing 100 ml. of the synthetic medium in which the phosphorus content was 10 μgm./ml. Samples were withdrawn for determination of cell population, inorganic phosphate (Pi), and alkaline phosphatase at the indicated time intervals. After 6 hr., the inorganic phosphate in the medium was completely utilized and the flasks were divided into two groups. One group received 50 μgm. phosphorus/ml., as potassium dihydrogen phosphate, while the other group received none. Determination of cell population and alkaline phosphatase was continued for another 6 hr. The alkaline phosphatase is plotted in relative units

The amount of phosphatase produced by *Staphylococcus aureus* has been correlated with coagulase production as a biochemical index of pathogenicity[10]. In a recent survey of more than 100 strains of this organism growing in a complex medium, Pan and Blumenthal (unpublished results) found that the average amount of acid phosphatase produced by coagulase-positive strains was about four times greater than that produced by the average coagulase-negative strains. To test the possibility that a phosphate-repressible acid or alkaline phosphatase system was operative in the staphylococci, 17 coagulase-negative and 3 coagulase-positive strains were tested. The results, presented in Table 1, indicate that a phosphate-repressible system could not be detected in any of the strains.

The results of an experiment with a wild-type strain of *N. crassa* are presented in Table 2. The

Table 1. DISTRIBUTION OF REPRESSOR EFFECT OF INORGANIC PHOSPHATE ON PHOSPHATASE SYNTHESIS IN STRAINS OF *E. coli* AND *S. aureus*

	Strain	Repressor effect	
		Alkaline phosphatase	Acid phosphatase
Escherichia coli	*B*	–	–
	B/r	+	–
	*ML*30	+	–
*K*12 strain	*K*40 *Hfr*	+	–
,, ,,	*CR*63 *F*+	–	–
,, ,,	173 (*W*3350) *F*–	–	–
,, ,,	179* (*C*600) *F*–	–	–
,, ,,	180* (112) *F*+	–	–
,, ,,	422* *Hfr*	–	–
,, ,,	444* (*U*38) *F*–	–	–
Staphylococcus aureus†	291 (coagulase +)	–	–
	198 (coagulase +)	–	–
	1-*D* (coagulase –)	–	–
	314 (coagulase –)	–	–
	B-21-6 (coagulase –)	–	–
	B-10-1 (coagulase –)	–	–

* Auxotrophic strains requiring thiamine and/or amino-acids.
† Total of 20 strains (17 coagulase-negative) tested with same results.

Table 2. EFFECT OF INORGANIC PHOSPHATE ON THE CELL GROWTH AND PHOSPHATASE-LEVELS IN *Neurospora crassa*

Time (hr.)	Mycelial weight (wet) (gm.)		Total acid phosphatase (units*)		Total alkaline phosphatase (units*)	
	–P†	+P†	–P	+P	–P	+P
12	0·320		2·4		1·0	
25	0·616	0·933	4·5	6·6	2·1	4·3
47	0·693	1·178	5·1	8·2	2·6	5·9
67	0·766	1·790	6·5	17·5	3·5	7·0

Cultivation of the mould is described in the text. In the control flasks (–P), no inorganic phosphate was detectable in the medium after 20 hr. of cultivation.

* One unit of phosphatase is defined as the amount of enzyme which can release one μ-mole of phosphorus per min. under respective assay conditions.

† –P, flasks to which no additional inorganic phosphate was added. +P, flasks to which 122 μgm. phosphorus/ml., as potassium dihydrogen phosphate, was added after 20 hr. of incubation.

amounts of both acid and alkaline phosphatases, which were present only in the mycelia, were found to be approximately proportional to the cell weight throughout the course of the experiment. The presence of extra-cellular phosphate did not repress the enzyme formation and the phosphatase formation did not increase significantly even after the depletion of phosphate from the medium. This strain of *N. crassa*, therefore, was a non-repressible strain.

Although it is likely that in the future other micro-organisms will be found with phosphate-repressible alkaline phosphatases, the absence of such a system in 20 strains of *S. aureus*, 1 strain of *N. crassa* and 7 of 10 strains of *E. coli* indicates that this is not a common property of micro-organisms. Even closely related strains, like those derived from *E. coli K*12, differ in this respect. It therefore seems unlikely that this particular repressor system will prove to be part of a general mechanism for the regulation of microbial phosphorus metabolism, although it may take such a role in those strains possessing this repressor system.

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INTESTINAL CARBOHYDRASES OF A NEW-BORN PIG

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THE activity of digestive carbohydrases of the new-born mammal differs from that of the adult animal. The development of the digestive carbohydrase system of the pig as well as the ability of the growing pig to utilize various dietary sugars have been the subject of several recent investigations[1-6]. It has been found that the lactase activity of the small intestinal mucosa is high at birth, and remains so for the first weeks of life[3,6]. It then gradually decreases, although some lactase is present in the small intestine of the adult pig too[7]. The intestinal maltase and invertase activities, to the contrary, which for many years have been thought to be due to a single enzyme, intestinal α-glucosidase[8], are weak or absent at birth and develop gradually during growth[3,6]. The amylase activity of the pancreas likewise is low at birth and increases during growth[2]. The new-born pig, therefore, is unable to utilize some dietary carbohydrates, as sucrose and starch, which are readily utilized by the adult animal[1,5,6].

Recently it has been found that the digestive carbohydrase system of the adult pig is more complicated than was believed earlier. Instead of a single intestinal α-glucosidase[8] there exist five or six different intestinal α-glucosidases[9], namely, three different maltases (maltase I–III), one of which (maltase I) also exerts all the intestinal invertase activity[10,11], a specific intestinal trehalase[12], an *iso*maltase[13] and a phenyl-α-glucosidase[13]. The two latter enzymes may, however, be identical[9,13]. Since lactase is also present[7], the adult pig has at least six different intestinal glycosidases. In addition, preparations of intestinal mucosa are known to hydrolyse β-glucosides[14]. An intestinal dextranase, distinguished from amylase, has also been found[15,16].

To define those enzymes present at birth, the carbohydrase activities of a homogenate of the small intestine of a new-born pig have been measured. The pig was slaughtered a few hours after birth, and the small intestine, which weighed 19 gm., was homogenized for 2 min. in an Ultra-Turrax homogenizer with 19 ml. of 0·01 *M* phosphate buffer, *p*H 6·0. After centrifugation in a laboratory

Table 1

	Activity of homogenate units/ml.	
	(*a*) New-born pig	(*b*) Adult pig
Invertase	0	78
Maltase I	0	55
Maltase II	13	248
Maltase III	7	66
*Iso*maltase (total)	0	30
Trehalase	0	36
Phenyl-α-glucosidase (total)	0·8	16
Lactase	104	42
Cellobiase	30	13
Gentiobiase	1·8	0·7
Phenyl-β-glucosidase	24	2·1
Amylase	26	1,800
Dextranase	0	2

Carbohydrase activities of homogenates of (*a*) the small intestine of a new-born pig, and (*b*) mucosa from the upper part of the jejunum of an adult pig. Protein contents of the homogenates (*a*) 44 mgm./ml. and 62 mgm./ml. The figures in (*a*) and (*b*) can be compared, but only approximately.

centrifuge for 5 min. the opalescent supernatant, which contains the carbohydrase activities[7], was analysed by means of methods described elsewhere[16,17]. Conditions for the carbohydrase activity determinations were such that 0·5 unit per ml. of homogenate could be detected.

The carbohydrase activities of the homogenate are seen in Table 1. For comparison, this table also includes the carbohydrase activities of a mucosal homogenate from the upper part of the jejunum of an adult pig.

The wall of the small intestine from the new-born pig was so thin that it proved impossible to scrape off the mucosa; hence, the entire small intestine was homogenized. This means, however, that the homogenate, made 1 : 1 w/v in buffer, was somewhat more dilute in mucosal protein than in the experiments with the adult pig, where only the mucosa from the intestine was homogenized. The values expressed are thus somewhat lower than would be found in a mucosal homogenate, but an approximate comparison with the adult pig values is possible.

No invertase activity could be detected in the homogenate from the new-born pig, which is in accordance with earlier findings[3,6] and also with the fact that new-born pigs cannot utilize dietary sucrose[1]. The homogenate from the adult pig had strong invertase activity (Table 1).

Maltase activity was found in the preparation from the new-born animal, although this activity was weak. The maltase activity was less than one-tenth that of the adult animal preparation (Table 1). Since it is known that the maltase activity of the ileum of adult animals is greater than that of the jejunum[16], the difference would have been still greater if the whole small intestine had been used in the experiments with the adult animal.

The maltase activity seems, however, to increase rapidly during the first phase of growth[3,5]. After two days of life pigs seem able to utilize dietary maltose[5], although they are still unable to utilize sucrose.

Heat inactivation experiments, performed as described earlier[9,17–19], indicated that the maltase activity of the preparation from the new-born pig was exerted by a mixture of maltase II (65 per cent) and maltase III (35 per cent). Apparently these enzymes appear in the small intestine of the growing pig earlier than maltase I, which hydrolyses sucrose in addition to maltase[9–11].

Neither trehalase nor *iso*maltase activity could be detected in the preparation from the new-born pig. In the small intestine of the adult pig there exist one trehalase and one specific *iso*maltase[9,12,13]. The weak *iso*maltase activity known to be exerted by maltase II and maltase III[10] was too weak to be detected under the conditions used.

The phenyl-α-glucosidase activity was less than one-tenth that of the preparation from the adult animal. This activity may very well be caused by maltase II and maltase III[10,17], and hence there is no specific phenyl-α-glucosidase[13] present in the intestine of the new-born pig.

Lactase activity, in accord with earlier reports[3,6], was greater in the preparation from the new-born animal, which is of obvious physiological importance. However, the β-glucosidase (cellobiase, gentiobiase and phenyl-β-glucosidase) activities were high although β-glucosides are not physiological nutrients for the new-born pig. The high β-glucosidase activities of this preparation suggest that these activities may be exerted by intestinal lactase (β-galactosidase) and that there exists no specific intestinal β-glucosidase. This idea is also supported by separation experiments which are in progress in our laboratory.

The phenyl-β-glucosidase activity may, however, be exerted by more than one enzyme, since the phenyl-β-glucosidase/lactase activity quotient of the preparation from the new-born pig was about 5 times that of the preparation from the adult pig, while the cellobiase/lactase and gentiobiase/lactase activity-quotients were the same in both preparations.

Amylase activity was low, compared with that of the preparation from the adult animal. Since the main source of amylase in the pig is the pancreas[16], however, no conclusions may be drawn about the ability of the new-born pig to digest starch. The pancreatic amylase activity of new-born pigs and their ability to digest starch have been studied by others[2,4].

Dextranase activity could not be detected. In contrast to amylase, this enzyme is localized to the intestinal mucosa of the adult animal[16], but its activity is quite weak.

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EFFECT OF MITOMYCIN C ON THE SYNTHESIS OF INFECTIVE VIRUS AND DEOXYRIBONUCLEIC ACID IN PSEUDORABIES VIRUS-INFECTED RABBIT KIDNEY CELLS

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THE antibiotic mitomycin *C* prevents the synthesis of deoxyribonucleic acid (DNA) without affecting the synthesis of ribonucleic acid (RNA) or protein in *Escherichia coli*.[1,2]. After infection with bacteriophages T_2, T_3, and T_5, cells treated with mitomycin *C* regain the ability to synthesize DNA, as shown by Sekiguchi and Tagaki[2]; the phage particles produced by these cells are, however, non-infective. These results are interpreted by the authors to mean that DNA synthesis proceeds in phage-infected bacterial cells through a pathway which differs from that of non-infected cells and which is resistant to the action of mitomycin *C*.

An alternative explanation of these results is that infection of the cells is followed by a massive induction of the enzymes connected with the synthesis of DNA, some of which are inhibited by mitomycin *C*. Previous experiments[3] have revealed that infection of rabbit kidney cells with pseudorabies virus causes an increase or decrease in the rate of DNA synthesis depending on the physiological state of the host cells. It was of interest, therefore, to examine the effects of mitomycin *C* using the pseudorabies virus–rabbit kidney cell system with particular emphasis on a comparison of the results obtained with rabbit kidney cells in the stationary and in the actively growing phases.

Primary monolayer cultures of rabbit kidney cells were prepared as described previously[4] and were cultivated in Petri plates in a medium composed of 96·5 per cent Earle's saline, 0·5 per cent lactalbumin hydrolysate, and 3 per cent bovine serum. Cultures were incubated at 37° in a humid atmosphere containing 5 per cent carbon dioxide. Virus titres were measured by the plaque method. The procedures used for the determination of RNA, DNA, and protein and for the incorporation of labelled nucleosides were the same as reported previously[3]. The number of virus particles was determined by electron microscopy. Virus preparations were mixed with a known number of polystyrene latex particles, the mixtures were sprayed on electron microscope screens, and counts were made from electron micrographs[5,6].

The action of mitomycin *C* on rabbit kidney cells is similar to its action on bacterial cells. The addition of 5 μgm. of mitomycin *C* per ml. to rabbit kidney cells results in an immediate reduction in the ability of these cells to incorporate thymidine into their DNA. After 4 hr. of incubation with the antibiotic the cells incorporate only 2–3 per cent as much thymidine as the untreated controls. The same concentration of mitomycin *C* has comparatively little effect on the incorporation of uridine into RNA: even after 24 hr. of incubation with mitomycin *C* this incorporation is reduced by only approximately 25 per cent.

The synthesis of DNA in pseudorabies virus-infected cells treated with mitomycin *C* is highly dependent on the physiological state of the cells at the time of addition of mitomycin *C*. (Previous experiments have demonstrated that cells in the stationary phase of growth are stimulated by pseudorabies virus infection to incorporate up to twenty-fold more thymidine than non-infected controls; infection of actively growing cells results, on the other hand, in a reduction in their capacity to incorporate thymidine[3].) Nine-day-old stationary phase cultures, as well as 4-day-old actively growing cultures, were incubated with 5 μgm. of mitomycin *C* per ml. for 18 hr. Part of the cultures was infected with an adsorbed multiplicity of 5 and incubation was continued in the presence of mitomycin *C*. Cultures which had not been exposed to the antibiotic (infected and non-infected) were used as controls. At various times after infection 3·3 × 10^4 c.p.m. of thymidine-2-^{14}C (specific activity 8·7 × 10^3 c.p.m./μgm. of thymidine) were added to the cultures and incorporation was allowed to proceed for 1 hr. The specific activity of the DNA was then determined. Fig. 1 summarizes the results of this experiment.

Nine-day-old cultures treated with mitomycin *C* regain the ability to incorporate thymidine after infection and for the first 4 hours incorporate thymidine at a rate similar to that of the untreated controls; after 4 hours there is a sharp decrease in the rate of this uptake. Four-day-old cultures treated with mitomycin *C*, however, remain unable to incorporate thymidine after infection. Thus, the action of mitomycin *C* on DNA synthesis is reversed by infection with pseudorabies virus only in cells in which the synthesis of DNA is thereby stimulated. These results suggest that this reversal may be a reflexion of the induction in these cells of enzymes (some of which are sensitive to mitomycin *C*) con-

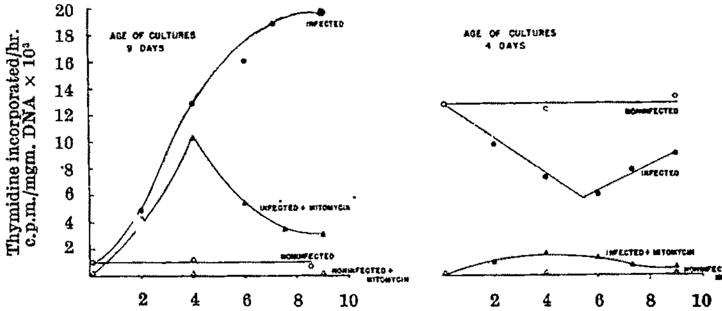

Fig. 1. The effect of virus infection on stationary phase and actively growing rabbit kidney cells, after treatment with mitomycin *C*, on their ability to incorporate thymidine-2-^{14}C

nected with the synthesis of DNA. Furthermore, the initial rapid increase in the ability of 9-day-old infected cultures treated with mitomycin *C* to incorporate thymidine, which is followed by a loss of this ability, seems to be due to the induction of these enzymes at a rate which, for the first 4 hr. after infection, exceeds the rate of their inhibition by mitomycin *C*. Thus, under certain conditions, infection with pseudorabies virus overcomes the block of the synthesis of DNA caused by mitomycin *C*, as infection with bacteriophage does in *E. coli* treated with mitomycin *C*. In the case of pseudorabies virus-infected rabbit kidney cells, however, this reversal is readily explainable in terms of a massive induction of enzymes connected with the synthesis of DNA.

Despite the ability of 9-day-old cultures treated with mitomycin *C* to synthesize DNA after infection with pseudorabies virus, the yield of infective virus produced by these cells is low. Non-infective virus particles are, however, formed by cells treated with mitomycin *C*, as shown in the next experiment. Cells were incubated for 4 hr. with 5 μgm. of mitomycin *C* per ml. They were infected and incubated for an additional 24 hr. in the presence of mitomycin *C*. (Cultures treated identically in the absence of mitomycin *C* were used as controls.) The fluids were harvested, the cellular debris was removed by centrifugation, and the clear supernatant fluids were subjected to 2 alternate cycles of high-speed (73,300 *g* for 60 min.) and low-speed (1,700 *g* for 20 min.) centrifugations. The preparations, suspended in one-tenth the initial volume, were assayed for infective virus (measured by plaque-forming units) and for physical particles (determined by electron microscopy). Table 1 shows the ratio of plaque-forming units to physical particles in these preparations. Although the number of plaque-forming units in the sample obtained from the cells treated with mitomycin *C* is more than 700 times lower than that of the control sample, the number of physical particles in both samples differs by only a factor of 3·2.

Table 1. EFFECT OF MITOMYCIN *C* (MC) ON THE ABILITY OF RABBIT KIDNEY CELLS TO PRODUCE INFECTIVE AND NON-INFECTIVE VIRUS PARTICLES

	MC-treated	Untreated	$\frac{\text{MC-treated}}{\text{Untreated}}$
Virus titre, PFU/ml.	$1 \cdot 5 \times 10^7$	$1 \cdot 1 \times 10^{10}$	1/740
Virus particles/ml.	$1 \cdot 8 \times 10^{11}$	$5 \cdot 5 \times 10^{11}$	1/3·2

PFU, plaque-forming units.

The next experiment shows that the non-infective particles produced by cells treated with mitomycin *C* can induce in the cells to which they are adsorbed the characteristic cytopathic changes (intranuclear inclusions and syncytia) which result from infection of rabbit kidney cells with normal infective virus particles. Cultures were incubated with 5 μgm. of mitomycin *C* per ml. for 4 hr. They were infected and incubated for an additional 24 hr. in the presence of mitomycin *C*. The fluids of these cultures, as well as of control cultures not treated with mitomycin *C*, were harvested and submitted to low-speed centrifugation to remove the cellular debris. One ml. of different dilutions of these fluids was added to rabbit kidney cultures (containing 4×10^6 cells ± 10 per cent per culture). After an adsorption period of 1 hour the cultures were washed to remove unadsorbed virus and were incubated in 5 ml. of medium for 3 hr. at 37°. The cells were then fixed with Bouin fluid, stained with hæmatoxylin, and the percentage of cells containing inclusion bodies determined. (Inclusion bodies start to appear in the nucleus 3 hr. after inoculation and increase in number with time. Previous experiments (unpublished) have shown that between 3 and 6 hr. after infection the number of inclusions is proportional to the multiplicity of infection. Since the latent period of pseudorabies virus in rabbit kidney cells is approximately 4–5 hr., the experiment was ended at 4 hr. to avoid the problem of recycling.) Assuming a Poisson distribution, the number of inclusion-forming units, which give rise to an inclusion during the first 4 hr. of incubation, was calculated from the number of intranuclear inclusions obtained with various concentrations of the samples. Table 2 shows the number of plaque-forming units and inclusion-forming units in mitomycin *C*-treated and untreated samples. Although the number of plaque-forming units produced by the treated cultures is 220 times lower than that of the untreated controls, the number of inclusion-forming units in both samples differs by only a factor of 3·4. The ratio of the number of inclusion-forming units produced by treated and untreated cells is comparable to the ratio of the number of viral particles in the two samples as determined by electron microscopy. Moreover, syncytia are formed in cultures infected with mitomycin *C* virus with a multiplicity of plaque-forming units per cell too low to induce these cytopathic changes with control virus. We conclude from these experiments that the non-infective virus particles which are formed in cells treated with mitomycin *C* are able to induce in rabbit kidney cells to which they are adsorbed the characteristic changes observed after inoculation of these cells with infective pseudorabies virus.

Table 2. NUMBER OF PLAQUE-FORMING UNITS (PFU) AND INCLUSION-FORMING UNITS (IFU) PRODUCED BY MC-TREATED AND UNTREATED RABBIT KIDNEY CELLS

	MC-treated	Untreated	$\frac{\text{MC-treated}}{\text{Untreated}}$
PFU/ml.	1×10^7	$2 \cdot 2 \times 10^9$	1/220
IFU/ml.	$8 \cdot 8 \times 10^7$	3×10^8	1/3·4

The action of mitomycin *C* on the rabbit kidney cell–pseudorabies virus system is thus similar to that previously reported for the *E. coli*–bacteriophage system. Our findings may be summarized as follows: the inhibition of DNA synthesis in cells treated with mitomycin *C* is overcome under certain conditions by infection with pseudorabies virus, probably as a result of an induction by the virus in the infected cells of enzymes connected with the DNA synthesizing mechanism; the DNA formed under these conditions does not seem to be functional and the virus particles formed are non-infective.

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LETTERS TO THE EDITORS

GEOPHYSICS

A Preliminary Model for the Variations of Upper Air Densities

SATELLITE observations have shown a significant variability of the density of the upper air above a height of 200 km. During the period 1957–60, several major effects must be distinguished, in addition to dependence on altitude. The construction of general representative atmospheric models is therefore extremely difficult, as has been found by several authors[1]. Hence it seems to be more useful to give a model for the variability, from which the density of the air can be calculated for special cases. Three influences seem to be predominant[2]: (1) The solar *W*-effect produced by the variable short wave-length solar ultra-violet radiation. This is demonstrated by a strong correlation with the sunspot number[3] or with the solar radio emission in the decimetre wave-length region[4]. (2) The day–night effect, the amplitude of which increases strongly with height[5]. (3) The annual effect. This effect shows a pronounced annual and a semi-annual period[2]. It is demonstrated by the fact that for different satellites the day–night variation is not constant in phase and amplitude if it is normalized for a constant solar *W*-effect[6]. This third effect is probably caused by the interaction with interplanetary plasma, the density of which varies along the Earth's orbit perhaps due to an interstellar wind[7].

Let F be the intensity of the solar ultra-violet radiation at 10 cm. wave-length; θ be the local time; a be the date in the year, $\rho_{\text{max.}}(\theta)$, $\rho_{\text{min.}}(\theta)$ be the diurnal and annual maximum density. Then the three influences above can be expressed by the following functions:

$$f(\theta) = \log \frac{\rho_{\text{max.}}(\theta)}{\rho(\theta)},\ g(a) = \log \frac{\rho_{\text{max.}}(a)}{\rho(a)},$$
$$i(F,h) = \log \frac{\rho(220,\ h)}{\rho(F,h)} \qquad (1)$$

$\rho(220)$ means the air density when the intensity of 10-cm. wave-length solar radiation is $F = 220 \times 10^{-22}$ W./m.^{2}c./s. The dependence on altitude of $f(\theta)$ and $g(a)$ may be expressed by the function $\theta(h)$ and $a(h)$. Then the air density $\rho(h)$ can be calculated as a good first approximation from:

$$\log \rho(h) = \log \rho_{\text{stand.}} - [f(\theta)\theta(h) + g(a)a(h) + i\,(F,h)] \qquad (2)$$

$\rho_{\text{stand.}}$ is given in Table 1, while the other functions are shown in Figs. 1 and 2. The function $\rho_{\text{stand.}}$ was extended up to 1,500 km. altitude by the observed acceleration of *Echo I* ($1960\iota_2$) (ref. 8). The plausible physical product of the combinations $f(\theta).\theta(h)$ and $g(a).a(h)$ has been proved by the observations of different satellites, which agree well with this assumption.

Table 1

Height (km.)	Density (gm./cm.^{3})	Height (km.)	Density (gm./cm.^{3})
180	$5{\cdot}9 \times 10^{-13}$	500	$4{\cdot}7 \times 10^{-15}$
220	$3{\cdot}0$	600	$1{\cdot}8$
260	$1{\cdot}2$	700	$7{\cdot}1 \times 10^{-16}$
300	$5{\cdot}9 \times 10^{-14}$	1,000	$7{\cdot}8 \times 10^{-17}$
400	$1{\cdot}5$	1,500	$8{\cdot}0 \times 10^{-18}$

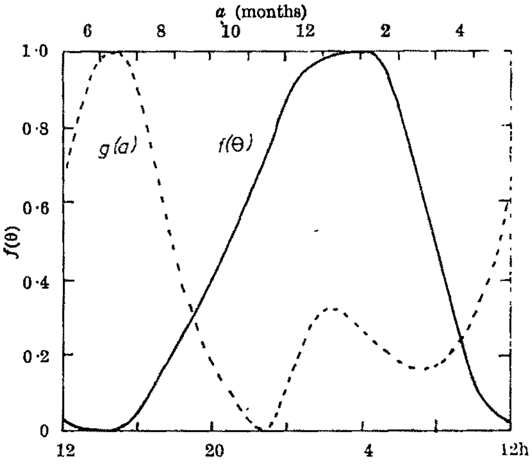

Fig. 1. Diurnal and the annual effects

Further, some minor influences exist. The magnetic activity enhances the air density by about 5 per cent if the planetary magnetic index Ap increases from 10 to 20, etc. Greater variations are only observed during magnetic storms, when the density may increase by about 30 per cent over 200 km. in height[9]. Finally, a weak influence of latitude and seasons has been found from observations of some of the *Discoverer* satellites, the orbits of which extended to polar latitudes (unpublished work). At an altitude of 200 km., the density of the air is about 10 per cent smaller in polar latitudes than in lower latitudes

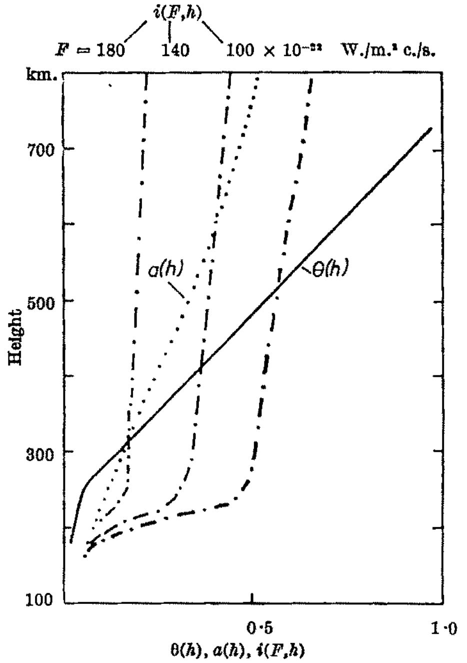

Fig. 2. Dependence on altitude of the *W*-effect, the diurnal and the annual effect

during the winter. On the other hand, a density gradient of about 15 per cent exists from the equator to the pole over the summer hemisphere.

The proposed model covers only the variations of density with a time-scale greater than one day. During strong solar flares the density of the upper atmosphere may increase considerably for a short time. The function $g(a)$ in Fig. 2 relates to the time November 1957–July 1960. Apparently in the yearly variation an annual and a semi-annual period are superposed[7]. The first has been already found in the variations of the F-layer[10] and recently also in the exospheric electron-density after whistler observations[11]. The semi-annual effect has been recently found also for the E-layer[12]. Because the annual and semi-annual effects are probably caused by the influence of the inter-planetary plasma on the upper atmosphere, its amplitude may vary with the solar cycle. This is shown by the last observations up to February 1961, when the sunspot activity was already weak. During this time the annual and the semi-annual effect was found to be considerably lower than that during the strong sunspot maximum of 1957–58. Further, some minor irregularities of the air drag seem to remain, perhaps due to inhomogeneities of the interplanetary matter. In summary, the fluctuations of the density (and temperature) of the upper atmosphere are so complex that any model may differ considerably sometimes from the real values.

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Palæomagnetic Evidence relevant to a Change in the Earth's Radius

Cox and Doell[1], applying a suggestion of Egyed[2] to test the reality of the expansion of the Earth through geological time, have calculated the radius of the Earth implied by comparison of Permian palæomagnetic measurements in the Maymecha–Kotuy region of Siberia and localities in western Europe, and find that although the standard deviation of the individual results is very wide indeed, the average of the computed radii of the Earth is close to the present radius. From this they conclude that the substantial post-Palæozoic expansion of the globe deduced by me on tectonic grounds is unlikely.

Cox and Doell state that "in the expansion of the Earth envisaged by Carey, Heezen and Egyed, the continents do not increase in area; hence, the distance between any two points on a stable part of one continent remains constant". When applied to the geotectonic synthesis presented by me in the paper quoted by them[3], this statement is certainly invalid. In each of the figures for the Asian continent in my paper I show the Asian block as composite, and elongation since the Palæozoic is explicitly shown on Fig. 9, and implicitly on Figs. 10, 39*b* and 39*d*. The Ob rhombochasm is discussed on pp. 203–204 and rhombochasm is defined on p. 192 as "a parallel-sided gap in the sialic crust occupied by simatic crust and interpreted as a dilatation". The extensions specified above fall between the sampling areas used by the authors and involve an extension of some 900 km. This is of the right order for the angular distance measured between these districts to remain unchanged if the surface area of the Earth had increased some 45 per cent since the Palæozoic.

The magnetic data presented by Cox and Doell are therefore what they should be if the tectonic synthesis presented by me is correct.

It would be possible to apply Egyed's method to places on the Earth which would provide a decisive test. This may be done, either by comparing localities which do satisfy the conditions stated by Cox and Doell, or alternatively by selecting localities on separated blocks such as Africa and South America or Australia and Siberia and testing whether the surface distance between them on the Palæozoic reconstruction, taken with their ancient latitudes implied by the palæomagnetic data, correspond to an Earth of the present radius or of a different radius. I examined the data superficially from this point of view in 1958, but concluded that the uncertainty within the magnetic data then available was too wide to give a useful result.

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It is important to note that if, during an expansion of the Earth, each point on the surface were to move radially outward, then all sampling areas would have the same relative geographical co-ordinates before and after expansion. Palæomagnetic results could not be used to detect an expansion of this type. However, an alternative model of expansion is that in which most or all of the increase in area is reflected by an increase in the area of the ocean basins. We considered Prof. Carey's model of Earth expansion to be of this general type, since he concludes[1] that the Atlantic, Indian and Pacific Ocean basins formed by dilatation attendant on expansion. If the ocean basins formed in this way, the method we used would show an increase in the Earth's radius, even if the continents had also grown a lesser amount.

The crucial point raised by Prof. Carey concerns a proposed separation of the two sampling areas used in our calculation. The 900-km. dilatation suggested by him would certainly mask an increase of 1,100 km. in the Earth's radius, provided the elongation took place along the meridian connecting the two sampling areas and the ancient pole. Implicit in Prof. Carey's reconstruction of the Eurasian landmass[2] there is, indeed, a 900-km. displacement of the Siberian sampling area with respect to Europe; its direction, however, is not along the ancient meridian, but rather at an angle of some 50° to it. The component of elongation in the ancient meridional direction in Prof. Carey's reconstruction is less than

600 km., not enough to mask an expansion of 1,100 km. Thus Prof. Carey's detailed model is not supported by the European and Siberian palæomagnetic results.

We join Prof. Carey in looking forward to future applications of this method. As he suggests, the method might also be applied to data from different continents after reassembling them into a single land-mass, assuming continental drift has occurred. However, such a calculation of the ancient radius would introduce additional uncertainties concerning the particular reconstruction used. We consider it preferable to use results from individual land-masses to test for expansion, and then proceed to compare results between continents as a test for continental drift.

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PHYSICS

Shock-excited Dark Discharge

THE existence of the so-called dark discharge region has been known from the earliest days of discharge in gases. Here, continuous current densities of the order of μamp./cm.^{2} involve no luminous effects and are attributed to the by-products of cosmic ray or other sources of weak ionization in the discharge space; as the current density is made to increase, the well-known 'glow' and 'arc' regions are successively established.

It is, however, possible to obtain current densities of about 5 amp./cm.^{2} for the 'dark' discharge condition when excitation is induced by the partial discharge of a small condenser bank in a somewhat critical pressure-range. We have found that in hydrogen, at pressures not exceeding 0·1 mm. mercury and using plane brass electrodes of diameter 2½ in., spacing 5¼ in., not heat-treated or specially cleaned in any way, shock excitation of the gas yields a dark discharge (that is, with no apparent visible glow) involving peak currents of some 400 amp. lasting for a time of the order of 1 μsec. The main features of the discharge can be summarized as follows:

(1) Although the upper limit of pressure appears to be critical around 0·1 mm. mercury, it is possible to excite the dark condition at lower pressures, if the potential is increased.

(2) Current densities range from about 2·5 to 15 amp./cm.^{2}, but repeatability was generally poor due to the presence of fatigue factors—these could be annulled by working at higher excitation voltages.

(3) The range of current values correspond with that expected from the approximate charge transferred, $2\cdot5 \times 10^{-4}$ coulomb as a minimum; values as high as 10×10^{-4} coulomb yielded little variation in peak current, but produced a longer discharge time.

(4) Above the critical pressure, an increase of pressure by a few per cent gave the standard glow in place of the 'dark' discharge at the higher voltages; however, even at this somewhat increased pressure, once the glow condition has been established, it is possible to induce a 'dark' discharge at about one-third of the glow voltage.

(5) There is no 'follow through' for this type of discharge—a certain amount of energy is drained away and the factors responsible for building up the more highly conducting condition disperse rapidly.

(6) The initiation of the 'dark' condition appears to be connected with the properties of the surfaces taking part in and exposed to the gas—'conditioning' by a succession of such discharges involving higher and higher energy dissipation makes it extremely difficult to reproduce the discharge under the conditions available to us.

(7) It was very occasionally possible to record a 'dark' discharge in air at a pressure of 0·01 mm.

The use of 'slow' mercury switching in these experiments is likely to introduce a variable and somewhat high-resistance path in the early conducting stages; it may well be that the 'build up' to the dark condition is a function of the manner and amount of the energy loss at the switch: in many cases the tube 'build up' is rapid enough to 'blow' one of the four switches (in parallel) in the bank.

An interesting parallel to the behaviour described in this communication followed some work reported in an early communication[1] on the scintillation discharge in neon. Here, one of the authors (L. J.) met the 'silent' discharge condition in neon and other gases—krypton, hydrogen, carbon dioxide, nitrogen—in the pressure range 1–10 mm., and where, in fact, the ratio of electrode separation to mean free path was of the same order as in the present experiments. Whereas current densities of some 100 amp./cm.^{2} were then recorded for the 'scintillation' condition, only some 5 amp./cm.^{2} was noted for the 'silent' discharge. The development of the 'scintillation' in hydrogen in the present experiments involved current densities of the order of 250 amp./cm.^{2} and generally followed a pre-breakdown glow stage; there appears to be no 'pinch' effect present. Some factors on which the heavy current condition depends—for example, surface properties of the electrodes, polarity of the discharge, ratio of electrode areas—are expected to affect the dark discharge condition in much the same way.

We are indebted to the Department of Scientific and Industrial Research for a grant towards this work.

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Spiral Etch-Pits of Ice Crystals

THOUGH the correspondence of etch-pits to dislocation has been investigated by many workers for various crystals, there have not been many direct observations of spiral structure of the etch-pit, which may be considered to be related to the screw dislocation. Spiral etch-pits on sodium chloride[1], on germanium[2,3], on silicon[4], on α brass[5], and on antimony[6] have been reported. The observed step-heights of etch-terraces are in the range 500 Å.–40,000 Å. Since these values of step-heights are large in comparison with a Burgers vector, the relation between these spiral etch-pits and the screw-dislocation is not as definite as that between the spiral growth pattern of crystal and the screw-dislocation.

Higuchi[7] succeeded in producing large etch-pits on the surface of ice by the use of plastic replica film. The size of these large etch-pits is about 0·5 mm., and the crystallographic basal plane appears in the etch-pits, when the surface of the ice is nearly perpendicular to the optical axis. By electron-microscope observations, it was found that minute hexagonal etch-pits are formed on the basal plane of these etch-pits by the evaporation process, and that some of them showed the spiral structure.

I used lake ice and on the ice surface nearly perpendicular to the c-axis. I produced large hexagonal etch-pits with basal planes by Higuchi's method. Then, the positive plastic replica of these basal planes was made with 'Formvar'[8], the concentration being 0·5 per cent. After shadowing with chromium vapour at an angle of 12°, this plastic replica was observed under an electron-microscope.

Figs. 1 and 3 show electron-photomicrographs of the minute etch-pits produced on the basal plane. Fig. 2 is a sketch of the stepped structure shown in Fig. 1. As seen in these figures, the stepped structure shows the spiral pattern. From many electron photomicrographs, the mean height of steps was estimated to be 200 Å.–2,000 Å. These values are nearly of the same order of magnitude as reported for the stepped structure of a growing ice-surface[9]; that is, 500 Å.

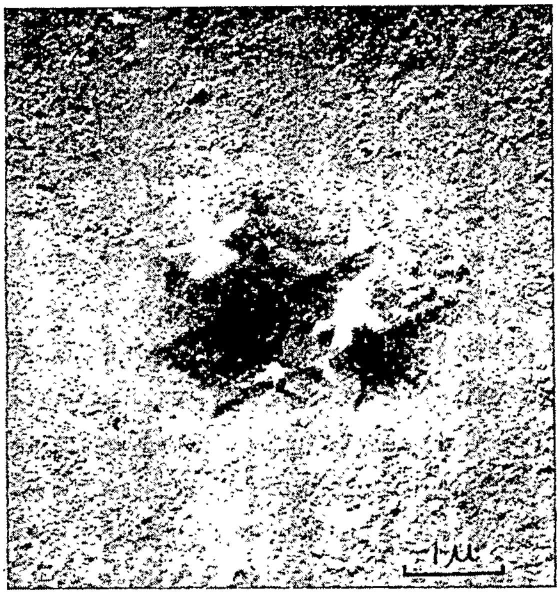

Fig. 1. Spiral etch-pits of ice crystal. (× 13,300)

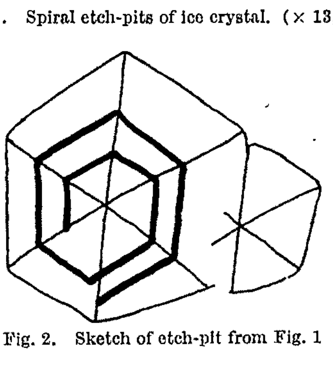

Fig. 2. Sketch of etch-pit from Fig. 1

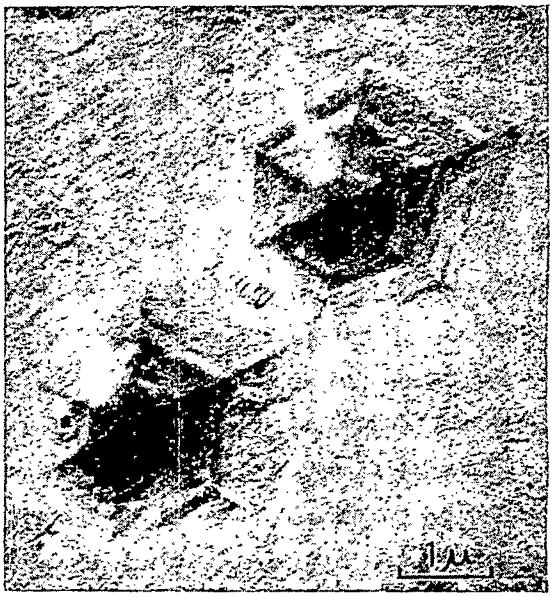

Fig. 3. Spiral etch-pits of ice crystal. (× 12,500)

This work was carried out in 1958 at the suggestion of Dr. K. Higuchi, and I thank him. I am grateful also to Prof. U. Nakaya, Dr. D. Kuroiwa and Dr. T. Mitui for their kind discussions.

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Discrimination between α-Tracks and γ-Background in Ilford Nuclear Emulsions

We have attempted to improve the discrimination between α-tracks and γ-background in Ilford 50μ nuclear emulsions by utilizing the Herschel effect[1]. The Ilford emulsions being sensitive to infra-red, direct application of the Herschel effect resulted only in a blackening of the emulsions. Therefore a desensitizer was necessary ; phenosafranine was chosen[2]. The emulsions were treated in the following way :

(1) Superficial irradiation with α-particles from a uranium-233 source.

(2) γ-Irradiation from a 17·5-MeVp betatron (doses varying from 2 r. up to 2,000 r.).

(3) Bathing in an aqueous solution of phenosafranine (for concentration see below) ; 1 hr. was necessary to allow the solution to penetrate into the emulsion.

(4) Rinsing with distilled water and drying in a slow air current.

(5) Infra-red irradiation : the light of a Philips infra-red lamp (220 V., 250 W., *E* 27, 13352 *E*/479), the filament of which was at a distance of 15 cm. from the emulsion, was passed through Schott filters *RG* 10 and *RG* 7. The filters must be placed with their esculine film side towards the lamp[3]. During the irradiation, the emulsion remained in a copper box, cooled by running water and two electric fans. An aspirator produced a cold-air current in the box. The irradiation time varied from 2 to 80 min.

(6) Immediately afterwards the emulsions were treated by temperature development (20 min. at 2° C., 15 min. at 22° C.) in a developer of the following composition :

Na_2SO_3, anhydrous	15 gm.	per litre of solution
Glycin (Johnson)	10 gm.	
Na_2CO_3, crystalline	23 gm.	
Ethyl alcohol, 94 per cent	10 c.c.	
Johnson 142	10 c.c.	

Then the emulsions were fixed in the following solution :

$Na_2S_2O_3$, crystalline	400 gm.	per litre of water
Na_2SO_3, anhydrous	12·5 gm.	
CH_3COOH 10, per cent	10 c.c.	

The emulsions were immersed in a fixing-bath at half the given concentration for 30 min., then in a fixing bath for 90 min. at full concentration, then again for 30 min. in a bath at half the concentration.

(7) Finally, the emulsions were rinsed in running water until all traces of colouring matter had disappeared ; and then they were dried.

This procedure gave the following results:

Emulsion *G*5 showed a clarification after 13 min. of infra-red irradiation and a bath in phenosafranine at a concentration of 10^{-2} gm./l., with the filter *RG* 10+*RG* 7. Discrimination improved. Higher concentrations of phenosafranine increased the clarification, but the α-tracks showed many gaps when irradiated for more than 13 min. with infra-red.

Emulsions *L*4 and *K*5 showed a clarification and an improvement of discrimination after a bath in phenosafranine at a concentration of 0·25 gm./litre and after 13 and 30 min. of infra-red irradiation with *RG* 10+*RG* 7.

With the *RG* 7 filter alone, we obtained a very noticeable clarification in all types except *C*2 ; but in each of the other types (*K*0, *K*5, *L*4, *G*5) the α-tracks showed many gaps, even after 2 min. of infra-red irradiation.

In emulsions *C*2 and *K*0, we could not improve the discrimination between α-tracks and γ-background.

A bath in phenosafranine alone was found to give a slight clarification, without impairing the quality of the α-tracks.

Another desensitizer, pinacryptol yellow, was tried, but gave no results whatever.

In Table 1, results are given for emulsion *L*4 ; *A* represents number of background grains per cm.^{3} of dry developed emulsion ; *B* represents number of gaps in the α-tracks per 100 blobs or grains in the track.

Table 1

Duration of infra-red irradiation	0	2m. 24s.	5m. 45s.	13m. 49s.	33m. 11s.
A	171×10^3	166×10^3	154×10^3	134×10^3	111×10^3
B	36·6	43·1	48·2	51·5	57·6

With G_5 and K_5 analogous results were obtained.

This work was part of the research programme of the Interuniversitair Instituut voor Kernwetenschappen-Belgium. We thank Prof. J. L. Verhaeghe. C. Beets and J. Fournaux for their interest in this work. We are also indebted to Prof. P. M. Van Vaerenbergh and Dr. J. Allewaert for the gamma irradiations.

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Radioactivation Analysis of Oxygen in Silicon by Irradiation with α-Particles in a Cyclotron

Although the determination of a trace of oxygen in transistor-grade silicon is very important, it cannot be done reliably by a chemical or by a neutron activation method. Radioactivation analysis using $^{16}O(t,n)^{18}F$, $^{16}O(\gamma,n)^{15}O$ and $^{17}O(d,n)^{18}F$ reactions were tried[1–3], but were far from satisfactory. Use of tritium in an atomic reactor resulted in low precision and sensitivity, owing to its short range and the inevitable presence of oxygen in lithium. Chemical separation of oxygen-15 is impractical because of its short life, and radioactivation of oxygen-17 is less advantageous because of its low abundance.

We have made use of the $^{16}O(\alpha,pn)^{18}F$ reaction for this purpose and succeeded in determining as small an amount of oxygen as 0·3 p.p.m. The excitation function for this reaction was determined by two of us[4]. A plate of silicon (30 mm. × 17 mm. × 1 mm., 1·2 gm.) was placed on a water-cooled holder with the aid of a spring washer for good contact, and irradiated with 40-MeV. α-particles in a cyclotron (5 μamp., 15 min.). Too high a flux and a poor contact caused slight fusion on the surface of the sample. The surface of the irradiated silicon was washed with dilute hydrofluoric acid containing a small amount of nitric acid to remove the surface contamination and pulverized in an iron mortar. The product was added in small portions to fused potassium hydroxide (25 gm.) containing sodium fluoride (10 mgm.) and disodium hydrogen phosphate (5 mgm.) as carrier. Sodium nitrate (0·5 gm.) was then added to convert radioactive phosphorus produced by $^{29}Si(\alpha,p)^{32}P$ and $^{30}Si(\alpha,pn)^{32}P$ reactions into phosphate, which served as an internal monitor for the flux of the α-particle beam. The cake was dissolved in sulphuric acid (200 ml.) and the fluoride distilled at 150–160° C., the distillate being caught by aqueous ammonia. The fluoride was precipitated as calcium fluoride together with calcium carbonate, filtered off, washed, dried and radioactivity measured. The time required for the separation was about 2 hr. and the overall recovery of fluoride was about 80 per cent.

The annihilation radiation was measured with a sodium iodide (1·5 in. dia. × 1 in. high and 1·75 in. dia. × 2 in. high) scintillation counter of a known efficiency, with and without a coincidence device.

The counting-rate was measured every hour for 10 hr. together with the γ-ray spectrum, which consisted only of the annihilation peak at 0·51 MeV. The decay curve agreed with that of fluorine-18. The oxygen content was calculated from the disintegration-rate of fluorine-18 by the absolute method, the contribution of $^{18}O(\alpha,p3n)^{18}F$ and $^{17}O(\alpha,p2n)^{18}F$ reactions being negligible under our conditions.

A raw silicon contained 4 p.p.m. oxygen, while a transistor-grade silicon had 0·3 p.p.m. By this method 10^{-3} p.p.m. oxygen can be determined. This method appears to be the most sensitive method for the determination of oxygen and is useful for the analysis of a solid sample having a high melting point and a low vapour pressure. The recoil effect, the evaporation of fluorine during irradiation and other details will be reported elsewhere.

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GEOLOGY

Analysis of a Supposed Clay Fulgurite from Ontario

THE existence of 'clay fulgurites' from Kingston, Ontario, was reported recently by Hawkins[1]. These were described as "partially fused ellipsoids" resulting from lightning striking clay sediments. The report was of considerable interest to us because it suggests that lightning may be responsible for the formation of tektites. At our request, Dr. Hawkins kindly sent us a sample of this material. The composition is as follows:

SiO_2	29·69
Al_2O_3	12·45
Fe_2O_3	30·97
FeO	1·63
CaO	0·47
MgO	1·72
H_2O	15·50
MnO	0·31
TiO_2	0·21
Na_2O	0·44
K_2O	1·84
Organic matter	4·18
	99·41

The specimen analysed was an irregular lump 3 cm. long with a yellowish-brown colour and streak, earthy lustre, and hardness of about 1 (Mohs). Portions of the specimen were covered with a shiny brown coating which was soluble in acetone and presumed to be organic (Fig. 1). In the analysis total water was determined by the Penfield tube method and organic matter by difference between loss on ignition and total water. The chemical analysis indicates that the specimen may be mostly a hydrated iron aluminium silicate or oxide. An X-ray powder diffraction pattern showed the specimen to contain some quartz, kaolinite, and additional material as yet unidentified. Petrographic examination disclosed no lechatelierite or glass. This specimen has no resemblance to any known fulgurite or tektite in composition. Therefore it appears to have no relation to the origin of tektites.

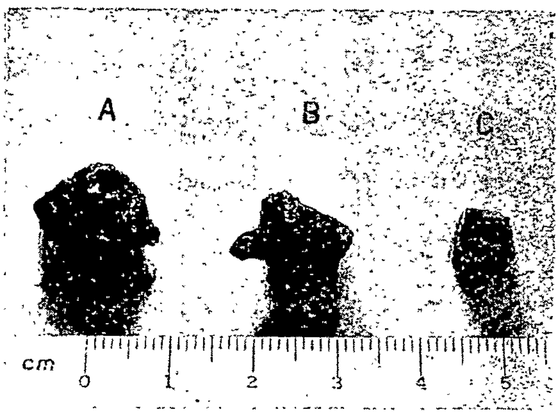

Fig. 1. Portions of the specimen marked *A* and *B* are untreated, showing their shiny coating of organic matter. The portion marked *C* was treated by immersion in acetone, exposing its dull surface

Thanks are due to E. C. T. Chao of the U.S. Geological Survey for furnishing X-ray data and to J. I. Dinnin, also of the U.S. Geological Survey, for determination of the alkalis.

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[1] Hawkins, G. S., *Nature*, **185**, 301 (1960).

Karroo Igneous Activity and Tectonics in south-east Southern Rhodesia

DURING the period 1956–60, members of the University of Leeds Research Institute of African Geology, under the direction of Prof. W. Q. Kennedy, have been investigating Karroo (early Mesozoic) igneous activity and tectonics in the south-east of Southern Rhodesia. Field-work has now been completed and it is intended in due course to publish the results in detail. In the meantime, we present here some aspects of the geology of the area which may be of general interest.

The Basement Complex underlying the area investigated (see Fig. 1) consists of highly folded gneissose rocks and represents part of the east-north-east trending Limpopo orogenic belt (*circa* 2,000 m.y.)[1], which separates the stable blocks of Southern Rhodesia and the Transvaal.

Warping and fracturing of the Dominion Reef, Loskop and Waterberg systems in the northern Transvaal indicate that the Limpopo belt continued to mark an important tectonic line at a later period (? late-Precambrian).

In the Karroo period, a relatively thick succession of sandstones, mudstones and shales, with some coal, was deposited in the vicinity of the present-day

(*Continued on page* 77)

Supplement to NATURE

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THE EXPANDING POPULATION

The Population Explosion and Christian Responsibility

By Dr. Richard M. Fagley. Pp. xii+260. (London: Oxford University Press; New York: Oxford University Press, Inc., 1960.) 28*s*. net.

DR. FAGLEY has written a useful and timely book which, it must be hoped, will be read by many thoughtful citizens. As the title indicates, it is directed to Christians, in the first place, with the purpose of arousing them to think seriously on the dangers inherent in the "Population Explosion", a picturesque name for the rapid expansion of the population of the world. In this it should be successful, for the writing is lively and clear, while the Christian background of the author is evident throughout. No one should dismiss it, however, as one more appeal to uninformed sentiment or theological dogma. The demographic facts are clearly set out with references to sources, and the probable consequences for the future of mankind are stated calmly and objectively. So far as I can judge, the author has put the case with due regard to scientific accuracy, and, when more than one estimate of the future development of populations is available, has chosen the least alarming among those offered by competent authorities. The Christian bias in this section of the book makes a welcome appearance at the conclusion of the factual and impersonal exposition, when the author reminds us of the human values which are in danger. "The main issue is the family caught in the ferment and upheaval of social change, bewildered, frightened, subject to disruptive forces, tending to lose its spiritual and social moorings. Here is found the most fundamental cost of the new pressures of population."

The book surveys various possibilities of mitigating the situation. A short discussion of the potentialities of improved methods of food production tends to the conclusion that they are unlikely to be adequate to do more than defer the catastrophe. The most controversial section of the book deals with birth-control, its methods, its sociological bearings and its ethical problems. In this, of course, we come squarely up against religion, and, to many readers, the author's summary of the different attitudes and teachings of the chief religions of the world will be most informative. Naturally by far the greatest space is devoted to Christianity, and, in spite of a few unimportant historical slips, the account of the chief traditions in the Church on the subject of birth-control is clear and fair. The chapter on the Roman Catholic doctrine is pre-eminently objective and sympathetic, which is no small achievement by a writer who cordially disagrees with it. His opinion, supported by documents, is that the rest of Christendom and its groups, with various velocities and degrees of clarity of thought, are moving towards an agreement on the subject of birth-control and the formulation of a moral theology for the guidance of their members. The present writer cannot forbear to point out that the Anglican Church, according to Dr. Fagley, has advanced further than any other in its thinking on this matter. But this is of small importance. The really important message of the book is that the time is short. We cannot wait for a century of discussion. The time to think and act is now, and Christians have a responsibility to reach an informed judgment and to persuade the politicians to lift their eyes to the future and consider the prospects of the human race.

W. R. MATTHEWS

A. V. HILL

The Ethical Dilemma of Science and Other Writings

By Prof. A. V. Hill. Pp. xiii+395. (New York: Rockefeller Institute Press; London: Oxford University Press, 1960.) 7·50 dollars; 52*s*. net.

THERE may be some, perhaps, who will expect the short title of Prof. Hill's book, as it appears on the cover, to indicate the general tone and purpose of its contents as a whole. They should be neither disappointed nor relieved to find that it provides, in fact, a meal of many different courses. "The ethical dilemma of science" must, indeed, for the author, as for any man of science concerned for the future of civilization, be a subject seldom absent from his thoughts. In this widely ranging collection of his writings, however, it figures primarily as the title of the noble presidential address which he gave to the British Association at its Belfast meeting in 1952; and it is then applied, more inclusively, only to the first chapter, consisting of various addresses dealing with the aims and prospects of science.

The internal title-page of the book adds "and other writings"; and such an extension is certainly required to give any inkling of the rich variety characterizing the products of his wide interests and agile brain which Prof. Hill has here collected. They range, indeed, from deeply considered and eloquently presented addresses, lectures and speeches, some of them in Parliament during his war-time membership, to typically generous and affectionate memorials and tributes, to men who have been his heroes, comrades and friends. They include full-blooded denunciations of social and political tyranny, and championship of its victims; claims for what science could do, criticism of its neglect and misuse, from first-hand experience in two world wars; and advocacy of what it could yet do for the Commonwealth, and especially for India's problems, based in the latter case on knowledge gained directly on a special mission.

Prof. Hill's personal researches in science have, of course, been centred on fundamental physiology, largely in terms of thermodynamics. These have no specific mention in this collection; but the enthusiasm for physiology, and for experimental science as a whole, which they have kindled in him, warms and colours all the wider interests and all the wise and generous human impulses which are here displayed. And, together with its major components, Prof. Hill, with an impatience for stolid conventions which his friends and admirers have learned to expect, has included in his book quite an array of the records of his random effusions and explosions—a journalistic *canard* involving the apparent loss by the late Prof. Donnan of his famous equilibrium, letters to *The Times*, squibs, lampoons, parodies, shafts of scornful humour aimed at many targets. These too represent, indeed, a part of the essential "A. V.". It is to be hoped that they will give enjoyment to many readers, widely beyond the circle of the professional scientists, but all capable of responding to the more serious challenge and stimulus of the book as a whole. My only personal complaint of it is that I have found it rather heavy in the hand for reading in bed.

H. H. DALE

EDDINGTON: PHILOSOPHER OR PROPHET?

The Philosophy of Science of A. S. Eddington

By John W. Yolton. Pp. xiii+151. (The Hague: Martinus Nijhoff, 1960.) 11.50 guilders.

MORE than fifteen years after Eddington's death there is still an Eddington problem. The problem has changed somewhat, it is true, but has not become less puzzling. At first—during the last ten years of Eddington's life—it seemed as if the right question to ask was, 'Has he done what he says he has done ?', and immediately after his death this assumed in many people's minds the less scientific form, 'Can he have done what he said he did ?'. Now both these questions seem to be answered by a more or less firm negative; the more interesting question, 'Is it possible to formulate the idea of a theory of Eddington-type, in such a way that it will be a significantly new type of theory giving a greater insight into science, but at the same time one which will indeed be possible ?', is that of most immediate interest. Roughly, a theory of Eddington-type would mean a theory which seeks to exploit to the full any limitations imposed on the propositions of scientific theories by the methods employed in making the experiments. Such a theory would need to discuss this kind of limitation very carefully: and, because we must understand 'proposition' here in a very general sense to mean a thought, sentence written or spoken or a manipulation with bits of the physical world, a theory of Eddington-type must itself be in a sense a philosophy of science—but not in the sense usually meant.

Before such a plan could be carried out we should need to get one 'white elephant' out of our path: that is, the fact that Eddington himself philosophized not 'in' what he was doing, but 'about' what he was doing, and not always in a very consistent manner. The present book is of some assistance here. It is a revised version of the essay which won first prize in the Belgian Eddington competition, and it seeks to show this self-conscious Eddington philosophy in its correct, essentially British, context. Locke and Hume are shown as the ancestors of a theory of knowledge, the most recent professional exponents of which include Russell. "Any analysis of Eddington's philosophy of science must recognize the twin strands of operationalism and dualism in his thought and must appreciate the possibility of uniting them . . . [by] . . . a careful analysis of dualist meaning." Such a discussion gets us no nearer to an appreciation of scientific theories of Eddington-type, but the author is clear and comprehensive in his purely philosophical task.

C. W. KILMISTER

HENRY CAVENDISH: HIS LIFE AND SCIENTIFIC WORK

Henry Cavendish

His Life and Scientific Work. By A. J. Berry. Pp. 208+4 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1960.) 35*s*. net.

IT is more than a century since George Wilson published (under the auspices of the Cavendish Society) his *Life of the Honourable Cavendish* in which he remarked on the paucity of information regarding the man himself. It is a careful and thorough piece of work, so it is not surprising that no further attempt was made to write a life of Cavendish, at least until after 1879, when there appeared Maxwell's edition of Cavendish's electrical researches, for the most part not previously published, hence unknown to physicists before that date. This revelation would have led, one might have thought, to a revaluation of the place of Cavendish in the history of science, adapted to the needs of those not having the detailed knowledge to read the *Collected Researches*, a further volume of which, *Chemical and Dynamical*, appeared with a revised version of the former in 1921. Mr. A. J. Berry's book can be said quite literally to fill this long-felt want.

Of Cavendish's life and personality little more could be added to what Wilson had written, but the new presentation of the evidence should at least dispel the common opinion that the sole occasions of Cavendish's emergence from Clapham Common were his attendances at the Royal Society Dining Club. Whenever and wherever a scientific matter was involved, Cavendish was an active and often a leading participant. Perusal of the Council minutes of the Royal Society reveals his very regular attendance at meetings of that body over two separate periods of several years; that he was chosen several times as one of the auditors of the Society's accounts; and was a member of the committees 'on measuring the attraction of hills and mountains', the protection from lightning of the Purfleet powder magazines, the disposal of meteorological and magnetic recording instruments at Somerset House, the flooring of powder mills such as Waltham Abbey, the 'erosion' of the gold coinage and the thermometric scale. Above all, he regularly took part in the 'Visitations to the Royal Observatory', and his name is usually prominent where a report on any special instrument was called for. Many of these duties, as also some of his private researches, involved a good deal of travel. Finally, the fact that a year before his death he was asked to join the group within the Royal Society known as the 'Society for the Promotion of Animal Chemistry' suggests that he was a colleague with whom other scientists, at least, found it a pleasure to work.

After a preliminary sketch of the state of science in the eighteenth century, Mr. Berry deals with Cavendish's researches under the separate heads of chemical, electrical, thermal, meteorological, and dynamical. In the case of the chemical he wisely devotes a separate chapter to the discovery of the composition of water. This *cause célèbre*—on the relative priorities of Watt, Cavendish, and Lavoisier—might have been thought to have been settled by the report of Lavoisier, Laplace, and others to the Paris Academy in 1790. But it is still necessary to express Cavendish's discovery in guarded terms, owing, as Mr. Berry reminds us, to the "question as to what Cavendish understood by phlogiston".

In his chapters on the research on electricity and heat (for the most part unpublished by Cavendish) Mr. Berry brings out admirably the peculiar nature of Cavendish's genius, revealed by his deep understanding of the distinction between what we should call the intensity and capacity factors of electricity and heat: the theory of divided circuits involving an implicit application of Ohm's Law; the equivalent conductivity of electrolytes; the better-known work on dielectrics; and, perhaps most remarkable of all, the thermal properties of heterogeneous systems, leading to the recognition of the existence of definite hydrates crystallizing at 'points of easiest freezing'.

Mr. Berry succeeds in bringing all this neglected work to life in a highly readable manner without historical distortion. The book is well produced; one must, however, regret that a book which should have a wide appeal must be sold at such a relatively high price. WILLIAM P. D. WIGHTMAN

HOMAGE TO SHERRINGTON

The Discovery of Reflexes

By Prof. E. G. T. Liddell. Pp. vi+174. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 30*s*. net.

THIS is a book of scholarship and insight that has been written as a "tribute to the life and work of Charles Sherrington. It is not a text-book and it is not a biography. . . ". As I have read the book, it has appeared to me to have more the dramatic quality of an epic. It is with consummate skill that Liddell has set out to survey the development of scientific thought in the attempt to understand the essential features of the structure and function of the nervous system.

This historical outline of neurophysiology falls into two parts: the first, before Sherrington's time, showing the system of beliefs that were current at the outset of his active life; the second, during the first twenty years of his active life—that is, up to the appearance of his great book *The Integrative Action of the Nervous System.* In the first three chapters of the book, Liddell traces the development of three separate modes of investigation which were to be fused together by Sherrington.

In these days of electron-microscopy, the investigations of histologists even a hundred years ago look extremely primitive. Yet a few years later Deiters, "the forgotten histologist" as Liddell calls him, was drawing pictures of nerve cells that have never been surpassed in accuracy and beauty. One can be confident that the classical flowering of neurohistology would have been a generation earlier, but for Deiters' tragically premature death at the age of twenty-nine. So the first chapter of the book closes in the last decade of the nineteenth century with the development of the twin concepts of the neurone and the synapse. The independence of the neurone, as established particularly by Cajal, led Sherrington to postulate the functional connexion at the zones of very intimate contact, the synapses, that had been seen by Held and Auerbach.

The third chapter, "Experimental Approaches", describes in great detail not only the experiments but also the controversies that abounded in nineteenth century neurophysiology. In retrospect, one can regret that so much heat was generated where there was often so little light, and one can at the same time be sobered with the thought of how poor our present efforts may look after a further century of retrospect. Bell, Majendie, Marshall Hall, Müller, Grainger, Pflüger are the dominant figures, and the young Sherrington must have recognized the muddled thinking and the unjustifiable arguments that characterized much of the literature. Yet Liddell surmises that the seeming confusion would have been mitigated, because Sherrington would have the advantage of Michael Foster's *Text-book of Physiology* and of a remarkable book by Vulpian, *Leçons sur la physiologie du système nerveux,* which Sherrington remembered with pleasure all his life. One can sense that Sherrington owed much to his early exposure to the thoughts of Vulpian on the nervous system.

By contrast with these two main influences on Sherrington, the second chapter, on "Animal Electricity", is of less interest, and this certainly reflects the impression it must have made on Sherrington. Electricity certainly provided him with convenient stimulating devices; but, even at the end of his scientific life, electrical recording was primitive and he made very little use of it. His great contributions were based on the analysis of muscular contractions.

After the three chapters that formed the background on which the performance of Sherrington is to be staged, Liddell introduces Sherrington in this remarkable way. "In the year 1879 two events took place, which, though quite unrelated at the time, may both seem now, seventy years after, to have been significant for the science of neurology. The first was the appearance of volume 1 of *Brain, a Journal of Neurology.* It contained, as it happened, a paper by George Henry Lewes on 'Muscle Sense', an old problem which in due course engaged the attention of Sherrington and others after him. The second event was the admission of Charles Scott Sherrington to Cambridge, at the age of twenty-one." . . . The chapter so introduced was to me of particular interest because Liddell himself naturally adopts a personal relationship to his subject. So much that he writes about Sherrington comes from that understanding which derives from the close personal relationship that existed between Liddell and Sherrington during the Oxford phase of Sherrington's life. We can see how quick Sherrington was to grasp the significance of the organization that was being revealed in the central nervous system by Cajal, von Kölliker, van Gehuchten, Golgi and others. The story that Liddell has told shows how the young Sherrington applied all this new knowledge with unerring intuition so that he was able to build up, in a few years, the basic concepts of reflex function that have formed the foundation of the neurology that we know to-day.

We are indeed grateful for a book with such insight and appreciation of the atmosphere in which Sherrington found himself in the latter nineteenth century and of his amazing creative achievements at that same time. Liddell has made this formative period of modern neurology live for us in a way that no one else could and we must be very grateful to him for it.

The book is very well produced, and there is a most valuable biographical appendix. J. C. ECCLES

FORM IN ART AND NATURE

Kunst und Naturform (Form in Art and Nature)
Herausgegeben von Prof. Georg Schmidt und Prof. Robert Schenk. Pp. 134 (68 tafeln). (Basel: Basilius Presse, A.G., 1960.) 68 francs.

THE problem of form in art and in Nature has been discussed in one sense or another at least since the period of the Egyptian dynasties, and one wonders whether the discussion had a counterpart even among the Aurignacian and Magdalenian artists. In the twentieth century there have been a number of works which approach the problem from various angles, commonly from a mathematical point of view. Among these we may recall Colman and Coan's *Nature's Harmonic Unity*, Cook's *The Curves of Life*, Hambidge's essays on *Dynamic Symmetry*, Irma Richter's *Rhythmic Form in Art*, P. E. Nobb's *Design: a Treatise on the Discovery of Form*, D'Arcy Thompson's *On Growth and Form*, Loran's *Cézanne's Composition* and others.

The book under notice provides a novel approach, because it makes a comparison between photomicrographs and modern non-representational paintings (or sculptures). It originated in an exhibition held in the Kunsthalle at Basle in 1958, and entitled "Form in Art and Nature", where the same comparison was made on a larger scale. The book provides short introductory articles by Willy Jäggi, Adolf Portmann, Georg Schmidt and Robert Schenk, followed by a series of well-printed plates, many of them in colour. The plates are arranged in pairs, so that on each left-hand page is a photomicrograph of some plant, animal or mineral subject, faced on the right-hand page by a reproduction of a work of art (usually a painting). The painters represented include Cézanne, Braque, Picasso, Mondrian, Matisse, Kandinsky, Klee, Giacometti, Jackson Pollock and various others, and the designs illustrated include a number of successful and attractive patterns and textures, mostly of the type covered by the term 'non-representational', though classified here under several headings. The aim of the exhibition and of the book has been to demonstrate a close similarity between forms revealed by the microscope, and those to be seen in the work of certain artists; and to comment on this resemblance. It is not claimed that photomicrographs or sections seen by artists under the microscope actually inspired the designs which resemble them (though this must have happened occasionally), but that the works of art came to resemble the microscopic preparations because of the "prevailing intellectual climate" of the twentieth century.

Now the pictures in this book are instructive, attractive, and intriguing; the text is sensible and thoroughly interesting; but I think none the less that the conclusions should be challenged. In the first place I believe that many of the pairs of pictures are not genuinely comparable. *Some* of them are (so much so that their independent evolution is remarkable); but in other cases comparisons are made which seem to me quite invalid. For example, Cézanne's "Sous-Bois" seems peculiarly unlike the photomicrograph of deformed brass printed opposite to it. The same applies to comparisons between Braque's "Paysage à l'Estaque" with the minute surface-structure of aluminium, and between a cubist Picasso and another aluminium surface. Again, an X-ray photograph of the human wrist-bones—hard, bony, three-dimensional and fibrous—is compared with a design in flat washes by Paul Klee, which shows a delicately coloured pattern of irregular shapes having no fundamental resemblance to the X-ray picture (Fig. 1). Further, a rigid, angular skeletal structure attributed to a marine isopod is compared with a pattern of coloured areas by Poliakoff which entirely lacks the hard, three-dimensional sculpture of the skeleton. But whether the painted pattern resembles a microscopic prepara-

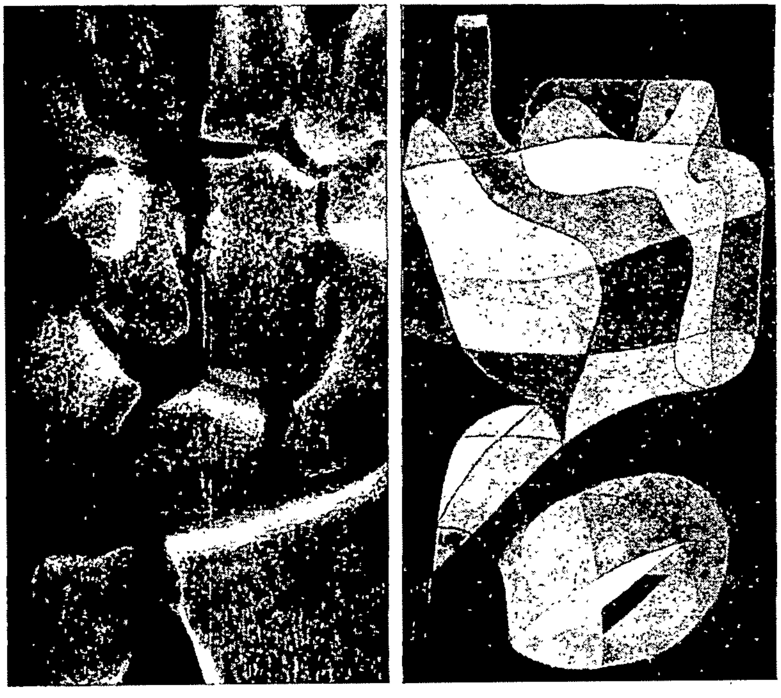
A *B*

Fig. 1. *A*, X-ray photograph of a human wrist. *B*, Paul Klee: "Lichtbreitung" (1929). (From *Form in Art and Nature*, pp. 106–7)

tion more or less closely, one feels that on the whole the comparison is fallacious because the natural object has a structure and function depending on certain peculiarities of its own such as rigidity, tensile strength or elasticity, rendering its fine structure (which is 'representational' of a certain architecture and function) fundamentally unlike the work of art which, being 'non-representational', is a pattern or texture representing no structure and function. To me it seems that the comparison is superficial and meaningless (although undoubtedly intriguing), that no conclusion can be based on it, and that it has nothing to do with the "intellectual climate" of our century. I feel that it is allied to the view so often presented to us by art critics that modern artists of certain types reveal to us 'fundamental natural forms' which underlie the real natural shapes which we see. A biologist who has studied plant and animal forms in great detail over many years may well agree that Nature has 'fundamental forms'; but he knows more or less clearly what these forms are, and is less likely to agree that modern art reveals them. For example, when Henry Moore carves something intermediate between a human figure and a pelvic girdle, I believe that he is making a plain mixture of superficial natural forms and not revealing anything fundamental: this is merely an interpretation, not a criticism. Similarly, I believe that the similarities in the book under review, in so far as they exist at all, are accidental, and reveal nothing fundamental.

This conclusion has no element of antipathy to the works of art in question, many of which I find fascinating or even beautiful in themselves, just as the photomicrographs are fascinating in themselves. But to me they are two independent series. I think that some of the earlier books dealing with form in art and Nature may have approached the truth more closely than this one, though nobody has yet solved the problem.

T. A. Stephenson

SCIENCE IN PROGRESS

Science in Progress, Eleventh Series

By Armand J. Eardley, Richard J. Russell, J. Tuzo Wilson, Serge A. Korff, C. M. Jansky, Jr., Gerald S. Hawkins, Alfred C. Redfield, B. F. Skinner, Roger J. Williams, E. Cuyler Hammond, J. W. Beard, John Turkevich and A. C. Zettlemoyer. Edited by Hugh Taylor. (The Society of the Sigma Xi devoted to the Encouragement of Research in Science. National Lectureships 1957 and 1958.) Pp. xii+379. (New Haven, Conn.: Yale University Press; London: Oxford University Press, 1960.) 60*s*. net.

THIS book will appeal to professing amateurs and to amateur-professionals—to those who, as a cultural exercise, are interested in science and what is happening in various fields; and to those scientists who are interested in developments outside their own specialities. Since it consists of essays which are surveys and re-appraisals, it will not tell those working in the selected subjects much that they did not already know.

A Greek scholar (say) would find the three contributions on the earth-sciences (Eardley's "The Cause of Mountain Building"; Russell's "Instability of Sea-Level"; and Tuzo Wilson's "Continental Growth") intelligible and informative. A professor of theology, bracing himself for the disputations on "steady state", would be well briefed on techniques of radio astronomy by reading Gerald S. Hawkins, with a side-interest in "Origins of Cosmic Radiation" (Korff), but he might feel that he was getting more insight into C. M. Jansky, jun., than into his brother Karl and "The Beginnings of Radio Astronomy" about which he was supposed to be writing. The student of geophysics would feel less rewarded, since the work reflects only the thoughts and activities aroused by the International Geophysical Year and little, if any, of the findings.

On the other hand, he, or any exact scientist, would surely find Williams's "Chemical Anthropology" engrossing and challenging. Here is the proposal for the "marriage of chemistry and anthropology". Neither physical nor social anthropology will be superseded but something new will be added. The study of human beings in terms of their biochemical individuality can be said to have started with Landsteiner's discovery of blood groups, but now, with insight into enzymes, endocrine glands and all the subtle differences in the body-chemistry, the chemist should be able to assemble the kind of information which would explain the 'eachness' of all of us. One thing which Williams shows is that there is no such thing as the 'average man'. Skinner complements this with "The Experimental Analysis of Behaviour".

Not only does the environment determine the conditions under which life exists, but also reciprocally the organisms influence the conditions prevailing in their environment. From that, Redfield proceeds to show how the chemical factors can be influenced biologically. Conversely, Hammond's masterly paper on the statistics of smoking and lung cancer might be said to show how biological factors can be influenced chemically. In the ultramicroscopic environment, we have Beard on "Viruses as a Cause of Cancer", and Turkevich on "The World of Fine Particles" and, to finish up, Zettlemoyer on "Molecular Interactions with the Surfaces of Solids".

Of course, as in any symposium, the quality of presentation varies, but in general it can be said that any intelligent person can have his ignorance of any of the subjects illumined.

Ritchie Calder

THE BRITISH ECONOMY IN TRANSITION

British Industry

Change and Development in the Twentieth Century. By Dr. J. H. Dunning and C. J. Thomas. Pp. 232. (London: Hutchinson and Co. (Publishers), Ltd., 1961.) 30*s*. net.

THIS survey of the changes in the British economy since 1914 manages to cover a very broad field in a little more than 200 pages. The topics include industrial structure, ownership and location, energy and power, research and trained man-power, new materials and processes, the State and industry, and international aspects. Drawing on a wide range of sources, the authors have given a clear quantitative picture of developments under each of their main headings. The book is intended for first- and second-year undergraduates reading economics, as well as the layman interested in British industrial development in general.

The authors find that British industry in 1914 was "structurally maladjusted in relation to the world economic trends then emerging", and it is inferred that this was a major reason for the relative stagnation between the wars. A comparison between the rate of industrial growth of 2 per cent per annum between 1880 and 1913 and 3–4 per cent per annum between 1815 and 1876 is taken to indicate a falling behind in the race; Britain had become "over-specialized"; her share of world trade in manufactured goods fell from 38 per cent to 27 per cent between 1876 and 1913; in this period, too, a large part of her investments were directed to the Dominions, South America and the United States, "principally at the expense of domestic capital formation". This assessment is not free from internal contradictions. The fall in Britain's share of world trade was simply a reflexion of the arrival of new large countries in a much-expanded world; and this share would have shrunk more if she had not indulged so liberally in foreign investment. Moreover, it is misleading to characterize capital exports over the period 1870–1913 as being principally at the expense of domestic capital formation. There was an inverse relation between long cycles in home and foreign investment in that period, and so one might just as well say that in certain phases of the cycle domestic investment was at the expense of foreign. It would have been better if the authors had inquired to what extent the organization of the British capital market in the pre-1914 period set a premium on capital exports.

Most readers will be interested in the contrast between recent industrial growth and the stagnation of the inter-war years; within its compass, the book gives a clear account of the most striking new features. It succeeds very well in describing technical progress in reasonably non-technical terms. It is salutory to be reminded that, at the peak of full employment in 1955, coal imports rose to £73·7 million (some £20 million more than coal exports), and that the revision of projected atomic energy investment in 1960 cut the earlier programme by half. There is one singular omission in the analysis of resources; nothing is said of the bearing of water supplies on the growth of industry and agriculture.

On Britain's external economic problems in recent years the argument is uneven. On p. 211 we learn that Britain has increased her share of the expanding group of export trades by more than that of any of her major competitors: on p. 217 we are told that two-thirds of Britain's export trade is with "the poorer and less highly industrialized countries whose people have comparatively simple tastes" and so it is not surprising that Britain's share of total world trade has been falling. The analysis would have been clearer if the authors had been less impressed by the significance of changing 'shares' and more concerned with the movement of the terms of trade corrected for productivity changes in the export sector. One would have liked to have, as a concluding section, a more thorough examination of Britain's dilemma concerning the European Economic Community.

BRINLEY THOMAS

STRUCTURE AND FUNCTION OF MUSCLE

The Structure and Function of Muscle

Edited by G. H. Bourne. Vol. 1: Structure. Pp. xvi+472. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 14 dollars.

THIS volume contains chapters contributed by a number of eminent workers. It is obviously impossible to deal with all aspects of muscle in any one book, but a better balance could have been obtained. Recent findings with the electron microscope dominate throughout. Apart from a description of the motor end-plate, there is little reference to the innervation of skeletal or smooth muscle. The difference between phasic, tonic and slow muscle is dealt with in less than a page.

The volume is entitled *Structure*, but it has proved difficult to separate structure from function. Indeed, the value of a book of this kind is in the integration of structure with function. The chapters in which this has been done are excellent.

Chapters 1 and 2 contain good general descriptions of the anatomy of whole muscles and their relation to movement, and of the micro-anatomy of muscle. Both appear to have been written with the student, rather than the experienced worker, in mind, and in this they are out of keeping with the rest of the volume. The development of striated muscle is dealt with in Chapter 3, which makes difficult reading; more diagrams would be helpful. Chapter 4 is a résumé of the histochemistry of developing muscle, with good illustrations. The significance of the findings merits attention in greater detail. Chapter 5 is a clear exposition of the structure and behaviour of skeletal muscle in culture.

In Chapters 6 and 7 the structure of skeletal muscle, as shown by the electron microscope, is described in detail, emphasis being placed on the sarcoplasmic reticulum in the former, and on the contractile elements in the latter. The presentation of Chapter 6 is outstanding, and forms an excellent pattern for an advanced reference work. Chapter 7 includes a clear description of the sliding filament theory of muscular contraction, and the figures are very good.

Chapter 8 deals with the difficult subject of smooth muscle, but is unnecessarily obscure. No clear picture emerges of what is, in fact, known, and detailed acquaintance with the literature is assumed. The problem of the relation and conduction between cells is not mentioned. Many figures are not referred to in the text and cannot be interpreted from the legends. A very good, comprehensive, review of the comparative anatomy, biochemistry and physiology of invertebrate and vertebrate muscle is given in Chapter 9. The subject is treated at a very advanced level, but is well presented, and a good summary is included.

Chapter 10 describes clearly the detailed structure of the motor end-plate, as demonstrated by standard staining techniques and by electron microscopy. There is no reference to the number of end-plates on different types of fibre, or to *en grappe* terminations. The structure and behaviour of muscle-spindles and other receptors in muscle are dealt with in considerable detail in Chapter 11, which is readily intelligible, nevertheless. The section on comparative physiology of muscle receptors is particularly good. Chapter 12 is a convincing description of the evidence obtained from high-resolution electron microscopy that the intercalated disks divide cardiac muscle into definite cell territories.

Some chapters have excellent summaries, and uniformity in this would be helpful to less experienced readers. The practice of having magnification scales on the plates should be used throughout. Printing errors are few; the author index page numbers referring to the last three chapters are two pages out. Several chapter page headings are not sufficiently specific.

The book is not, and is not intended to be, a textbook on muscle; it is an encyclopædic reference work. The aim of providing a balanced source of information has not been achieved fully, and more uniformity in presentation is desirable. Nevertheless, the volume contains much excellent material of value to those who work with muscle, and Chapters 7, 10, 11, and the summary to Chapter 9, should be read by all advanced students and teachers.

I. A. Boyd

CELL TISSUE AND ORGAN

Notions de Cytologie et Histologie

Par Prof. M. Chèvremont. Réimpression 1960. Pp. iv+991. (Liège: Éditions Desoer, 1960.) n.p.

During the past twenty years or so, the microscopic investigation of form and function has made tremendous strides through the invention of new methods. Their influence on cytology and histology is reflected strongly in this interesting and important book.

The text is divided into three main sections, and of these the most noteworthy is perhaps the first; it certainly imparts to the rest a special, if not a unique, flavour. It comprises a series of chapters on the cell. Since the author has had long experience of work with the ciné-camera and tissue culture, it is not surprising to find that reference is continually made to events in the living cell and that dynamic aspects of structure are preferred to discussion of morphological minutiæ. In this section are many striking illustrations taken from the author's motion picture studies. Among the early chapters is a full and detailed account of histochemical techniques, the results of which are illustrated by many excellent colour plates. Unfortunately, owing to rapid developments since the book was written, this is already out of date; nevertheless, it indicates the general aims and potentialities of histochemistry. Since original publication there has also, of course, been an almost explosive growth of information from the electron microscope following on perfection of techniques for ultra-thin sectioning. The next edition will call for the inclusion of better illustrations and more mention of such topics as the endoplasmic reticulum and the newer views on the structure and function of the Golgi apparatus.

The second part deals with the basic bodily tissues, and the third with organs and cell systems. The approach is consistently dynamic; attributes of the living tissues are discussed continually and further accentuated by description of their behaviour *in vitro*. A chapter in the third section which will naturally be read with great interest concerns the 'histiocyte' system, to use the word preferred by the author for 'reticulo-endothelial'. Prof. Chèvremont's intriguing views on the transformation of comparatively specialized cells, even muscle or epithelium, into wandering phagocytes are here expressed in a stimulating fashion.

Minor criticisms are that although many names are quoted in the text there are few bibliographical references, and that some of the photomicrographs might with advantage be replaced by diagrams. However, there is no doubt that the postgraduate student and junior staff member particularly will find the book invaluable, as would anybody wishing to assess the present status of the subject. Inevitably, since they have held the field so long, comparison of this book must be made with the trans-Atlantic manuals. It would be fair to say that the illustrations on the whole are inferior; however, the text is written in a more adult style than some; but the principal difference, stemming from the first section, seems to lie in the continual reference back to the functional attributes of individual cells. Indeed, the inclusion of such a lively and interesting introduction to cytology within the covers of a book on histology raises questions about the relationship between the two. For example, How much cytology ought the student to know before embarking on histology? The latter, of course, deals mainly with the arrangement, maintenance and function of cell populations, and seeks by experiment to identify the controlling factors. In the majority of standard books the cell itself is still dismissed quite shortly. Neglect of the brick when little was known about it and concentration on the edifice may have been unavoidable a generation ago. Now, however, there is a mass of knowledge about cells which is of absolutely basic importance; basic for the graduate in biochemistry, physiology, biology, and basic for the medical student with his additional interest in abnormal cells. Prof. Chèvremont has erected a signpost. In most histology laboratories in Britain, unfortunately, it cannot be followed until additional curriculum time and staff are provided.

N. M. Hancox

ANIMAL HUSBANDRY

Animal Husbandry Heresies

By Dr. Allan Fraser. Pp. 200. (London: Crosby Lockwood and Son, Ltd., 1960.) 16*s*. net.

In twenty short essays the author criticizes in a most interesting way many current beliefs in animal husbandry. Both the practical and scientific aspects of animal production are covered under four main headings—inheritance, environment, husbandry and purpose. After discussing early breed improvement and Bakewell's methods of inbreeding and progeny testing which led to the formation of breed societies, the latter are criticized for paying too much attention to the trade-marks of colour and form rather than to commercial qualities. It is pointed out that the recent extension of such breeds as the Clun sheep and Friesian cattle are due to the latter. Exhibits at shows of dairy bulls, poultry and sheep without production records are considered futile. Dr. Fraser is critical of the authenticity of many pedigrees in the past, but does not mention the new blood-group test.

Geneticists come under fire. From a production point of view the postulation of new genes gets us nowhere, and, although progeny tests are useful, the method of their application needs further research. In man the differences in physique at different class-levels are due to the environment of rearing and but little to genetics; this is also true for animals. Certain environmental effects, such as vitamin deficiencies, may be passed on to the next generation. as may maternal effects such as size in horses. His main criticism of present-day genetics is that it does not give enough credit to environment, both external and internal, for it is only the provision of proper nutrition which allows the full genetical potential for production to be expressed.

The science of animal nutrition places too much faith in rationing on tables of food analysis which are based on chemistry and not enough on the biological value to the animal; this is the case with non-protein nitrogen. The present distinction between maintenance and productive rations needs a new outlook, for a cow can draw on her body for milk production. The transition from peasant to mechanized animal husbandry, which has not yet proceeded far enough in Great Britain, is outlined and the losses and gains assessed. On the medical side, it is doubted whether the protective value of foods, such as milk and eggs, is as good as it used to be before intensive methods of production were employed. The author's description of hill ewes at the end of winter as being "pregnant skeletons concealed in wool" is typical of the force with which he makes his points.

The book is provocative, for there is just cause for some of his criticisms, while for others, one hopes that they will stimulate replies from the specialists concerned. JOHN HAMMOND

BROADLEAVED TREES OF BRITAIN

Guide to British Hardwoods

By Dr. W. B. R. Laidlaw. Pp. viii+240. (London: Leonard Hill (Books), Ltd., 1960.) 30*s*.

THE main purpose of this handbook is to provide students, foresters, and others interested in the subject with a ready and accurate means of identifying the broadleaved trees of Britain. This is achieved with notable success for, although the range of subject-matter is wide enough to attract the interest of those who have a fairly extensive knowledge of botany, the presentation is so clear that the intelligent novice should have little difficulty in using it to identify all but the least commonly found trees and shrubs.

To some the title of the book will seem misleading. Does not the term hardwood suggest a discussion of timbers and the trees which produce them? But here is a field approach to the whole range of native broadleaved trees and shrubs together with those which have become so well established in Britain that they form a regular feature of its countryside, parks, and gardens, and there is also a carefully made selection of less frequently seen introductions. Only brief references are made to the qualities of the timber for which many hardwoods are of importance (Are all the major hardwoods really in such short supply as the writer would have us believe?), and none to structural features.

All characteristics not considered essential for identification of the species in the field in all seasons have rightly, in a book of this kind, been discarded for the sake of clarity, but no trouble has been spared to show how the essential features may be used, through several lines of approach, for accurate identification. There are, for example, separate keys based on winter bud and twig details, summer characters, and fruits, summarized field characters in a concise tabular form, a table of seasonal characteristics, and the short, but adequate, descriptions of each species. One of the most outstanding features of the book must be the beautifully drawn and reproduced illustrations of leaf, flower, etc., in fine detail but with commendable clarity. The omission of any scale is, however, most regrettable.

For the beginner there is a short account of botanical anatomy and morphology. This is necessarily very condensed, and the beginner may find a little difficulty at first in absorbing parts, but such features as leaf- and flower-form, used extensively in identification of species, are given more fully. Together with the glossary of terms elsewhere in the book, the student should have sufficient background information to enable him to make full use of the main part of the work.

Those who are familiar with the commoner trees will find much of interest in the less-known species which the writer was, fortunately, persuaded to include, and will be most grateful for the more complete treatment given to the complex problem of identifying the willows and poplars.

J. L. DAVIDSON

ADVANCES IN PLANT PHYSIOLOGY

Annual Review of Plant Physiology

Vol. 11. Edited by Leonard Machlis, in association with Winslow R. Briggs. Pp. vii+468. (Palo Alto, Calif.: Annual Reviews, Inc., 1960.) 7 dollars.

WITH the present volume of the *Annual Review of Plant Physiology* this useful publication enters the second decade of its existence. As in past years the subjects dealt with range widely over the whole field of plant physiology. This volume of the *Review* has very much an international character, for the contributors have been drawn from no fewer than ten countries. Five of the contributions are from the United States, three from England, two from Germany and one each from Australia, Canada, Denmark, France, Japan, New Zealand and the U.S.S.R.

Of the seventeen articles, nine are concerned with various aspects of metabolism, which include the primary processes of photosynthesis discussed by A. A. Krasnovsky, and energy transfer through phosphorylation mechanisms in photosynthesis by O. Kandler. Nitrogen metabolism is represented by a short article by C. S. Yocum in which recent advances in knowledge of nitrogen fixation are considered, while the special metabolism of the succulent Crassulaceae is reviewed by S. L. Ranson and M. Thomas. Articles on transport in the xylem and in the phloem are contributed by E. G. Bollard and M. H. Zimmermann respectively, while the relationship between salt uptake and water absorption by intact plants is dealt with by R. Scott Russell and D. A. Barber. A less-familiar subject, that of heat transfer between the plant and its environment, is discussed by K. Raschke. Another topic to which not much attention has been previously given in the *Review*, and the account of which by A. C. Neish is therefore of particular value, is that of the formation and inter-conversion of aromatic compounds in plants.

Articles on growth and development again include accounts of certain aspects of auxin and gibberellin action, the former by A. W. Gelston and W. K. Purves and the latter by B. O. Phinney and C. A. West, while P. Chouard contributes an interesting discussion on vernalization and its relation to dormancy.

An innovation in the present volume is the grouping of the articles in the list of contents under a number of main headings. Under the heading "Cell Structure and Function", F. Mercer writes on the submicroscopic structure of the cell. In this, particular attention is naturally directed to the contributions made by electron microscopy to the elucidation of the fine structure of the cytoplasm and its inclusions. Another subject which might well have been included under the same heading, but which actually appears under the heading of "Special Topics", is the physics and chemistry of protoplasmic streaming by that authority on the subject, N. Kamiya. Another special topic considered is the productivity of the oceans, discussed by E. Steemann Nielsen. The two remaining articles concern tobacco mosaic virus and pectic and cellulolytic enzymes in plant disease, the former contributed by A. Siegel and S. G. Wildman and the latter by R. K. S. Ward.

What will be found particularly useful in this volume is an index of all the articles which have appeared in the eleven volumes of the *Review* that have so far appeared. The value of this index is enhanced by the grouping of the titles of the articles under twelve main subject headings, which makes reference easy. WALTER STILES

THE FLORA OF THE PACIFIC STATES

Illustrated Flora of the Pacific States

Washington, Oregon, and California. By Leroy Abrams and Roxana Stinchfield Ferris. Vol. 4: Bignoniaceae to Compositae (Bignonias to Sunflowers). By Roxana Stinchfield Ferris. Pp. vii+732. (Stanford, California: Stanford University Press; London: Oxford University Press, 1960.) 140*s*. net.

IN North America, at least, it has been a relatively rare phenomenon for an incomplete Flora to survive the death of its author and be brought to completion. Asa Gray's *Synoptical Flora* and N. L. Britton's *North American Flora* come to mind as examples of great works, valuable even in their incompleteness, but incomparably less valuable than they would be had they been finished.

It is, then, a real pleasure to record the completion of Leroy Abrams's *Illustrated Flora of the Pacific States*, for its author died in 1956, and had been in poor health since 1948. It is some measure of the respect that he commanded, and of the affection that he inspired, that this fourth and last volume was carried through to completion by a number of distinguished contributors and by Abrams's friend and assistant of forty years standing, Roxana S. Ferris.

In 1910 the flora of the Pacific coast of the United States (California, Oregon, and Washington) was not well known. Nevertheless Leroy Abrams, at that time assistant professor (that is, a lecturer) in botany at Stanford University in California, began work on an illustrated Flora which he hoped would do for the Pacific Coast what Britton and Brown's *Flora* had done for the north-eastern United States. Abrams was not unacquainted with the flora of western North America and the difficulties that its study entailed. He had already published a *Flora of Los Angeles and Vicinity* and had just completed *A Phytogeographic and Taxonomic Study of the Southern California Trees and Shrubs* for which Columbia University in New York City had awarded him the Ph.D.

As a research student in New York, Abrams had known N. L. Britton, and had become acquainted with Britton and Brown's *Illustrated Flora of the Northern United States, Canada, and the British Possessions*, a work which aimed ". . . to illustrate and describe every species, from the ferns upwards, recognized as distinct by botanists and growing wild within the area adopted . . . within such moderate limits of size and cost as shall make it accessible to the public generally. . . ." 'Britton and Brown', as it is affectionately known, may be taken as the culmination of a century of botanical study in the north-eastern United States. That the first volume of the first edition (1896) was issued within six years of the inception of the work, and that it was completed with a third volume two years later, is some indication of the amount of taxonomic knowledge that was available to the authors. The first edition of 1896–98 included 4,162 species. The third edition of 1952 covered about 4,680 species. This is another indication of the relatively complete knowledge of the flora of the north-eastern United States that existed at the turn of the century.

Despite the very considerable difficulties inherent in the study of an essentially unknown, though not unexplored, area, Abrams set himself, in 1910, to the production of an *Illustrated Flora of the Pacific States*. In contrast to the speed with which 'Britton and Brown' was written, the first volume of Abrams's *Flora* was not published until 1925, and the fourth and last volume did not appear until 1960. That the illustrations, the literature citations, and the text are accurate and that the keys work are a result of the integrity of Leroy Abrams. That there were many distinguished collaborators is freely acknowledged. The uniformly high standard of scholarship and the relative uniformity of treatment must be attributed to Abrams, who, alone or with the help of Mrs. Ferris, supplied about half the text.

In common with many, if not most, present-day American Floras, the plant families are treated more or less in the sequence adopted by Engler for his *Die Naturlichen Pflanzenfamilien*. Thus, Volume 1 of the *Flora* covers the ferns, the fern allies, the Gymnosperms, the Monocotyledons, and the beginning of the Dicotyledons from the Saururaceae to Aristolochiaceae; Volume 2, the Polygonaceae to Krameriaceae (the end of the Leguminosae, *sensu* *latu*) ; Volume 3, Geraniaceae to Scrophulariaceae; and the present Volume 4, the Bignoniaceae to Compositae. Two bits of data appear noteworthy. First, the ratio of Monocotyledons : Dicotyledons is 1,019 : 4,884, or roughly 1: 4·8, as contrasted with the north-eastern United States where the ratio is 1,415 : 3,955 or roughly 1 : 2·7, and with the south-eastern United States with 1,479 : 4,045 or also roughly 1 : 2·7. The statistics for the Gramineae are 78 genera and 389 species (1 : 5) on the west coast, 110 genera and 487 species (1: 4·4) in the north-east, and 100 genera and 476 species (1 : 4·8) in the south-eastern United States. Thus it appears that the west coast of the United States is significantly poor in numbers and diversity of species of Monocotyledons. Secondly, Abrams lists 939 species of Compositae in 176 genera (1 : 5·3) as compared with the north-eastern United States with 703 species in 115 genera (1 : 6) and the south-eastern United States with 770 species in 150 genera (1 : 6). It appears that although there are more species and genera of Compositae (though there are fewer grasses) on the west coast of the United States, the generic and specific diversity is little, if any, greater there than in the east. This does not seem to support the thesis that semi-arid regions have richer floras, in terms of diversity, than more mesic areas. The relative paucity of grasses must also cast some doubt on the general validity of this thesis.

Abrams's *Flora* comprises 6,069 species of higher plants in an area of about 324,000 square miles. It is rather startling, for botanist and layman alike, to discover that a recently published Flora of California comprehends something more than 5,000 species in slightly less than half the area (about 158,600 square miles). A close comparison of the two works shows, however, that this does seem to be the case. For example, in the first 420 pages of the fourth volume of Abrams's *Flora* there are only about 100 naturally occurring species (out of 770) which do not occur in some part of California. This fact suggests that the primary job of cataloguing the flora is virtually completed, for the west coast as it is for the north east, and the taxonomist may now turn his attention to some of the other fundamental questions which Abrams's inventory has exposed (one cannot help wondering how long it will be before the flora of the other two-thirds of the United States is equally well catalogued).

The completion of Abrams's *Flora*, then, enables us to formulate some of the fundamental problems presented by the flora of the west coast of the United States. Why are the Monocotyledons so poorly developed in the area ? What, precisely, is the real taxonomic status of the many 'endemic' species that occur in the area ? How are they to be compared with other, generally accepted, species in the area and outside it ? What, indeed, is a species ? How are we to recognize one when we see it and how are we to measure it ? Why have we been led to regard the west coast flora as an exceptionally rich one, when, on the testimony of its published Floras, it is no richer than many other comparable floras in its same latitude, both in North America and elsewhere ? These, and many more, questions will present themselves to the thoughtful student of the flora of western America. He will be grateful that he has so reliable a guide as Abrams's *Illustrated Flora of the Pacific States*.

G. P. DeWolf

HYPOGEAN FAUNAS

Biologie des Eaux Souterraines, Littorales et Continentales

Par Claude Delamare Deboutteville. (Actualités Scientifiques et Industrielles, No. 1280.) Pp. 740. (Paris: Hermann, 1960.) 60 NF.

ORGANISMS which are to be found in the cracks and fissures of rock or within the voids of sands and gravels form the subject of this important new book. Delamare Deboutteville's *Biologie des Eaux Souterraines, Littorales et Continentales* brings together for the first time in a comprehensive and readable account the work carried out in the past thirty years by European and American zoologists on these little-known but widespread and important faunas.

He treats this niche as similar in character, whether it occurs beneath the ground, beneath the sea, or beneath the intertidal zone. Moreover, this environment is continuous between sea and land, so that organisms have been able to spread along it in the course of evolution. This view is shared by a number of other workers such as Angelier, Chappuis and Spooner, the last having coined the collective term 'hypogean', which may usefully be applied to all such faunas.

The book is divided into six parts. The first concerns the environment. After a useful survey of collecting methods it gives a well-illustrated account of the physical geography and hydrology of the habitat, together with a summary of what little is known of its physical and ethological conditions. Finally there are examples of the localization of the fauna on certain beaches.

The second and third section of the book are the most valuable for the general reader, and indeed should be read by all students of marine zoology. They include a fascinating account of the adaptations of hypogean forms, followed by descriptions of the wide range of animal life, with representatives of most of the invertebrate phyla, which are to be found in the interstices of sand and gravel. Some of the figures which illustrate only parts of such animals might, with advantage, have been replaced by figures of complete animals, but otherwise the survey is excellent.

The fourth and fifth sections are in monograph form, reprinted from *Vie et Milieu*. Though of some value to the specialist, their content could with advantage have been summarized for the general reader.

In the final part there is an interesting account of the segregation and speciation of faunas, showing how continental forms have been derived from marine species by passing along the continuous subterranean habitat.

Deboutteville does not pretend to include a complete bibliography, and even some text references are not cited ; nevertheless, it will form a valuable starting point for new workers and research students.

This important and, for the most part, attractive work should help to bring to the notice of zoologists, especially in Britain where the subject has been neglected, the existence of a fascinating and important group of organisms which have as yet been studied only from the faunistic point of view. Their physiology, ethology and bionomics are virtually unknown.

D. J. Crisp
P. J. S. Boaden

FISH CULTURE

Traité de Pisciculture

Par Prof. Marcel Huet. Troisième édition. Pp. xii+369. (Bruxelles: Éditions Ch. de Wyngaert, 1960.) 450 francs; 9 dollars.

It is a pleasure to welcome the third edition of Huet's treatise on fish culture. The new material in this edition consists of some expansion of the section on the culture of *Tilapia* in tropical Africa, and new methods (using new synthetic herbicides) for controlling excessive vegetation in ponds.

The book is written for fish culturists in temperate climates, and this in effect means largely carp culture and trout culture. Carp culture has had adequate treatment elsewhere, for example in Wunder's *Fortschrittliche Karpfenteichwirtschaft*, (reviewed in *Nature*, July 8, 1950), so Huet is right in giving the most detailed treatment to trout culture. He devotes 63 pages of text and 62 plates to the subject—the largest section in the book. But he has by no means neglected other fish which are cultured, and has chapters or sections on carp, on tench and other fish which can be cultured with the carp, and on the culture of the pike, of coregonids, black bass, eel, and of *Tilapias* in tropical Africa. Huet interprets 'fish culture' in the wide sense, dealing with the raising of fry and fingerlings for the stocking of natural waters, as well as for raising to table size in ponds. Only the fancy-fish trade is omitted, and this has its own extensive literature.

The sections on general principles are useful and well illustrated. Chapter 1 deals with the construction and management of fishponds, on the choice of site, the levelling to be done, the sources and quality of the water, the soil, and the engineering work needed. The diagrams are especially useful. This theme is continued in Chapter 7, with accounts of pond maintenance and improvement, including the control of weed-growth, which may not only much diminish fish production, but can also lead to complete filling up of the pond.

There follow accounts of the 'dry period' and alternative crops, the use of lime and the reasons for it, and the use of fertilizers to increase production. In my opinion, these sections could have been expanded; for at a time when labour costs are rising, and suitable fish-fodders are in many cases becoming more costly, the correct use of fertilizer is still the cheapest way of securing a large increase in the fish crop, and the possibilities are by no means fully explored.

Chapter 2 covers the food of fish in ponds, with some good illustrations of the most important food organisms. The treatment includes a brief account of the ecology of a pond and the nutritive cycle, and ends with the practice of supplementary feeding and a list of the most important supplementary fodders, their preparation and their nutritive quotients. Huet repeats the view that at least 50 per cent of the food of carp in ponds must consist of natural food, because the latter contains enzymes which assist the fish in the digestion of the supplementary food; but this is surely no longer accepted. The stomach-less carp nevertheless possesses a very effective range of digestive enzymes; in fact, recent work in Israel suggests that the carp is as efficient a digester of fodder as cattle. The unquestioned value of a high proportion of natural food lies partly in its high protein content, which means that supplementary fodder can consist largely of cheap carbohydrates, and partly in something resembling the 'animal protein factor' of the stock-raisers and poultry farmers, which may well be associated with vitamin B_{12}.

Chapter 3 deals with carp culture, Chapter 4 with trout culture, and Chapter 5 with the culture of other fish. Chapter 6 deals with rates of stocking and productivity, and emphasizes, among other things, the difficulty in assessing the probable natural rate of productivity of newly constructed ponds. A final Chapter, 8, covers the methods of fishing out of a pond, and the handling and transport of the fish.

This is a lot of ground to cover in 270 pages of by no means closely printed text (there are 93 pages of photographs), and I have no doubt that Huet has been obliged to exert rigorous selection of his material. The obvious success of the book has justified his treatment. The paper and format are excellent, and the 280 photographs are very well done. It is regrettable that the book has no index, and that the references to the literature are inadequately given. Perhaps these could be supplied in the next edition. C. F. Hickling

ECOLOGY OF SOIL FUNGI

The Ecology of Soil Fungi

An International Symposium. Edited by D. Parkinson and J. S. Waid. Pp. xi+324. (Liverpool: The University Press, 1960.) 42*s*. 6*d*. net.

Nearly fifty years ago Waksman raised the problem of whether the soil is the home of an indigenous mycoflora, or merely the sink for fungus spores floating in the atmosphere. The answer, which he and subsequent workers gave, has been so clear that it has even been suggested that the fungal load of the atmosphere is largely derived from the soil population. The next phase of research on the soil fungi was largely floristic: the components of the mycoflora were identified and enumerated; the world-wide distribution of many species was established; floras characteristic of different soils and also of environments within soils (especially the rhizosphere and the roots of hosts susceptible to pathogenic fungi) were recognized. More recently, attention has been directed to research on the activity of fungi in the soil, and during 1958 a small group of workers was convened at the University of Liverpool for a symposium on the ecology of soil fungi. The publication of the proceedings of the symposium in this book emphasizes the value of such specialized meetings, although the value of including verbatim reports of the discussions is doubtful, as such matter is impermanent and often inconsequential.

The general picture of the soil fungi emerging from this symposium is one of populations consisting largely of resting organisms in a mosaic of micro-environments with micro-zones of antagonism, making little mycelial growth, but bursting into activity when some event brings fresh nutrient to resting cells able to exploit it. Small soil animals, root growth and litter accumulation play an important part in producing such events. Much of our present knowledge of the activity of soil fungi comes from dilution-plating techniques, the limitations and merits of which, as well as those of alternative methods, are discussed in detail. All workers recognize that the traditional methods are highly selective, little able to discriminate between passive and active states of the organisms under study; hence the attempts to supplement plating techniques with visual microscopic methods, difficult as these are with an opaque medium such as the soil. Slowly we are gaining knowledge about the presence and mode of life of Basidiomycetes in the soil. It is even possible that a future book on the ecology of soil fungi would be mainly about the Basidiomycetes and Actinomycetes instead of the moulds which occupy the centre of interest at present.

Despite the unusually abundant misprints (not even insertion of the customary accents would remove the odd appearance of some passages in French), the large amount of original research presented here, with reviews of progress and methods, makes this a valuable and stimulating work.

P. H. Gregory

ILLUSTRATIONS OF FUNGI

Iconographia Mycologica

By O. Verona and T. Benedek (*Mycopathologia et Mycologia Applicata*, Volumes XI, XII, & XIII). (I) Plates *A*1–18; *B*1–10; *C*1–18; *D*1–4. (II) Plates *A*19–46; *B*11–23; *C*19–25; *D*5–6. (III) Plates *A*47–84; *C*26–33; *D*7–10. (Den Haag: Uitgeveij. Dr. W. Junk, 1959–60.)

THREE supplements to the journal *Mycopathologia et Mycologia Applicata* have recently appeared from the pens of O. Verona, plant pathologist, and T. Benedek, medical mycologist, under the title *Iconographia Mycologica*. These supplements consist of a series of folded sheets each containing drawings of characteristic features and a brief description of one or more selected genera of fungi. It is stated that the sheets are separate so that the owner has a choice in the manner in which he decides to arrange the collection. In fact, two systems of classification are given, one strictly orthodox and taxonomic; the other into the four categories: saprophytes, plant parasites, animal parasites and industrially important fungi. As a system of filing, this second arrangement would soon become impossible since some genera, for example, *Aspergillus*, fall into at least three of the four categories. Therefore the taxonomic system of filing is the only one that could be useful.

What, then, is the advantage in a loose-filing system here? It does allow additions to be fitted into the collection as these appear, but this is a dubious advantage since, if the rate of appearance of additions continues at a high level for long, the work as a system of identification is going to be incomplete and useless for just that long. If it is soon complete and up to date, then I see no advantage over the preparation of a complete book published at one time and ready for use, and in which the occasional minor change can easily be entered in hand by the owner. It seems, then, as though the work is not intended to assist in identification. This supposition is supported by the fact that there are no keys to groups, and while in some genera some species are mentioned, in others no specific epithet is given. Moreover, there are no references to works by which specific identity could be confirmed. One is left with the single conclusion that the main use for the loose-filing system is that sheets can be taken out as wanted with the aim of demonstrating or illustrating some point, presumably to an undergraduate class. The sheets here, then, serve the same functions that used to be performed by lecture-room wall charts and diagrams. It is doubtful that they perform these functions better for being smaller and monochromatic.

In fairness, it should be stated that the standard of drawing here is considerably higher than that found in illustrations to the more usual works of general identification such as Gilman's *Manual of Soil Fungi* and Barnett's *Imperfect Fungi*, but it is not so high as that which one has become accustomed to from the type of Commonwealth Mycological Institute publication issued under the names of E. W. Mason and S. J. Hughes, from which, incidentally, many of the illustrations in the present supplements appear to be taken.

David Park

HURRICANES

Atlantic Hurricanes

By Gordon E. Dunn and Banner I. Miller. Pp. xx+326. (Baton Rouge: Louisiana State University Press; London: Interscience Publishers, Ltd., 1960.) 75*s*.

THIS book has been written for the layman. The authors say that it is desirable he should have some meteorological background, and indeed already at the beginning of the second chapter he will encounter streamlines, contour lines and isotachs (should he feel at a loss, he can refer to a glossary of terms in an appendix), and then easterly waves and the Intertropical Convergence Zone. Still, his enthusiasm may have been stimulated by the first chapter, an eye-witness account of one of the most intense hurricanes of this century, and it is better that he should come to feel that his background could be improved than that the authors underestimate him.

The book continues as a well-illustrated, non-mathematical and very interesting account of practically all that is known about hurricanes and the related hazards of storm floods and tide surges, including how to prepare for, and ride out, these disasters. It becomes evident how much their study is now in the hands of American scientists, and in particular how the modern techniques of radar and reconnaissance by aircraft, and doubtless soon also by satellite, are being developed under the impetus of the National Hurricane Research Project, in which the authors work. It seems a far cry from the days, more than a century ago, when Englishmen took a leading part in the study of tropical storms. It may be said that the Americans have a special interest: each year they suffer a hundred million dollars' worth of damage from hurricanes, a sum equivalent to several times the expenditure on the entire meteorological service of Britain. But similar storms in the Bay of Bengal last October drowned at least

12,000 members of the Commonwealth; there, tropical storms are about as frequent as in the Caribbean, and there it was that Henry Piddington studied them and invented for them the name 'cyclone'. About the same time, the engineer, Major-General William Reid, after arriving in Barbados to restore Government buildings blown down by a hurricane, took the lead in the study of Caribbean storms. In the present book we read that his own, first published in 1838, "is still held in high esteem as a book of reference". Of course, in those days a spirited man, even on his own, might make some impact; to-day it requires a group of men, handling facilities too expensive to be provided by other than Governments. Nevertheless, the facilities exist; Can it be the spirit which is missing ?

F. H. Ludlam

EARTH HISTORY

The Age of the World

Moses to Darwin. By Prof. Francis C. Haber. Pp. xi+303. (Baltimore, Md.: The Johns Hopkins Press; London: Oxford University Press, 1959.) 40*s*. net.

In the not very distant past the question of the age of the world was not the sole concern of scientists and naturalists, but one about which theologians, the informed public, and even the uninformed public were deeply concerned. As recently as 1838, the Rev. Baden Powell, Savilian professor of geometry at Oxford, declared that the most inconceivable assertions of the astronomers and chemists were admitted and even applauded, whereas, on the other hand, the geologist was regarded as visionary and presumptuous if he attempted to draw the obvious conclusions from the rocks he studied and to claim that millions of years ago the Earth was governed by the same physical laws as to-day. The reason for this attitude was, of course, that at that time geologists were attempting to establish natural laws that contradicted the account of the Creation given in the book of Genesis, as well as the statement carried by every Bible then (and for many years afterwards) that the date of the Creation was 4004 B.C.

To-day it is accepted as axiomatic that the age of the superficial rocks of the Earth's crust is of the order of several thousand million years, and that living organisms must have existed on the Earth's surface for at least 500 million years, and probably very much longer. Yet, historically speaking, this conception of the length of the geological time-scale is quite recent.

The complete revolution in scientific thought that has led to the unquestioned acceptance of age figures of such magnitude by scientists and laymen alike has seldom formed the subject of special study, but it has now received the examination it deserves by Prof. F. C. Haber, of the University of Florida, in his book *The Age of the World*. The author is a professional historian and, following the modern tendency in studies in the history of science, he deals with his subject as a special aspect of human history. The sub-title of Prof. Haber's book, *Moses to Darwin*, indicates the period that he covers in detail. So far as the Christian world is concerned, this limitation is justified. Once the teaching of Christianity spread, the Mosaic account of the Creation came to be accepted as literally true throughout Christendom, and this belief was not successfully challenged until the nineteenth century. It not only determined lay opinion for centuries but also exerted an inhibiting effect on the development of more rational views by scientists and naturalists.

In the latter half of the seventeenth century, however, and during the eighteenth century, many advances were made by both geology and cosmogony. In this period, the author points out, doubts were expressed from time to time as to the validity of the Mosaic dogma. For example, in 1681, Thomas Burnet, sometime Master of the Charterhouse, published the first volume of his *Sacred Theory of the Earth*, a book very widely read at the time and for many years afterwards. While of little scientific import, it had the merit of conforming to the teachings of the Bible. Yet even Burnet, himself a divine, ventured to suggest in a later book that the Biblical days of the Creation might be considered as allegorical rather than literal days. As a result of this heresy he lost his position at Court. A further instance, not noticed by Prof. Haber, was the publication by the astronomer Halley, in 1715, of a rather cautious suggestion that the Biblical account of the Creation could not be taken literally. Halley also made what was probably the first suggestion of a scientific method for determining the age of the Earth—by measuring the rate of increase of the salinity of the oceans—a method that was actually used some two hundred years later[1].

In 1749 the great French naturalist Buffon published a *Theory of the Earth*[2] in which he expressed unorthodox views about its age. In consequence the Faculty of Theology in the Sorbonne forced him to retract certain statements and to express a belief in the scriptural account of the Creation. In 1788, and again in 1795, the geologist James Hutton published his well-known dictum that in the rocks of the Earth's crust he could find "no vestige of a beginning —no prospect of an end". He was followed in 1802 by Lamarck, who, in his *Hydrogéologie*, emphasized that the geological processes of Nature must have required vast periods of time. His views were not well received at the time, and were soon overshadowed by the more popular doctrine of successive catastrophes advocated by his fellow countryman Cuvier.

These and other occasional expressions of enlightened opinion had no very marked effect on the broad stream of scientific thought at the time they were published. During the first two or three decades of the nineteenth century, however, geology and related sciences rapidly progressed so that the views of geologists about the age of the Earth could no longer be dismissed as entirely imaginative and had to be given serious consideration. The struggle between Church and science which, so far as geology was concerned, had been smouldering for a century or more, now flared up into the open. Not surprisingly, the statement by Hutton had added fuel to the fire, for to have accepted it would have done away entirely with belief in the Mosaic account of the Creation. Geological science had to contend with religious beliefs that had been firmly held for some eighteen centuries and were not to be overthrown easily, more especially since they were held by many, probably most, scientists of repute, some of whom were in Holy Orders themselves. For a time at least geologists were reluctant to declare, openly and unequivocally, opinions directly contrary to their religious beliefs, and some prominent geologists, notably Buckland in England and Cuvier in France, attempted to reconcile their geological views with these beliefs. A contemporary publisher's advertisement of an English translation of Cuvier's *Discours sur les Révolutions du Globe*, published in 1813, sums up the situation in the following notice: "The Christian may furnish himself, from the publication of a Parisian philosopher, with armour to defend his faith against those writers who have endeavoured to overturn it by objections against the Mosaic account of the Deluge, and the age of the human race"[3]. The tide had turned, however, and more rational views about the age of the Earth gradually gained ground, even though theological opposition persisted for many years.

Two landmarks stand out on the road of progress towards modern views on the subject. One was the publication of Charles Lyell's *Principles of Geology* (1830–33), which, as a clear, logical and well-documented exposition of the new geology, had a profound and lasting effect on geological thought. The second was the appearance, in 1859, of Darwin's *Origin of Species*, which provided a new and compelling reason for extending geological time. Once the doctrine of evolution was accepted as a reality, there remained only one obstacle in the minds of those who were reluctant to abandon completely the Mosaic account of the creation of the world. This was the strongly held belief that man was a special creation. Evidence from other sources, anthropology, archæology, ethnology and philology, had by this time accumulated to an extent that made it unlikely that this belief would survive long in scientific circles. In any event, it did not effect the main issue, that of the length of geological time, which was already widely accepted as being of immense, though uncertain duration, and the long struggle for recognition of this fact was now over.

This, briefly, is the story discussed at length and with an appropriate historical background by Prof. Haber. He also points out how other branches of knowledge gained from the changed ideas about secular time. Thus, it became possible to explain the occurrence of archæological remains of cultures that, apparently, had been at their height at a time when, hitherto, it had been thought the world had not been created; and it was also possible to accept the suggestion of philologists that the development of languages seemed likely to have commenced in times much earlier than the date assigned by Biblical scholars as that of the Creation. Another anomaly done away with was that produced by the astronomical conception that light emanating from distant sources in the universe had been travelling so long that it had, in the view of the astronomers, left its source before the universe was created.

Prof. Haber's book contains five chapters. In the first he discusses especially the ideas about time held since the dawn of the Christian era and shows how, for centuries, no progress was made towards rational thought on the subject, until the breaking of what he calls the "6,000 year time scale" set up by Archbishop Ussher and others. In Chapter 2, entitled "Historicism and the Scientific Revolution", he considers the growth of geological ideas in relation to the development of science as a whole, up to the end of the seventeenth century. He traces the history of ideas about the origin and significance of fossils. From the time of Aristotle the problem had remained unsolved. Although there appears to be little that is new in his account, he justifies his conclusion that "by the end of the seventeenth century the fossil enigma had been brought into the mainstream of European thought about cosmogony". Consideration is also given in this chapter to the contributions to astronomy and cosmogony made by Descartes, Newton and Leibniz, more particularly regarding the conception that the Earth had cooled from a molten mass. This assumption constituted a considerable advance in geological thought and was later used as a means of calculating the age of the Earth.

In Chapter 3 the author deals with the outlook on his subject that developed during the eighteenth century, a period in which the biological sciences came to the fore, and the idea of perfection of design in Nature was developed. This latter conception, however, did not preclude belief in the Mosaic chronology and cosmogony. In this chapter Prof. Haber also discusses the importance of Buffon's contributions to the advancement of natural history, and gives an account of the remarkable series of experiments, little noticed in Britain, which he carried out in an attempt to make a positive estimate of the age of the Earth. Buffon prepared a series of balls of each of the materials of which the Earth is composed, including rocks, minerals and metals, and after raising them to white heat, determined the rate at which they cooled to room temperature. On the assumption that the Earth had once been a molten mass he calculated the duration of several periods in its history, allotting about 40,000 years as the time that had elapsed since the Earth had cooled sufficiently to allow life to exist on its surface. Buffon published a full account of these experiments in 1778, in his *Epoques de la Nature*[4]. Though, by present-day standards, his results are quite unacceptable, they indicate an advance in the thought of that period.

Chapter 4 is entitled "The Biblical Age of the Earth Surrendered", the first part having the subtitle "The Flood Triumphant". At the beginning of the nineteenth century it became evident to geologists in both Europe and America that vast land areas were covered with unconsolidated deposits, sometimes containing mammalian remains, that could easily pass for material left by a retreating flood. This fact was seized on by theologians and many geologists, as proof that a more or less universal Noachian flood had been a reality in the not too distant past. The author goes on to describe what he calls the "Lyellian Revolution", which played a large part in the final overthrow of belief in the Mosaic chronology among scientists. The chapter concludes with an account of "Mosaic Geology in America". In his final chapter the author discusses the effect of the publication of the *Origin of Species* on ideas about geological time; and, lastly, the origin of man.

Among scientists the overthrow of belief in the Biblical chronology of the Creation was now virtually complete, and the only question that remained a live issue was not one of geology versus theology but what, in more precise terms than had as yet been proposed, really was the age of the Earth. During the nineteenth century attempts were made to estimate this in various ways. Prof. Haber does not enlarge on them, and he makes only a very brief reference to the work of Sir William Thomson (Lord Kelvin), who, shortly after the publication of the *Origin of Species*, attempted to solve the problem by means of astronomical and physical data. The story of his intervention, the concern it caused among biologists, particularly Darwin, and among geologists, too, and the revolutionary effect the discovery of radioactivity had on the resolution of the problem, leading, as it did, to an entirely new method of calculating the Earth's age, is an interesting one. It would have rounded off Prof. Haber's story in a very satisfactory way if he had continued it in rather more detail to the time when the new radioactive methods of determining the age of the Earth were first used, some fifty years ago. This criticism apart, he has written an extremely interesting book, which is not just a record of isolated opinions, events and discoveries but a continuous narrative of the development of ideas on his subject. There is no bibliography, but extensive footnotes provide a valuable guide to source material.

V. A. EYLES

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[1] *Phil. Trans. Roy. Soc.*, 29, 296 (1715).

[2] De Buffon, G. L. L., in *Histoire Naturelle*, 1, 65 (1749).

[3] The advertisement occurs in a copy of J. J. Berzelius' *Mineralogy*, translated by J. Black, London, 1814, in the possession of the reviewer.

[4] First published as Supplément 5 to Buffon's *Histoire Naturelle* (1778). Preliminary results of the experiments were published in Suppléments 1 and 2 (1774) of the *Histoire Naturelle*.

GEOPHYSICAL METHODS

Methods and Techniques in Geophysics

Vol. 1. Edited by Prof. S. K. Runcorn. Pp. ix+374. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 10 dollars; 72*s*.

MANY books and review articles have been written which are concerned with the observational data of geophysics and the deductions from them; Prof. S. K. Runcorn's aim in presenting this book is to provide a comparable account of the methods and techniques which are used to obtain the observations, which will be useful to those who wish to start a particular type of experimental work and to those who wish to evaluate critically the methods and the results.

It contains accounts of the techniques used in a variety of investigations: there are two articles on heat-flow, one by Sir Edward Bullard and the other by A. D. Misener and A. E. Beck; articles on borehole surveying by T. F. Gaskell and P. Threadgold; the geomagnetic elements by K. Whitham; palæomagnetism by D. W. Collinson and K. M. Creer; gravity at sea by J. C. Harrison; earth movements by P. L. Willmore; earth currents by G. D. Garland; the properties of rocks under high pressure and temperature by D. S. Hughes; and the secular motion of the pole by W. Markowitz.

The accounts are good, and admirably fulfil their purpose; obviously, it is not possible to give a complete account of all the experimental techniques involved in every type of measurement, but the general principles and sufficient practical details and difficulties are related, and quite detailed references have been given. The measurement of heat-flow is an important geophysical technique and this has been recognized by two articles on it. Harrison's account of the measurement of gravity at sea includes an account of the cross-coupling effect, and points out that in the case of a beam-gravimeter stabilized along the true vertical the error due to it in an extreme case could be as high as 500 mgals; in actual operation, however, the error with the Graf instrument can be maintained less than 15 mgals and a correction can of course be made for it. The errors involved in gravity at sea are still essentially navigational.

The article on measurements in palæomagnetism shows a curious balance in that so much is concerned with the astatic magnetometer. It might perhaps be argued that the original papers in which the instrument was described are not easy to obtain, and that it is the fundamental instrument used in the study of rock magnetism; nevertheless, it is curious that there is no account of the effects of pressure on the magnetic properties of rocks, or an account of alternating-current bridge methods of measuring susceptibility.

These criticisms are trifling, however; this is an excellent book and is well produced. It is bound to be of use to many geologists and geophysicists.

M. J. KEEN

POLYSACCHARIDES OF MICRO-ORGANISMS

Polysaccharides of Micro-Organisms

By Prof. M. Stacey and Dr. S. A. Barker. Pp. vii+228+8 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 30*s*. net.

IT was a courageous undertaking on the part of Prof. Stacey and Dr. Barker to attempt to review the extensive field of microbiological polysaccharides in one small volume of more than 200 pages. They have, however, achieved this task, and have produced an elegant little book which is both up to date and readable, and will be much appreciated and enjoyed by those who already have a good grounding in carbohydrate chemistry.

Early in the book there is a short account of carbohydrate nomenclature and conformation, which is followed by a brief description of the various monosaccharide components encountered and their biosynthesis. There follows an account of the structural components of cells, of the function of capsular and cell-wall polysaccharides and of biological activity; and, in an attempt to appeal to the non-specialist reader, the authors have included two chapters in which the older (classical) and modern approaches to the isolation, purification and structural analysis of polysaccharides are given. These chapters are of necessity concise, but are timely in that they emphasize the value and importance of employing the results of physical, chemical and serological measurements to establish the homogeneity, or more correctly, the lack of demonstrable heterogeneity, of the materials under investigation. The establishment of the suitability of polysaccharide preparations for structural studies is an aspect of polysaccharide chemistry which has been much neglected until recently.

The remaining seven chapters are devoted to the polysaccharides associated with Gram-negative and -positive bacteria, viruses, rickettsiæ, moulds, yeasts, yeast-like fungi and protozoa.

The bacterial heteropolysaccharides have been longest and most thoroughly studied; but, in spite of all the effort since many of these important substances were discovered in the early part of the century, the progress in elucidating their detailed structure and, in some instances, even the qualitative composition, has been disappointingly slow. However, recently there has been a renewed attack on these materials in many different laboratories and already startling results are being obtained, especially in determining the fine structural details which are primarily responsible for the outstanding and characteristic serological specificity of many of the microbial polysaccharides. Indeed, it is the use of enzymic and serological inhibition studies and the more modern techniques of carbohydrate chemistry, such as periodate oxidation, that has enabled so much progress to be made in such important fields as the association of a known chemical structure with the delicate and specific serological reactions as developed in the outstanding Kauffmann-White scheme for *Salmonella* classification. Here the important new work of Westphal and of Staub and their associates is rightly described. It is, however, unfortunate that the closely related and pioneering work of McCarty in this field has been overlooked.

The nature of the dextrans produced by many strains of *Leuconostoc mesenteroides* and closely related organisms is considered in detail, and a very valuable table (17) is included, in which are given the amounts of 1–6, 1–4 or 1–3 glycosidic linkages which may be expected to be present in a particular bacterial dextran.

Little can yet be said about the polysaccharides of rickettsiæ and viruses. The chapter on the polysaccharides of the so-called 'Higher Bacteria', which deals largely with the products derived from human, bovine and avian strains of *Mycobacterium tuberculosis*, makes fascinating reading and contains much useful information. The polysaccharides of moulds have been much studied, but, in many instances, apart from some knowledge of the qualitative composition of the polysaccharides obtained, little or nothing is known of their structure. Similarly, with a few exceptions, the carbohydrate complexes produced by yeasts and protozoa are, as yet, largely uninvestigated.

Two appendixes, one containing the melting points and specific rotations of the monosaccharides most frequently encountered in the polysaccharides considered, and a second giving a brief account of some of the more important papers which have appeared since the manuscript was completed, are most useful additions.

The book is beautifully produced, and the paper, printing and binding are all that could be desired. The formulæ given in the text are well set out and clearly produced, and the selection of those references believed to be of key importance are remarkably complete up to 1958. The book is reasonably priced and the authors and publishers are to be congratulated on producing a short, well-written and up-to-date account of polysaccharides synthesized by micro-organisms.

W. T. J. Morgan

THE GROWTH OF ORGANIC CHEMISTRY

Advances in Organic Chemistry

Methods and Results, Vol. 1. Pp. ix+387. 90*s*. Vol. 2. Pp. vii+504. 113*s*. Edited by Ralph A. Raphael, Edward C. Taylor and Hans Wynberg. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.)

"THE subject of organic chemistry exercises a perennial fascination for the scientific publisher." It is with this rather naïve admission that the editors introduce the present series and presumably seek to justify its existence. There is no denying the advances in organic chemistry, in methods and results. But is this series really necessary when, in addition to the recognized sources of information, a parallel series covering progress in organic chemistry is already well established and has gained popular acceptance?

Six topics are reviewed in Volume 1. These are: Kolbe electrolytic synthesis (B. C. L. Weedon); polyphosphoric acid as a reagent in organic chemistry (F. Uhlig and H. R. Snyder); the Wittig reaction (S. Trippett); hydroxylation methods (F. D. Gunstone); selective degradation of proteins (E. O. P. Thompson); optical rotatory dispersion and the study of organic structures (W. Klyne). Of these, four have been the subject of reviews elsewhere within the past few years and an authoritative text-book on one of the themes has recently appeared. Still, this new publication may be taken as an indication of the widespread interest in and the vigorous development of the fields in question. Volume 2 certainly strikes a newer note. Here, the editors have been more discriminating in the selection of topics, which include: alkenylmagnesium halides (H. Normant); dialkoxydihydrofurans and diacyloxydihydrofurans as synthetic intermediates (N. Elming); ethynyl ethers and thioethers as synthetic intermediates (J. F. Arens); ketene in organic synthesis (R. N. Lacey); nuclear magnetic resonance in organic structural elucidation (H. Conroy); hydrogenation-dehydrogenation reactions (L. M. Jackman): ultra-violet photochemistry of simple unsaturated systems (P. de Mayo); muscarine (C. H. Eugster).

Normant's review will be warmly welcomed. His discovery that alkenylmagnesium halides are formed in excellent yield by the Grignard reaction when solvents such as tetrahydrofuran are used in place of ether constituted an important advance in preparative organic chemistry. The reaction has been exploited in many directions; it has proved particularly useful in the field of organometallic chemistry. The elegant electrolytic method for the dialkoxylation of furans due to Clauson-Kaas has made available a group of new and versatile intermediates. These dialkoxydihydrofurans and the related diacyloxy derivatives have been widely used for many synthetic purposes. Elming, who has been closely associated with this work, gives an admirable survey. Arens provides an excellent review of the preparation and reactions of ethynyl ethers and thioethers. This is a valuable addition to the literature on acetylene chemistry. Although earlier reviews on certain aspects of ketene chemistry have appeared, Lacy, in his contribution, gives a unified presentation of the reactions of ketene and its derivatives—diketene, *iso*propenyl acetate and β-propiolactone—compounds which are of considerable industrial importance. Despite the existence of several standard works on the subject, the inclusion of a chapter in this series on the applications of nuclear magnetic resonance in structural elucidation is not surprising; such are the potentialities of this new tool. As the author rightly points out, its intelligent use demands more of an understanding of the principles involved than does any other form of spectroscopy in popular use by organic chemists. However, within this brief compass, the author is only able to deal with those aspects of theory which have direct structural analytical significance. The treatment is concentrated almost exclusively on proton resonance, and solid-state spectra are omitted. Despite these limitations, the review has its obvious merits and will be appreciated by practising organic chemists.

There are occasions when the use of hydrogen-transfer reactions offers striking advantages over catalytic hydrogenation and dehydrogenation and, in consequence, they have their established place in the armoury of the organic chemist. The advantages arising from the usually complete stereospecificity of biochemical hydrogen-transfer reactions had led to some subtle and elegant applications. Jackman's survey of new developments in this field is timely, and many would wish that the section dealing with biochemical systems could have been amplified. Recent years have witnessed a renaissance in photochemistry, and preparative photochemical methods are rapidly gaining favour in certain fields. De Mayo does a signal service by focusing attention on recent developments and suggesting further areas where photochemical processes might prove useful and profitable. Finally, Eugster presents an interesting account of the chemistry of the alkaloid muscarine, the relatively simple, but unique, structure of which has only recently been unravelled, despite the combined endeavours of many illustrious organic chemists over many years.

The series has a truly international flavour, and a very high standard is maintained throughout. The inclusion of some details of experimental procedures adds to the value of the series. If the more individual and original character of the second volume can be maintained in future issues, the series is likely to earn for itself a worthy place on the private book-shelves of many organic chemists as well as in their institutional libraries.

J. Idris Jones

THE PROTON IN CHEMISTRY

The Proton in Chemistry

By R. P. Bell. (The George Fisher Baker Non-resident Lectureship in Chemistry at Cornell University.) Pp. vii+223. (London: Methuen and Co., Ltd., 1959.) 42*s*. net.

It is perhaps ungenerous to complain that the title of this book is wider than the contents, when the author himself has admitted this in his introduction and made his excuses. The book is, in fact, mainly about the properties of acids and bases, or at least about the transfer of protons from one molecule to another. Within this chosen field, these lectures, given at Cornell University while the author was George Fisher Baker non-resident lecturer, provide a readable, accurate and lucid account of acid-base theory and associated phenomena, and the book can be wholeheartedly recommended to students of physical chemistry as a supplement of their regular texts.

In the final chapter the author has done much to clarify what is known about the isotope effect, that is, the change of properties resulting from the substitution of the proton by the deuteron, in acids and bases.

There are, of course, many other chemical phenomena with which protons are concerned. No doubt some of these would have taken the author too far from the general theme of the book, but some, such as hydrogen bonding, which is only briefly mentioned, might well have been treated at greater length. In view of the great importance of hydrogen bonding in organic and biological compounds, some consideration of the strength of hydrogen bonds would have been welcome.

An account of the present state of knowledge of the structure of liquid water would also have provided a useful background to the properties of the hydronium ion. Another rather surprising omission, since energy curves for proton transfers are given, is any reference to the neutralization of the proton in electrolysis, one of the most direct and simple ways of observing such transfers. It would also probably not be stretching the matter too far to include those redox potentials in which protons are involved.

Another matter of considerable interest, which might well be dealt with in a future edition, might be the circumstances in which a proton, free or hydrated, can capture a free electron and the properties of the hydrogen radical so formed. In view of recent work on the chemisorption of hydrogen at metal surfaces, the polarization of hydrogen atoms and their reactivity, it is not out of the question that this subject might also find a place among the phenomena which might reasonably be included.

It is only necessary to add that the book is very well produced and, under present conditions, reasonably priced.

J. A. V. Butler

SIALIC ACIDS

The Chemistry and Biology of Sialic Acids and Related Substances

By Dr. Alfred Gottschalk. Pp. ix+115+8 plates. (Cambridge: At the University Press, 1960.) 22*s*. 6*d*. net

This book deals with a class of naturally occurring substance which has attained great biological importance in the past few years. Although the initial chemical work on the carbohydrate components of mucous secretions was done before the beginning of this century, it was not until about ten years ago that biochemical interest in the mucins of epithelial secretions was awakened by the discovery of their role in blood-group-specific substances and in the inhibition of influenza virus hæmagglutination; Dr. Gottschalk is closely associated with the latter work.

The book starts out with a clear exposition of the historical development of the subject and is followed by a chapter (28 pages) on the chemistry of sialic acids. A section (25 pages) on the preparation and determination of sialic acids is valuable for its discussion of the advantages and disadvantages of the various methods used. The next two chapters (23 pages) deal with what is to me the most exciting part of the book: the linkage and occurrence of sialic acids in biologically active mucoproteins, mucolipids and other natural products. The following chapter, on the sialic acid content of tissues, usefully collects together scattered data into a readily accessible form. Other chapters deal with enzymes acting on sialic acids and a comparison with bacterial muramic acid.

Minor points are that a structural formula of N-acetyl neuraminic acid would, I think, be helpful on the first page of the historical development, and the section on milk oligosaccharides could well include mention of the work of György and of Malpress.

These are, of course, trifling criticisms of a book which is well written, beautifully produced and, at the price, is good value as an introduction to the sialic acids.

T. F. SLATER

TOWARDS PHYSICAL CHEMISTRY AT HIGH TEMPERATURES

Tables of Thermodynamic and Transport Properties of Air, Argon, Carbon Dioxide, Carbon Monoxide, Hydrogen, Nitrogen, Oxygen and Steam

By Joseph Hilsenrath, Charles W. Beckett, William S. Benedict, Lilla Fano, Harold J. Hoge, Joseph F. Masi, Ralph L. Nuttall, Yeram S. Touloukian and Harold W. Woolley. Pp. xiii+478. (London and New York: Pergamon Press, 1960.) 140*s*. net.

Thermochemistry for Steelmaking

Vol. 1. By Prof. John F. Elliott and Molly Gleiser. (Sponsored by the American Iron and Steel Institute.) Pp. viii+296. (Reading, Mass.: Addison-Wesley Publishing Company, Inc.; London: Pergamon Press, 1960.) 75*s*. net.

Physicochemical Measurements at High Temperatures

Edited by Dr. J. O'M. Bockris, Dr. J. L. White and Dr. J. D. Mackenzie. Pp. viii+394. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1959.) 75*s*.; 13.50 dollars.

THE publication of three books serves to emphasize the flow of research and development at present, in regard to systems at high temperatures. Probably the most detailed and most extensive information already within reach concerns the thermodynamic and transport properties of gases. *Tables of Thermodynamic and Transport Properties of Air, Argon, Carbon Dioxide, Carbon Monoxide, Hydrogen, Nitrogen, Oxygen and Steam* presents a valuable compilation of mutually consistent data for temperatures from 10° to 3,000° K. and from 0·01 to 100 atmospheres, for the gases named and for steam and air. Experimental measurements for the various gases were considered to varying degrees in choosing molecular force constants. From the best values, equations of state were derived in the form of virial expansions; a large number of mutually consistent results have been derived from such equations of state. Thermodynamic properties of the ideal gases were derived from spectroscopic data using standard formulæ of statistical thermodynamics. Transport properties were calculated either from empirical data or using force constants based on the Lennard-Jones 6–12 potential. Properties tabulated include (with few exceptions) the compressibility factor, density, entropy, enthalpy, specific heat, specific heat ratio, and velocity of sound, as well as the viscosity, thermal conductivity, and Prandtl number, and vapour pressure of liquid and solid. Other quantities derived for the ideal gases include specific heats, entropies, enthalpies and free energies. In these Tables the only quantities which may still be described as crudely empirical are vapour pressures.

This bold and systematic use of statistical thermodynamics, combined with spectroscopic data and evaluations of molecular force constants, shows very well how the thermal properties of gases at high temperatures can be mastered by the mechanization of thermodynamic calculations. The results are of immediate importance in the conduct of aerodynamic research, and applications to other fields may be predicted with confidence. From these *Tables*, any remaining obstacles to the development of high-temperature thermodynamics of gases seem likely to be overcome without much difficulty. (Librarians should note that these *Tables* were originally published as *U.S. Bur. Stand. Circ.* 564. This edition is a 1960 reprint with revisions. The publishers do not indicate how extensive these are.)

More specialized experimental information required for other fields is by no means available in the same massive way. There has been a need for data at high temperatures in the steel industry for a very long time. One outcome is that much experimental information of use in high-temperature technology is being collected and collated as the result of sponsorship by the American Iron and Steel Institute. *Thermochemistry for Steelmaking* constitutes the first fruits of a large programme of work, and covers a field even wider than steel-making. Physical properties tabulated include density, thermal conductivity, viscosity and surface tension of the liquid state of the elements. Tables are given of standard thermodynamic functions of elements and selected compounds which have been computed up to 3,000° K. Vapour pressures are also given for the more common elements and for selected compounds. Such compilations should prove very valuable in high-temperature chemistry generally, and are likely to stimulate advances not only in steel-making but also in other fields.

The third publication, *Physicochemical Measurements at High Temperatures*, deals with methods of laboratory investigation at high temperatures. In this field the advancement of knowledge must still be described as of uneven quality. Because of their technological implications for extraction metallurgy, certain problems such as the study of chemical equilibria in melts and in slags have received much attention, and considerable mastery has been achieved. A considerable body of fundamental scientific information has also been accumulated through laboratory research on glasses and on refractories. Much still needs to be done, however, to bring the most recent ideas about condensed states of matter to bear on problems which are also of technological importance.

The plan of the book is to present a collection of articles by various experts concerned predominantly with solids and melts. Only brief reference is made to flames or gases at high temperatures. It is to be regretted that the interaction between gases and condensed states of matter is not dealt with. Such interaction is the origin of many difficulties in making measurements at high temperatures, and its study is of great practical as well as theoretical interest.

In its chosen fields, this book is a useful guide to techniques of research at high temperatures. It contains chapters on temperature measurement and control, on refractory materials, on various equilibria, and on applications of calorimetry, densitometry and viscosity. Measurements are described on surface tension, on vapour pressures, on light scattering, on ultrasonic velocities, on diffusivity, and on various electrochemical parameters. Probably unavoidably at the present stage of high-temperature studies, individual sections of this text differ considerably in critical quality. Some of the authors attempt to survey the field in perspective, and to arrive at conclusions, however provisional, that may at least guide new entrants into the field. Others tend to provide catalogues of work at high temperatures, without much discriminating comment.

Physico-chemical properties have generally been studied at high temperatures only because of some compelling technological requirement, or because new opportunities for making fundamental measurements have become available by the development of new techniques not too difficult for laboratories of general physical chemistry. Further progress in the very important field of high temperatures depends on both kinds of research stimuli. It can be argued that the development of research at high temperatures on an adequate scale in Britain awaits the provision of more specialized research laboratories with really adequate resources, closely allied with technological development projects. However this may be, the publication of books such as the present triad offers valuable help in mastering present achievements and in planning for the future. A. R. UBBELOHDE

UNNATURAL PRODUCTS

Silicones

By R. N. Meals and F. M. Lewis. (Reinhold Plastics Applications Series.) Pp. xi+267. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1959.) 48*s*. net.

Organosilicon Compounds

By Dr. C. Eaborn. Pp. x+530. (London: Butterworths Scientific Publications, 1960.) 80*s*.

Unnatural Products

New and Useful Materials from Silicon. By Prof. Eugene G. Rochow. (Thirty-fourth Annual Priestley Lectures.) Pp. vii+117. (University Park, Pennsylvania: Phi Lambda Upsilon, Pennsylvania State University, 1960.) 2.75 dollars.

TWENTY years ago, organo-silicon chemistry would have been identified largely with Kipping's monumental life-work. Since then there has been an enormous accretion of new knowledge, partly because of a general broadening in the scope of organic chemistry, but largely stimulated by the development of silicone polymers and novel methods of synthesis. These three books are all concerned with the chemistry and technology of organo-silicon compounds, but they present it from three quite diverse points of view and for different circles of readers.

Meals and Lewis set out to provide a readable and self-contained account of the silicones for those whose primary interest lies in the silicone oils and resins as technological materials: engineers, chemists in industry and students of technology. The essentials of silicone chemistry and the structural basis of the useful properties of the polysiloxanes are concisely set out; about half the book is devoted to the manufacture of the monomers, the fabrication of materials such as resins and rubbers, based on the silicone polymers, and typical applications of these materials in industry. Due attention is given throughout the book to the properties that confer or limit their usefulness and, within its clearly visualized terms of reference, the book contains a great deal of information.

Dr. Eaborn's book, by contrast, is very definitely written for the research chemist with academic leanings, and he deliberately—and rightly—cuts down to the bare minimum his account of the application of alkyl silicon halides and their derivatives. He has produced an authoritative and comprehensive account of the organic chemistry of silicon (as at late 1958), which will serve as the standard work of reference for all who are interested, as active workers or as teachers of organic (and inorganic) chemistry, in this and cognate fields. To keep the subject within bounds, he imposed some restrictions on his work—the omission of most technical aspects and a self-denying omission (which many readers will regret) of comparisons between organo-silicon chemistry and the organic chemistry of other elements. A commendable feature of the book is the way the author has introduced order into the great mass of factual material by means of three rather general chapters, together making up about a third of the book, and dealing respectively with methods of forming silicon-carbon bonds, bond properties (stereochemistry, bond character and the mechanism of substitution reactions) and the characteristic reactions of silicon–carbon bonds in different molecular environments. A great deal of information, with the relevant references, is summarized here in tabular form.

Much of the fascination of silicon chemistry lies in the analogy that may be drawn with carbon, and the extent to which carbon is really unique. Eaborn emphasizes particularly that the essential difference between carbon and silicon lies in the availability of 3*d* orbitals; this has several far-reaching effects in the chemistry of silicon. It confers a considerable amount of double-bond character on bonds formed between silicon and the halogens, nitrogen and other elements; it enables silicon to attain a five- or six-co-ordinate state in stable compounds or in transient reaction intermediates; it influences profoundly the reaction path in substitution reactions at the silicon atom. The critical way in which these physical-organic concepts are developed will make these general chapters profitable reading for many chemists who are not directly concerned with the remainder of the book. This reviews systematically the formation, structure and properties of the main classes of organo-silicon compounds—halides, hydrides, oxygen compounds of different types, cyclic compounds, etc. The disparities in length between chapters may perhaps reflect the extent to which some classes of derivative are ripe for investigation. Ample use is made, throughout, of the general concepts developed in the first part, though the organization of the material largely follows the traditional organic chemist's approach. Every chapter is followed by the appropriate bibliography, and the total coverage of the literature is very extensive indeed.

If the first two books are devoted principally to facts, *Unnatural Products*, which embodies Dr. Rochow's Priestley Lectures before Pennsylvania State University, is concerned with ideas. In it he lets his ideas range widely, and develops a theme that he stated briefly in his foreword to the book by Meals and Lewis. In brief, the abundance of silicon in the universe is the direct consequence of its nuclear structure; the chemistry is determined by its electronic structure. The very abundance of silicon challenges technological man to explore and exploit the differences between the familiar compounds of carbon and substances, having no counterpart in Nature, based on the chemistry of silicon. Only by extending our chemical knowledge, so as to make kinetically possible the sort of reaction paths that look attractive thermodynamically, will it be feasible to make the best use of the world's resources of raw materials and energy. In his lectures, Rochow ranged over the modern inorganic chemist's approach to his subject, embracing everything from classical descriptive chemistry, through thermodynamics, to molecular spectroscopy. The lectures afforded an unusual opportunity for a distinguished chemist to show how he thinks about his work. The result (except for one lecture on intramolecular motions and energy barriers, which must have made singularly hard going for his audience) is a very readable little book.

J. S. ANDERSON

TRILOGY OF CHEMISTRY

Inorganic Chemistry

An Intermediate Text. By C. W. Wood and Dr. A. K. Holliday. Pp. viii+393.

Organic Chemistry

An Introductory Text. By C. W. Wood and Dr. A. K. Holliday. Pp. xxii+321.

Physical Chemistry

An Intermediate Text. By C. W. Wood and Dr. A. K. Holliday. Pp. viii+322.

(London: Butterworth and Co. (Publishers), Ltd., 1960.) 21*s*. each.

THESE three attractively produced books form a series intended to meet the chemistry requirements at Advanced and Scholarship levels of the General Certificate of Education of the various examining boards. They should be useful also to first-year undergraduates (not only those reading for a degree in chemistry) and to students preparing for Higher National Certificate, graduateship of the Royal Institute of Chemistry, etc., in colleges of technology.

The authors give no hint of a later volume to cover the concomitant practical work (qualitative and volumetric analysis, typical preparations, etc.), but these already published give concise qualitative tests for each element, and précis the chief organic preparations.

It may be said at once that the volumes more than cover their field, yet not so amply as to make them intimidating to the good advanced-level student. Their large pages and carefully considered typographical plan make them easy to read and to refer to: diagrams, though on the small side and drawn in 'stencil' fashion, are sufficiently clear. Equations are prominently displayed, mainly in structural form in the organic volume, and ionically, where appropriate, in the inorganic.

Each volume is complete in itself: thus, physical chemistry topics, atomic structure and valency, etc., are fully treated in the physical volume, but enough of each is carried into the others to illuminate discussions there. The historical approach is pleasing, without ever becoming oppressive. Much information of interest to the keen student, and not otherwise accessible save by very wide reading, is to be gleaned from the footnotes. The up-to-date treatment may be judged by the adoption of C=12 as the basis for atomic weights: few school text-books have reached this yet.

Misprints are negligible, though the close formulation of ions as, for example, SO_4^{2-} and $N_2H_6^{2+}Cl_2^{2-}$, instead of in the more open standard form, is rather irritating.

Collaboration between an experienced schoolmaster and a university lecturer seems most effective for work at this 'frontier' level. My own preference is for the physical volume; I feel some slight regret that the authors did not take this opportunity to develop in print the simple approach to organic chemistry recommended by Sir Robert Robinson in a presidential address to the Science Masters' Association about eight years ago, which many of us have found by experience to be more easily assimilable than the wholly systematic impact.

CHARLES HOLT

TREATISE ON ANALYTICAL CHEMISTRY

Treatise on Analytical Chemistry

A Comprehensive Account in Three Parts. Edited by I. M. Kolthoff and Philip J. Elving, assisted by Ernest B. Sandell. Part I: Theory and Practice, Vol. 1. Pp. xxvi+809. (New York: Interscience Encyclopedia, Inc., 1959. Distributed by Interscience Publishers, Inc., New York; and Interscience Publishers, Ltd., London.) 133*s*.

THIS *Treatise on Analytical Chemistry* is to be a major work extending to many volumes. Part I, which is to consist of eight or nine volumes, is devoted to theory and practice. Part II, the analytical chemistry of the elements, will deal extensively with organic as well as inorganic analysis in a series comprising some ten volumes. Part III, which is as yet not fully planned, will be concerned with the analyses of industrial products. The first volume of this last part will be divided into two sections, one dealing with the philosophy relating to the significance and organization of analytical chemistry and the other with specifications and standards;

subsequent volumes of Part III will describe analytical procedures associated with plastics, fabrics, fine chemicals, etc. The final volume of each of the three parts will contain an index for all volumes in that part. There is no index in individual volumes.

The senior editor, Prof. Kolthoff, is noted for his pioneer work on the systematization of analytical procedures and for his ability, both as an investigator and a teacher, to interpret analytical processes on a sound physico-chemical basis. Many of the authors contributing to this first volume are his co-workers and former pupils, and the continuity of his influence can be observed clearly throughout these pages. The approach of the seventeen authors of these first nineteen chapters is remarkable for its uniformly high standard. The clear descriptions of essentials and fine details are outstanding.

The subject-matter of this volume may be listed briefly as follows: methods of analytical chemistry; errors, their detection, determination and control; evaluation and interpretation of accuracy and precision; sampling, rationale and techniques; elements and compounds, an account of modern atomic and molecular theories; atomic weights; thermodynamics of chemical equilibria; graphical presentation of data; electrode potentials; concept and determination of *p*H; acid-base theories; acid-base strength and aqueous titration curves; non-aqueous acid-base titrimetry; complexometry; mechanism of redox titrations; redox equilibria; solubility; precipitation; equilibrium in precipitation reactions.

Thus this volume is chiefly concerned with the basic principles of analysis and more particularly with classical analytical chemistry. But this is not just old material in a new guise, for a fresh approach to the topic is made in almost every chapter. For example, the reader might justifiably expect a conventional treatment of the theory of acid-base titrations, but much new material is to be found in both chapters devoted to this subject.

It is perhaps not entirely fair to single out individual sections for praise, but a special commendation might be given to the chapters on solution and precipitation phenomena, which constitute a lucid account of modern theory written with a strong analytical bias.

To sum up, this volume is a notable beginning to a monumental work which, if the present standard is maintained, will become the standard reference-book of analytical chemistry. R. BELCHER

A HISTORY OF PLATINUM

A History of Platinum from the Earliest Times to the Eighteen-Eighties

By Donald McDonald. Pp. x+254. (London: Johnson, Matthey and Co., Ltd., 1960.) 35*s*.

FOR many years the author of this fascinating book has been investigating the history of platinum and its associated metals, and speaks with unrivalled knowledge and authority. The result is a most important study which, within the boundaries which Mr. McDonald has set himself, must remain for very many years at least the final statement of fact. In his own words: "the story recounted is that seen by the refiner and fabricator engaged in commerce. It does not concern itself with the recondite parts of the geology of the deposits, nor with the abstruse chemistry which has grown up round the complex series of compounds into which the platinum metals enter so freely. . . . The account is remarkable in its continuity and slow development over the best part of two hundred years and it has never before been told as a continuous tale". The present volume carries the story down to 1880–1890, before which time "the work on the metal was of a fundamental nature and was largely conducted by scientists who published their results. The few people who were tempted into manufacture were either scientists like Wollaston and Deville, who sooner or later published, or they were people like Chabaneau and Bréant, whose secrets eventually leaked out. The facts therefore are available to the historian". To me this would appear to be a remarkable under-statement of the author's task, the whole book bearing the marks of long-continued and most pertinacious research.

"After the period of 1880–1890 the position is very different. By then the platinum industry, at any rate as far as Europe is concerned, had moved out of the laboratories of the scientists into the factories of industrialists working in competition with each other." This competition resulted somewhat naturally in great reluctance to publish details of their processes, and the facts have either never been recorded or, if recorded, have since been lost.

Of the ancient civilizations the only one from which undoubted finds of platinum occur appears to be the Egyptian, whither it had found its way mixed with gold imported from Ethiopia where platinum is known to occur even to this day. A seventh-century B.C. box from Thebes with 'silver' characters on one side was sent to Berthelot, who demonstrated beyond doubt that one of these characters consisted of native platinum alloyed with small amounts of gold and iridium. No evidence was found that the Egyptian craftsmen had noticed any difference between this piece of metal and the silver used for the other characters; it had been hammered out in the same way as the rest, and its occurrence was in all probability purely fortuitous.

Other references from Roman times onwards which have been suggested as applying to platinum are of doubtful applicability, and in the author's opinion "there seems no doubt that the platinum which about the middle of the eighteenth century began to reach Europe from the Spanish Colonies in South America was the first to be recovered in any quantity and the first to attract the scientific curiosity of mankind". The story of the discovery and exploitation of the metal there is told in great detail from the time that the Spanish colonists threw it away as a nuisance in their search for gold, though a few samples were sent back to Spain. The earliest known rumour of its existence based on some solid foundation occurs in the writings of della Scala (1484–1558), an Italian scholar and poet, but how unimportant the metal was considered is shown by the fact that the first mention of it in European literature appears to be a book published in Madrid in 1746. From then onwards the list of scientists who became interested contains the names of almost everyone of renown: Lavoisier and Bergman, Faraday and Wollaston, Wohler, Berzelius and very many others, and more than half the book is devoted to this section of the history; not merely the history of platinum, it amounts almost to a history of contemporary science.

The author's long association with the firm of Johnson, Matthey and Co., Ltd., to which more than to any other body the commercial development of this and its allied metals is due, has enabled him to use records which have proved invaluable in the elucidation of the later stages of his work. Percival Norton Johnson—of whom Mr. McDonald has already published an outstanding biography—published an account of work on the separation of gold and platinum in 1812 and from then, until he retired in 1860, he and his partners played a major part in both the scientific as well as the industrial evolution of the platinum metals.

Mr. McDonald has presented his story in a most scholarly yet delightful and readable form, and his firm has published a volume which it is a pleasure to read, and which constitutes an outstanding contribution to the history of the precious metals.

F. C. THOMPSON

THE ADVANCE OF FLUID MECHANICS

The Scientific Papers of Sir Geoffrey Ingram Taylor
Edited by Dr. G. K. Batchelor. Vol. 2: Meteorology, Oceanography and Turbulent Flow. Pp. x+515. (Cambridge: At the University Press, 1960.) 75*s*. net.

THE second of four volumes of Sir Geoffrey Taylor's collected works has now appeared, and we gasp at the achievement represented by this one volume only. Its fields, of meteorology, oceanography and turbulent flow, are in fact almost one, for the main theme is turbulence, whether in the atmosphere, the oceans or in the laboratory, and whatever the medium discussed, the growth of ideas is essentially continuous. Of the 45 papers presented, however, some five or six deal with gravity waves in fluids and these have their own special appeal.

Theoretical work on atmospheric turbulence began with Taylor's classical *Philosophical Transactions* paper of 1915, "Eddy Motion in the Atmosphere", in which he invented the 'mixing length' concept of turbulent exchange and applied it to the thermal and moisture modifications of air masses and the distribution of wind in the terrestrial boundary layer. Moreover, he was able to put flesh on to the bones of his theory from observations he had taken only a year or so earlier from the ice-scout ship *Scotia* and, in so doing, established a way of working which always kept theory and observation reacting closely on each other. In fact, there is about one paper only on turbulence in this volume which is not in some sense immediately framed by observation. That is the famous 1921 paper, "Diffusion by Continuous Movements", in which the mixing length concept was generalized in terms of the Lagrangian autocorrelation function and a notable relation between certain statistical properties of a turbulent field was first stated. The development of this approach was, however, hindered by lack of observations on the statistical properties treated, and from 1921 until 1935 the pace of Taylor's original work on turbulence slackened somewhat; though this period contained the very thoughtful work on the difference between momentum transfer, in which pressure forces are concerned, and the transfer of inert 'substance' such as heat (in some circumstances) and matter. Then, during 1935–36, came the great surge, the beginning of the modern era, with the "Statistical Theory of Turbulence", Parts 1–5, growing out of the 1921 paper, but now testable because of the introduction of more readily observed Eulerian statistics and because of advances in observational techniques, particularly the hot-wire anemometer, over the previous few years. The strength of this work largely derives from the results which Taylor was able to deduce when the turbulence is isotropic and essentially dissipative; it left the structure and behaviour of the large, energy-containing and transferring eddies mainly as a subject for further inquiry and that remains more or less true to-day. In this connexion it is of interest that the penultimate paper, of 1956, deals in a sense with one aspect of the latter problem, the behaviour of large eddies, produced by buoyancy, in a stably stratified fluid.

The other work in this volume, on gravity waves, has a special contemporary interest in our present age of 'big bangs'. In 1929 Taylor showed that the travel of the pressure wave from the Krakatoa eruption would not support the thesis, deriving from Kelvin, that the marked semi-diurnal variation of pressure in the atmosphere could be ascribed to resonance with the 12-hour tidal force. But he later showed that the atmosphere might have free periods other than that associated with the Krakatoa wave, and he suggested that these might arise from the complex thermal structure of the upper atmosphere. That, as Pekeris, and Weekes and Wilkes later showed, was in fact the case, and tidal resonance was 'saved'. But the theory of the semi-diurnal variation of pressure is not, on that account, complete.

This volume is to be prized on a number of counts: not least, of course, as a record of the growth of ideas, but more, I believe, as providing the possibility of finding out how the record has been achieved, and, perhaps, of doing likewise. There is only one Sir Geoffrey Taylor, but his recipe for cooking could mightily help a good young chef if he will read the recipe carefully enough. To any with the love of the tantalizing study of fluid motion it will bring enjoyment and enrichment, and a new knowledge of the art of science.

P. A. SHEPPARD

REVIEWS OF NUCLEAR PHYSICS

Progress in Nuclear Physics
Vol. 8. Edited by Prof. O. R. Frisch. Pp. vii+304. (London and New York: Pergamon Press, 1960.) 90*s*. net.

THE latest volume in Prof. O. R. Frisch's review series contains six articles, three written by experimentalists and three by theoreticians. The subjects embrace cosmic rays, high-energy nuclear physics (well represented this time in both its theoretical and experimental aspects), and low-energy physics, where the emphasis is on nuclear models and theoretical interpretation. As in previous volumes in this series, the articles are all of a high standard in their primary function of collating and discussing critically the relevant published work on their particular subjects. Most of them are also commendable for their clarity of presentation and their provision for readers not already experts in the specific fields.

In the first article, "The Composition of the Primary Cosmic Radiation", C. J. Waddington presents a masterly and clear discussion of the present data on fluxes and the charge and energy spectra of light and heavy nuclei in the primary cosmic radiation. From the sometimes conflicting information available, he extracts a table summarizing what appear to be the present best flux-values, and points out the interest and importance of future work with balloon- and satellite-borne equipment.

"The Interaction of Polarized Nucleons with Nuclei" is treated by E. J. Squires, who considers the various theoretical approaches which have been made to the interpretation of polarization data. The approximations involved in deriving the theoretical formulæ are discussed, and the limitations which they impose are pointed out. Squires's treatment, which is thorough and includes copious references, is intended primarily for the specialist and may be found somewhat heavy going by readers inexperienced in this field.

"Collective Motion in Nuclei" by D. M. Brink is a survey of the present status of the rotational, vibrational, and unified nuclear models. The author first summarizes the essentials of the subject as covered in previous reviews. He then goes on to discuss in somewhat greater detail more recent advances, particularly particle coupling schemes and the attempts which are being made to justify the models theoretically.

"Dispersion Relations for Elementary Particles" by J. Hamilton is food for theoretical physicists. It is, moreover, an excellently constructed review article, presenting the subject in an interesting and lucid fashion. The introduction shows clearly what a dispersion relation looks like and its uses in simple examples. There is a brief but instructive section explaining the importance and scope of dispersion relations as a technique for studying the properties and interactions of elementary particles. Then follows the main substance of the article, in which a variety of scattering and related problems are ably discussed.

In "Cherenkov Detectors" G. W. Hutchinson gives a clear and up-to-date review of recent developments in these detectors as applied to the physics of high-energy machines. The main emphasis is on progress which has been made since the appearance of previous reviews on the subject. Particularly valuable are the accounts of various forms of the gas counter and of the solid–liquid critical reflexion counter. Total absorption counters are discussed briefly; there is very little mention of cosmic-ray applications.

In the final contribution, on "The Photoproduction of Pions", E. H. Bellamy describes the experimental techniques used for investigating the production of charged and neutral pions by high-energy photons, and surveys the data at present available on cross-sections and angular distributions. In a series of useful diagrams the data are summarized and compared with theoretical curves. However, some sections, particularly in the first half of the article, are not very clearly presented.

JOAN M. FREEMAN

A FEW NUCLEONS

Nuclear Forces and the Few-Nucleon Problem

Proceedings of the International Conference held at the Physics Department, University College, London, 8–11 July, 1959. Edited by T. C. Griffith and E. A. Power. Vol. 1: Pp. xiii+1–340. Vol. 2: Pp. vii+341–712. (London and New York: Pergamon Press, 1960.) 200*s*. per set of two volumes.

A GOOD way to stimulate activity in a particular field of research is to hold an international conference. Such a conference was held in July 1959, at University College, London, to discuss the nuclear few-body problem (reported briefly in *Nature*, 184, 1113; 1959). The two volumes under review are the official Proceedings.

The difficulty in understanding systems of a few nucleons is mainly mathematical; it is very hard to calculate their properties reliably from given nuclear forces, or to learn about the latter from the former. Except in the two-nucleon system, there are too many independent variables for comfort and yet too few to justify taking averages (as in the many-body problem). The conference produced no spectacular solution, but it directed attention to the problem, summarized the present situation and indicated some lines for further research.

The conference began with the two-nucleon problem and what has been learned about the basic two-body forces. This occupies a quarter of the Proceedings: rather a large share, but a fair indication of where most progress has been made. Next came high-energy scattering of nucleons from light nuclei, much of which can be reduced approximately to two-body interactions, and a session on photonuclear reactions. The conference then reached the core of the few-nucleon problem: binding energies and low-energy scattering and reactions, involving three to eight nucleons. Here the true difficulties emerged and all manner of approximations were used to overcome them. Some of the latter look rather crude to an outsider, but clearly solutions which are anything like exact are not feasible. The full calculations are too much for present fast electronic computers; a relatively simple example was estimated to need several hundred hours on an I.B.M. 704 computer. Of course, as a man from Los Alamos remarked, it depends on how many 704's you have. The conference finally ended on the border of the many-body problem, with systems of up to sixteen nucleons.

Each topic was introduced by a review paper, followed by more specialized papers and periods of discussion (the last have been edited for brevity). Altogether there are about ten reviews and seventy other contributions, divided between theory and experiment in the ratio of two to one. The authors include such names as Blatt, Breit, Brueckner, Chew, Marshak, Massey and many other well-known physicists. The Proceedings are thus an authoritative document. They are also much more readable than the learned journals; the style is often that of the spoken word, and most speakers come quickly and concisely to the point. The discussion provides a stimulating commentary on the main text, raising fresh questions and points of view, and bringing some touches of humour. The volumes are handsomely produced, the layout is clear and the errors commendably few. All this is in their favour.

On the other hand, there is inevitably some confusion of notation: the first six authors to write the two-nucleon scattering matrix do so in six different ways. More regrettable, however, is the lapse of some seventeen months before these Proceedings appeared, especially since this kind of literature declines rapidly in value with time. Through special efforts, other conference proceedings have appeared within six months or even—a recent spectacular case—in two. Nevertheless, these volumes retain considerable value and will be read with interest by many physicists.

R. J. N. PHILLIPS

A PANORAMA OF SCIENCE

The Neutron Story

By Dr. Donald J. Hughes. (The Science Study Series, Book 1.) Pp. 158. (London: William Heinemann, Ltd., 1960.) 4*s*. 6*d*.

Magnets

The Education of a Physicist. By Prof. Francis Bitter. (The Science Study Series, Book 2.) Pp. 155. (London: William Heinemann, Ltd., 1960.) 4*s*. 6*d*.

Soap Bubbles and the Forces which Mould Them

By C. V. Boys. (The Science Study Series, Book 3.) Pp. 156. (London: William Heinemann, Ltd., 1960.) 4*s*. 6*d*.

Echoes of Bats and Man

By Prof. Donald R. Griffin. (The Science Study Series, Book 4.) Pp. 156. (London: William Heinemann, Ltd., 1960.) 4*s*. 6*d*.

How Old is the Earth?

By Dr. Patrick M. Hurley. (The Science Study Series, Book 5.) Pp. 160+8 plates. (London: William Heinemann, Ltd., 1960.) 4*s*. 6*d*.

The Wider Universe

By Paul Couderc. (Arrow Science Series.) Pp. 128+4 plates. (London: Arrow Books, Ltd., 1960.) Bound, 10*s*. 6*d*.; Paper, 5*s*.

Matter and Antimatter

By Maurice Duquesne. (Arrow Science Series.) Pp. 127+4 plates. (London: Arrow Books, Ltd., 1960.) Bound, 10*s*. 6*d*.; Paper, 5*s*.

Intelligence

Its Evolution and Forms. By Prof. Gaston Viaud. (Arrow Science Series.) Pp. 127 + 4 plates. (London: Arrow Books, Ltd., 1960.) Bound, 10*s*. 6*d*; Paper, 5*s*.

The Anatomy of Plants

By Prof. P. Font Quer. (Arrow Science Series.) Pp. 128. (London: Arrow Books, Ltd., 1960.) Bound, 10*s*. 6*d*.; Paper, 5*s*.

TO deal with nine volumes at a time may seem more like a march-past than a review. The essential qualitative information can even be dealt with smartly at the double; they are all good, some distinguished, and one a revered classic. One group was written in the United States to serve a specific purpose, at a certain level in schools, as part of a planned programme of physics teaching; I do not know of anything comparable of English origin at present, and regard this as a very valuable series. For the other group, it does seem fair to ask whether we need have gone so far afield, and whether translation in any case is worth while, at least from French; but this also is the start of a series, of which the later members are to be written by the British authors.

The five volumes of the Science Study Series published by Heinemann are the first transplantations of the readers sponsored by the Physical Science Study Committee in the United States. This enterprise, backed by high-level scientists, imagination—and funds—has been conducting a major operation in vitalizing the teaching of physics, and the provision of background reading to supplement the formal course is one of its many sides. These books are intended to be read by very intelligent pupils of about seventeen, and in relation to sixth-form pupils in Britain, it must be said at once that they will not help anyone to get 65 per cent at Advanced level. But they will convey an appreciation of the ideas and methods of science, and a sense of contact with first-rate scientists who have tried to communicate their enthusiasm as well. The difference between them and, say, the excellent books that Pelicans provide is in the type of reader foreseen.

The Neutron Story, by the late Donald Hughes, deals with the discovery of the neutron, its properties and uses, the problems of its decay, and nuclear power. Francis Bitter's book is an introspective account of how the concepts of magnetism take root in a person's mind; he mentions that his interest in science was first aroused by a course in celestial mechanics, an opening gambit which the Ministry of Education's recent Pamphlet 38 would endorse. He reminisces of his work at Cambridge with Kapitza, and of war-time degaussing, and goes on to explain the principles of the work at Massachusetts Institute of Technology on nuclear magnetic resonance. Boys's *Soap Bubbles* is a welcome reprint of a masterpiece; and very timely now that we can get liquid detergents that will supplant the elaborate recipe with Price's glycerin and Castile soap. Griffin's *Echoes of Bats and Men* is a simplified version of *Listening in the Dark*, and is the easiest of the batch to read. I have done some consumer research on Hurley's book, which I used for half a term as the backbone of work with a non-science sixth form; we found it rather difficult, chiefly because I was trying to explain radioactivity concurrently instead of doing this first. But it did its job well, stimulated participation, and as it concentrates on a single problem and its pursuit, gives an excellent example of the way in which scientific ideas develop. I shall do this again, and can recommend it. For the rest, I would make them available for sixth-form pupils to read; so far, nothing else quite at this level and price seems to be published in Britain.

The Arrow Series are translations, three from France and one from Spain. Couderc's *The Wider Universe* is a very good survey of recent progress in astronomy, leading up to the modern theories of cosmology. Duquesne's *Matter and Antimatter* is less satisfying, chiefly because the early chapters deal with familiar work which is in all the text-books, and the discussion of anti-particles (up to 1956) seems a sort of tail-piece. Gaston Viaud's *Intelligence* is an experimental psychologist's approach to the development of conceptual thought, and the processes behind the analytical technique for breaking down an apparently insuperable problem into lesser and manageable units; I found it clear, stimulating and enjoyable. Font Quer's *The Anatomy of Plants* really scarcely belongs to the group, for it is, in fact, part of the section on morphology from a major work; it is well illustrated, and one's only criticism is that the examples chosen seem to include many plants that are common in Spain but unfamiliar in Britain.

These four books, though probably breaking no new ground in the paper-back field (except by offering a non-paper-back edition as well) are sufficiently distinctive to be recommended for a library as a group; with a special recommendation for Couderc and Viaud. G. R. NOAKES

PLASMA PHYSICS

Plasma Physics

By S. Chandrasekhar. Pp. 217. (Chicago: University of Chicago Press; London: Cambridge University Press, 1960.) 14*s*. net.

DURING the past few years there has been growing interest in the dynamics of fully ionized gases, much of it stimulated by the hope of releasing energy by the controlled thermonuclear fusion of light elements. Although the classified research in this field was released at the Second Geneva Conference on the Peaceful Uses of Atomic Energy, there is a serious shortage of comprehensive reviews of recent developments, which this collection of Prof. Chandrasekhar's lectures will do much to alleviate. The lecture course was chiefly concerned with the most interesting approach to plasma dynamics—one that lies between a detailed consideration of individual particle orbits, a method which is usable in only the simplest geometries, and the crude but widely applied magnetohydrodynamic method in which the plasma is represented as an electrically conducting fluid. The method is valid for a diffuse plasma in a strong magnetic field and is based on the assumption that the Larmor radius of a typical particle orbit is much smaller than any other length in the problem, and the corresponding gyrofrequency is much greater than any other frequency, including the molecular collision frequency. It is then possible to use a perturbation treatment of particle orbits and make use of a number of adiabatic invariants to simplify the self-consistency problem for the macroscopic fields.

Chandrasekhar's lectures begin with a brief review of the Maxwell–Lorentz theory of the interaction of an assembly of charges with an electromagnetic field, then proceed to a discussion of the orbits of charged particles in slowly varying fields, introducing and considering rather carefully the significance of the adiabatic invariants of the motion. The first-order orbit theory is then applied to a number of problems, including the equilibrium and stability of plasma in a magnetic field, and the propagation of low-frequency oscillations. There is a brief chapter on plasma oscillations, and a final section in which the possibility of intermolecular collisions is admitted and certain transport processes considered.

The subject is necessarily mathematical in character; but formalism is not allowed to get out of hand. There is no mention of experiment, a particularly unfortunate circumstance in a subject as full of surprises as the physics of ionized gases. The preface confesses that Prof. Chandrasekhar has not read the material, and it is not clear that he would have published it in its present form; however, although not the authoritative monograph for which one might have hoped, this work will be welcome to everyone interested in plasmas, and it should be read by anyone studying theoretical aspects of the subject. W. B. THOMPSON

THERMAL STRESSES

Theory of Thermal Stresses

By Prof. Bruno A. Boley and Prof. Jerome H. Weiner. Pp. xvi+586. (New York: John Wiley and Sons, Inc.; London: John Wiley and Sons, Ltd., 1960.) 15.50 dollars; 124*s*.

THE authors give a comprehensive survey of methods of calculating stresses and deformations in a solid body due to temperature variations. Elastic, visco-elastic, and plastic materials are considered under static, quasi-static, and dynamic conditions, and sections are included dealing with thermodynamic fundamentals and heat conduction.

The book is divided into four parts, of which the first is concerned with basic thermodynamics, and derives the stress-strain and heat-flow equations. For most practical problems, the terms in the heat-flow equations, representing convertibility of mechanical and thermal energy, may be neglected, so that the calculations of temperature distribution, and of stress and displacement distribution, may be made separately. Most of the problems treated in later sections of the book are of this type. The remainder of the section considers various ways of formulating the thermoelastic problem in 2 and 3 dimensions.

The second part is devoted to the solution of the Fourier heat conduction equation for various boundary conditions. Methods described include the superposition and imaging of sources and sinks and the use of Green's functions, characteristic function solutions, the use of Laplace transforms, conformal mapping, approximate solutions using finite differences, electrical analogues, the variational methods of Kantorovich and Green, and Biot's method, obtaining the equations in Lagrangian form. Static solutions may be extended to cover dynamic cases using Duhamel's theorem.

The third and largest section of the book deals with the determination of stress and displacement for a given temperature distribution for various structural elements. Curved and straight beams, trusses, rings, cylinders, plates, reinforced sheet, and shear-lag problems are dealt with mainly by using the approximations of strength of materials theory, St. Venant's principle being invoked in the treatment of end effects. Exact and approximate theories are compared for the case of the rectangular beam. Semi-inverse methods are used for most of the exact solutions described, energy methods being used to obtain approximate solutions. Other methods described are Goodier's method, the use of centres of dilatation, stress functions, and finite difference and influence coefficient methods. Thermally induced vibrations of plates and beams are briefly considered and there is a chapter on stability.

The fourth part, dealing with inelastic systems, serves as an introduction to a rapidly developing field. Laplace transforms are used to solve linear visco-elastic problems, and creep buckling is discussed. The final chapter gives examples of plate and cylinder analysis for plastic materials.

The book covers a wide range, from classical thermoelasticity to recent developments in visco-elastic theory, and it is pleasing to report that the cross-referencing is good. Parts 2 and 3 are largely self-contained, a fact which will be welcomed by readers concerned with practical aspects of the subject. The numerous references to published work are listed, with comments, in footnotes. I feel that a list of references at the end of each chapter, and a list of symbols at the beginning of the book, would have been helpful. Despite these minor imperfections, the book meets a real need for a reference work in English on this subject, and should prove to be of great value to research and design engineers working in this field. B. S. Owen

FLAME PHOTOMETRY

Flame Photometry

By Prof. John A. Dean. (McGraw-Hill Series in Advanced Chemistry.) Pp. viii + 354. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1960.) 89*s*.

THE application of flame photometry to the determination of microconstituent elements in biological, mineralogical and related materials has been of continuing interest to chemists since the pioneer work of Lundegaardh on flame excitation, and this interest has increased enormously in the past decade.

The present book by Prof. Dean is a comprehensive account of investigations and of instrumental developments in this field. A general, but adequate introduction dealing with atomization, flame characteristics and the nature of emission spectra is followed by a chapter of particular interest which deals with the use of organic solvents, either added to aqueous solutions to enhance emission by their effect on such physical properties as surface tension and viscosity, or in selective extraction methods to increase the concentration of the test element and reduce interference by extraneous ions.

Following a full description of modern flame photometers, burner systems and photo-sensitive detectors, there is a detailed discussion on interferences arising from flame background, cation and anion enhancement and suppression and spectral coincidences.

Individual chapters are devoted to specific periodic groups giving spectral emission lines and band-heads for various elements, comments on possible interferences and specific applications. Scandium, yttrium and the rare-earths are dealt with, and original spectra of these elements are given. Atomic scientists will notice that beryllium is not mentioned, and that the sensitivity of boron is poor as compared with, for example, the fluorimetric method. Strontium and barium receive detailed treatment.

The principles of atomic absorption spectrophotometry are only briefly discussed, and one hopes that, in view of the interest shown recently in the possibilities of this technique, it will not be long before Prof. Dean deals more fully with this subject.

The later chapters are concerned with the determination of non-metals and with agronomic, clinical and botanical applications. An extensive bibliography of 780 references is given which covers a wide range of materials and analytical procedures.

The book will prove a most valuable working volume for the laboratory and has much to offer the student of spectrophotometry. H. L. Bolton

INTERIOR BALLISTICS OF ROCKET MOTORS

Rocket Propellant Handbook

By Boris Kit and Douglas S. Evered. Pp. xiv + 354. (New York and London: The Macmillan Company, New York, 1960.) 12.50 dollars; 87*s*. 6*d*.

Liquid Propellant Rockets

By David Altman, James M. Carter, S. S. Penner and Martin Summerfield. (Princeton Aeronautical Paperbacks, No. 1.) Pp. v + 189. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 2.95 dollars; 24*s*. net.

Solid Propellant Rockets

By Clayton Huggett, C. E. Bartley and Mark M. Mills. (Princeton Aeronautical Paperbacks, No. 2.) Pp. v + 167. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 2.45 dollars; 20*s*. net.

Solid Propellant Rocket Research

A Selection of Technical Papers based mainly on a Symposium of the American Rocket Society, held at Princeton University, Princeton, January 28, 29, 1960. Edited by Martin Summerfield. (Progress in Astronautics and Rocketry, Vol. 1.) Pp. xix + 692. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 6.50 dollars.

ROCKET motors are mechanically simple devices; that they are nevertheless difficult to design and expensive to develop is due to the fact that present-day quantitative knowledge of the relevant chemical and physical processes is still hopelessly inadequate. The upper limit to the performance of a rocket is relatively easy to predict: only the laws of thermodynamics are required, coupled with the availability of the thermodynamic properties of the propellants, the assumption of equilibrium, and the ability to obtain numerical solutions to a dozen or so simultaneous non-linear algebraic equations. It is the rate processes which present the difficulty: how large the motor must be for a given approach to the maximum performance depends, for example, on rates of chemical reaction, on the fluid mechanics of droplet break-up, and on the interactions between finely divided solids; none of these processes can be accurately computed at present.

In these circumstances, design must proceed mainly by trial and error. The role of theory is reduced to that of investigating models which are no more than caricatures of reality. Such investigations are successful if they correctly predict the trends of the performance variations resulting in changes to the design; for they can then guide the development engineer in the most hopeful directions and assist him to distinguish between major and minor influences. Such models are needed both for 'normal' and 'abnormal' operation, that is, for the desired smooth and steady burning, and for the unsteady phenomena, known as 'chugging', 'chuffing' or 'screaming', which all too often intervene at the last moment to destroy a promising design. It appears likely that steady burning in liquid-propellant motors is best represented by models in which the flight and vaporization of droplets play the major parts with the atomization process, one which must be studied experimentally. Steadily burning solid-propellant rockets conform well to the supposition that the burning-rate per unit area of propellant surface area depends mainly on pressure, and secondarily on initial temperature and transverse gas velocity; these dependences have, however, to be determined experimentally for each propellant. The most disastrous type of unsteady burning, 'screaming', is known to involve, for both classes of rockets, large-amplitude acoustic vibrations within the combustion chamber; but the nature of the interactions with the combustion process by which the vibrations are driven remains an almost complete mystery.

To turn now to the four books under review, the first, by Kit and Evered, is a contribution to the simplest aspect of the problem: the thermodynamic prediction of maximum performance. The authors have collected all the relevant thermodynamic data they could find, and presented it in the form of tables with connecting discussion. The enterprise is a useful one, and the result will be accepted gratefully by propellant-system designers; the gratitude of the latter will, however, not be unmixed with dismay, and occasional fury, when they find that vital information has carelessly been omitted. To take the first entry of Table 1–1 as an example, the authors state that the heat released in the hydrogen–oxygen reaction is 57·80 kcal./gm. mole; temperature and phases are not indicated. Two pages later we are told that such figures 'usually' relate to 1 atmos. and 25° C., and that the phase of the H_2O "must always be stated"; but they still fail to give that information. There are numerous such small but paralysing omissions in the book.

The two paper-back books, *Liquid Propellant Rockets* and *Solid Propellant Rockets*, are reprints from the Princeton series of volumes on high-speed aerodynamics and jet propulsion; they have been made available for the benefit of students. I am not enthusiastic about them for this purpose, for they lack the formal organization, carefulness of definition and freedom from distracting speculative material which students justifiably expect; they are also, of course, considerably out of date. The article on liquid-propellant motors by Summerfield may serve as useful background reading, but that on thermodynamics by Altman and Carter can only confuse, while the solid-propellant sections are chiefly of historical interest.

Solid Propellant Rocket Research falls into an entirely different category. It contains the papers presented at the symposium held at Princeton in January 1960, under the auspices of the American Rocket Society, together with a few additions. Sections included are: mechanical properties of grains (that is, of solid-propellant charges), steady-state burning mechanisms, combustion of metals, theories of unstable combustion, experiments on unstable combustion, and solid-propellant ignition. The book gives an impressive view of the magnitude and vigour of the present effort being made by the United States in this field; it also adds emphasis to the remarks about present ignorance made at the beginning of this review. To cite two examples, though both Nachbar and Summerfield *et al.* present theoretical models for the burning process of composite propellants, none of the authors would claim that the models can be made to fit experimental findings for more than a very restricted range: the relation of the theories of oscillatory burning to the experimental facts is still more tenuous. Nevertheless, if money, man-power and professional expertise can find solutions to these problems, few can doubt that it is the American workers who are most likely to succeed. D. B. SPALDING

THE NEW LOOK IN MATHEMATICS

Mathématiques Générales

Algèbre—Analyse. Par Prof. Charles Pisot et Prof. Marc Zamansky. (Collection Universitaire de Mathématiques, 4.) Pp. xxiv+648. (Paris: Dunod, 1959.) 4,500 francs.

TO those brought up on a *Cours d'Analyse* of the classical French tradition, the new look is austere. Pisot and Zamansky have planned their book for students just beginning a university course in mathematics, of honours standard, working on a basis acquired at school, which, the authors assert frankly, is insufficient in some aspects and useless in others, though they are optimists enough to believe that, in France at least, this state of affairs will be remedied in a few years.

There are four main sections. The first introduces logical notions and the elements of set theory, with illustrations to show that many of these ideas are known at school, if not clearly identified there. The second section is on algebra, mainly linear algebra: the fundamental ideas of a vector space and the linear mapping of one vector space on another serve to give, in compact form, the basic theory of matrices, determinants and related concepts. The section on analysis is based on two main principles: first, the Cauchy sequence $\{x_n\}$ in which $x_p - x_q$ tends to zero as p and q tend to infinity; secondly, the doctrine, somewhat subtle at this stage, of uniform convergence. Early introduction of this latter concept allows much use of piece-wise constant functions. Thus, for example, an integral can be defined more or less intuitively for these functions, and then extended to limits of sequences of such functions; this establishes the existence of integrals for all the functions ordinarily encountered, though it does not quite give the Riemann integral. But most analysts would now maintain that the persistence of the Riemann integral is due to "the stubborn conservatism of academic tradition", and the approach to integration in this volume would carry over quite easily to Lebesgue integration. Most of the technique of analysis is reserved for the final section, which includes some work on differential equations.

How would this book fit into a British course? Some of the ideas are already incorporated in our courses, though perhaps not as first-year work, and more will undoubtedly follow. But how much? A comparison of this book with the new volume by Dieudonné, *Foundations of Modern Analysis*, shows that the former is itself still a compromise between the classical and the contemporary in analysis. Dieudonné, we may suppose, represents the pure gospel according to Bourbaki, when he insists on the modern emphasis on conceptual notions, as opposed to the computational aspect expounded, for example, in Whittaker and Watson. His is the remark above about the Riemann integral. If the older analyst should doubt whether the concept-conscious undergraduate would succeed with, say, some of the stiffer examples in Whittaker and Watson, it would certainly not be quibbling to suggest that there is no reason why he should: indeed, the epsilon-conscious undergraduate of the 1920's might have been no more successful with some of Wolstenholme's teasers on conics. Mathematics changes in mode as it grows in content, and the undergraduate must be taught in the style and idiom of living mathematics. Yet this may not be a complete answer, save as regards the training of pure mathematicians. It is true that certain concepts of modern abstract mathematics have found a place in branches of mathematical physics, but too much abstraction might hamper the student whose chief interest in mathematics lies in its physical applications. Are we then to impose an early separation between the pure and the applied mathematician? Before accepting this harsh doctrine, we may remember that Maxwell, Rayleigh, J. J. Thomson were all products of the old Tripos, and hope that some British mathematician will set himself the task of writing a text for our universities which will weave together the modern abstract concepts with the older computational technique, which will absorb Bourbaki without exorcising Whittaker and Watson, which will save us from the sterility of ideas without technique and from the superficiality of technique without ideas.

T. A. A. BROADBENT

PICTORIAL GUIDE TO THE LANGUAGE OF SCIENCE

Mathematics in the Making

By Lancelot Hogben. Pp. 320. (London: Macdonald and Co. (Publishers), Ltd., 1960.) 50*s.* net.

"SCIENTISTS need to be taught mathematics", Sir Cyril Hinshelwood has said, "as a language they can actually speak"; one implication of this is that those non-scientists who wish or need to know what modern science is doing should have some knowledge of the mathematical language, though a phrase-book may serve them instead of a complete grammar and dictionary. Hogben has given us a vivid and stimulating phrase-book, built up in roughly chronological order, with a wealth of coloured plates, diagrams and pictures. The representations of three-dimensional surfaces, for example, are so admirable that some irrelevancies—Samurai, the mosque at Cordova—can be forgiven. The team-work which has gone into the making of the book deserves all the praise which Hogben bestows on it.

Neugebauer and Needham have thrown a completely new light on early mathematics in Egypt, Mesopotamia, China, India, and Hogben's account is clear and illuminating. He is rather less at home with the Greeks, since he under-estimates the importance of the Greek contribution to the concept of mathematical proof, and over-estimates the role of measurement in mathematics. Hogben says that we must learn to communicate in language which the electronic brain understands; the mathematician would say that the electronic brain has been constructed to obey the commands given to it in our language.

The influence of the Moslem civilization bridges the gap in the Western World from the Hellenic legacy to the seventeenth century. Here Descartes showed us how to describe geometrical properties in algebraic language, Fermat groped towards the Newton–Leibniz calculus, Pascal and Bernoulli initiated the doctrine of probability. On probability, the author is at his best; his enthusiasm is infectious, his excellent diagrams and pictures are genuinely constructive, and his somewhat old-fashioned obsession with visual aids is pardonable. By way of contrast, there is much clumsy symbolism and heavy-handed manipulation in dealing with algebraic geometry and calculus. More modern mathematics is dismissed briefly: hasty references to topology, non-Euclidean geometry, an ugly version of vector algebra lacking any motivation for the non-commutative product, Boolean algebra and recent views on logic and the nature of mathematics. The author is willing to deal sketchily with these topics because, to use a favourite phrase of his, they have as yet had little or no 'pay-off'. But it may be fair to suggest that there is a further reason; Hogben has no interest in or liking for mathematics as a creative art. This may well diminish the value of his book to the many amateur students of mathematics who, outside their surgeries, court-rooms, offices, enjoy the delights of mathematical investigation without worrying very much about the material 'pay-off'.

Success for the book is certain, and, on the whole, well deserved. There is a warmth and vitality which over-ride some minor irritations. Ideological epigrams about Roman gangsters, remittance men in Kenya and the third person of the Trinity can be ignored.

T. A. A. BROADBENT

ELECTRON MICROSCOPY

Vierter Internationaler Kongress für Elektronenmikroskopie

Berlin, 10–17 September, 1958. Verhandlungen Band I: Physikalisch-Technischer Teil. Herausgegeben von G. Möllenstedt, H. Niehrs und E. Ruska. Pp. xix+851. 228 D.M. Verhandlungen Band II: Biologisch-Medizinischer Teil. Herausgegeben von W. Bargmann, D. Peters und C. Wolpers. Pp. xv+649. 196 D.M. (Berlin: Springer-Verlag, 1960.)

BETWEEN them, these two volumes contain more than 1,500 pages and nearly 1,700 illustrations, mostly electron micrographs very well reproduced. Of the 400 separate contributions, just one-half are in English, one-tenth in French and the remainder in German; the authors come from 26 different countries (as the foreword tells us), and the subjects dealt with run into almost every conceivable branch of science and technology. Volume 1, devoted to the physical basis and technological applications of electron microscopy, is almost half as long again as Volume 2, which comprises the biological and medical applications. This uneven division confounds the view that the development of the instrument is more or less complete and that the subject is now mainly concerned with its uses, particularly in biological investigations. In fact, more than half the first volume is taken up with papers on the principles, design and operation of the electron microscope, and with basic preparative methods, such as shadow-casting and object supports. The interaction of the electron beam with the specimen is now also receiving considerable attention, especially the removal of material from embedded sections. Field emission microscopy, to which a special symposium was devoted, is represented by sixteen papers.

Compared with the previous conferences, the most striking change is the increase in metallurgical applications. More than sixty papers are concerned with crystal growth, the origin and movement of dislocations, and transition and precipitation processes in metals. This increased activity is largely due to the methods developed in the past few years of preparing, from bulk metal, films thin enough for electron microscopy, an advance comparable with the revolution in biological work when the technique of cutting very thin sections of tissue was devised. The metallurgist is in a better position, however, in that he can carry out meaningful experiments on his specimen while it is in the electron beam. Several papers describe attachments for heating, cooling and straining a metal film, and a number of cinematographic records have been made of the resulting changes. We may now expect to see the same rapid extension of electron microscopy into metallurgical laboratories, as occurred earlier in biology.

A fair number of ultra-microtomes is now available for cutting sections down to about 100 Å. thickness ; one or two are described in Volume 2 and more were on show at the conference. Biologists of all kinds are now busily engaged in sorting out the unexpectedly rich micro-anatomical detail revealed by this new technique in all tissues and micro-organisms. Almost the whole of Volume 2 is taken up with papers describing observations on thin sections, under the headings of cell morphology, anatomy, pathology, botany and micro-biology. The latter section also includes much work on the structure of isolated bacteria and especially of viruses. In general, biologists are now engaged with the electron microscope in the same sort of rapid extension of structural knowledge as was made possible by improvements in the optical microscope in the last half of the nineteenth century. To complete the picture, active efforts are being made to discover suitable means of staining particular cell components with compounds of high density, so as to show them up in the electron image.

The Berlin conference was by far the biggest of the four international meetings on electron microscopy so far held. Since for much of the time there were four parallel sessions, it is most valuable to have a permanent record of the contributions. The editorial committee of the German Electron Microscopical Society has gone to great trouble to make the record complete, and the publishers have given us a work which is in the best traditions of German book production. However, one cannot but ask if it is not too much (in bulk and in cost), and too late. This standard of presentation is right and proper for a subject which has reached a certain state of finality : the *Handbuch der Physik* is all that one could require. But electron microscopy is still in rapid flux : many of the findings reported here are provisional and most of the authors are likely to have published one or more further papers in specialist journals during the two years which it took to produce these proceedings. As a survey of the state of electron microscopy in 1958 they are a most admirable record. For the working scientist it would be more useful to have a much quicker, but technically speaking a poorer, printing of the manifold contributions, so that he could refer to them while they were still relevant to work in progress, and also, if this be not too vain a hope, so that the price could be low enough to have a copy on his own shelves as well as in the library. Active exploration of alternative ways of publishing conference proceedings are urgently necessary ; one such experiment is now in progress in connexion with the European conference on electron microscopy held in Delft in 1960. V. E. Cosslett

X-RAY DIFFRACTION PHOTOGRAPHS

The Interpretation of X-ray Diffraction Photographs
By Dr. N. F. M. Henry, Prof. H. Lipson and Dr. W. A. Wooster. Second edition. Pp. x+282. (London : Macmillan and Co., Ltd. ; New York : St. Martin's Press, Inc., 1960.) 63*s*. net.

THE battered condition of the reviewer's copy of the first edition (1951) of this book testifies to its value to generations of impecunious research students. In the second edition, the authors have maintained the high standards set by the first, and in their preface they explain that parts of the book are corrected, but substantially unchanged, and new sections have been added to deal with techniques which have come into general use in recent years, for example, the counter diffractometer.

The main emphasis is the same as that of the first edition, namely, on explaining crystal lattices, the geometry of X-ray diffraction and how to index good X-ray photographs of various kinds. These topics are treated in considerable detail and explained clearly by means of fully worked examples. An outstanding feature of the treatment is that the examples chosen illustrate the difficult cases encountered in practice, and the explanations are explicit. Triclinic and rhombohedral examples are given in detail and not avoided by the not uncommon practice of assuring the unwary reader that "extensions to systems of lower (or higher) symmetry are obvious". Only very elementary knowledge of mathematics is assumed ; normally, explanations are given in geometrical terms aided by extremely good diagrams. The problems, with answers in an appendix, are a most useful feature for the beginner. Appendixes contain tables of formulæ, of multiplicity factors and of crystal classes and their physical properties.

For the chapters devoted to detailed explanation, the book can scarcely be bettered ; unfortunately, apart from a masterly chapter on the properties of X-rays, those in which topics are outlined are disappointing. For example, a reference (1930) is given for the application of a correction to apparent intensities measured on oscillation photographs, but none for the other kinds of photographs now in more common use. Similarly, there is some vagueness about problems concerning crystals which are unsuitable for accurate morphological measurement. Thus, although the unit cell dimensions of monoclinic crystals have actually been measured accurately by purely X-ray methods, the beginner might not realize this from "but, no doubt, by suitably choosing reflections, it should be possible to devise methods similar to those described in 13, 4·2". It seems a pity that, after so much trouble has been taken to make the reader familiar with the reciprocal lattice, the tables of correction factors are given in terms of θ instead of sin θ.

In a book of this kind it is extremely difficult to avoid numerical typographical errors, and even three authors have not been sufficient to prevent some from appearing.

These are minor criticisms of a book which is, on the whole, extremely good. The printing and paper are good and the diagrams excellent. Both beginners and experienced crystallographers will find much of value in this book, which is not unreasonably priced.

M. R. TRUTER

MODERN MINERAL TECHNOLOGY

International Mineral Processing Congress, 1960

Proceedings of a Congress arranged by the Institution of Mining and Metallurgy, held on 6th to 9th April, 1960, at Church House, Westminster, London, S.W.1. Pp. xiii+1118. (London: Institution of Mining and Metallurgy, 1960.) 100s.; 14.50 dollars.

THIS volume contains the inaugural address, the fifty-two papers and their discussion at the 1960 International Mineral Processing Congress, the fifth of these meetings to be held since the War. The change of title from 'ore dressing', 'mineral dressing' and 'mineral engineering' still leaves this ebullient engineering science inadequately described. A stroke of genius is needed which will bring its activities in metallurgy, chemical engineering, physical chemistry, electromagnetics, nuclear physics and the rest to a titular focus. As with the previous congresses, held in Britain, France, Germany and Sweden, papers from leading laboratories and industrial operators in many mining countries are roughly grouped—in this case in nine divisions. Detailed review of such a mixed bag is impossible. The work recorded shows that the challenge of industry continues to be met at all levels, from improvement of established processes to creation and development of the new ones required by the insatiable demand of modern civilization for metals, earths and high-purity mineral concentrates.

The first group of five papers deals with comminution, the step in ore treatment in which valuable minerals are exposed for surface attack or detached from the accompanying gangue. New methods of grinding, some with a discipline of statistical mathematics, are described. Papers 6–10 deal with particle dynamics in aqueous media, both under conditions of free fall and of centrifugal acceleration. X-ray scanning of opaque slurries is described. By the aid of these techniques large tonnages of 'pulp'—an aqueous suspension of small particles, mostly less than 20 μ in diameter—are controlled as to size, shape and/or mass into equal-responding fractions. This, coupled with the comminuting processes, is an essential preliminary to concentration (separation of the various mineral species in the ore).

In papers 11–18, fundamental physical chemistry is related to flotation research. Improved methods of micro-auto-radiography are described in connexion with adsorption of tagged reagents to mineral surfaces. Relative rates of reaction in response to surface agents, the mechanisms of complexing, physical stability of sorbed layers and reactions in the zeta zone which surrounds a particle in aqueous suspension are investigated, using ultrasonics, photomicrography and thermochemistry. The transporting role of air is considered, using contact-angle measurement or over-gassed water. The depressive effect of typical accompanying minerals in sphalerite ore undergoing flotation is shown. In the next group of papers (19–23) advances in flotation practice are described and process economics receive some attention.

Interest in the older processes of concentration by gravitational force continues to stimulate research (papers 24–30). Jigging of transparent objects in organic liquids and the study of stratifying action show the value of statistical appraisal in complex problems of dynamics. Dense-media separation finds a novel control valve in the shape of a magnetic field which constrains the ferro-magnetic media at the under-flow end of the separating vessel. Tetrabromo-ethane (specific gravity 2·963) is proposed as a dense separating liquid in place of magnetite or ferro-silicon pulped with water, the usual fluid mixture. Cyclone physics continues on its scientifically turbulent course. In commercial practice it has established its value everywhere, but agreement as to how it works is not yet complete.

Papers 31–38 describe advances in the magnetic, electronic and high-tension electrical treatment of ores. The effect of moisture and surface conditioning on the behaviour of small particles in an electrostatic field promises to extend the use of electrostatic separation by improving control. A novelty is the use of a refracted beam of light to trigger a mechanism by means of which diamonds can be automatically removed from passing gravel. Papers 40–44 are concerned with the chemical processing of galena, scheelite, wolframite, some silicate minerals and uranium ores, and the use of autoclaves to aid solvation. These are followed by the last eight papers (39 and 45–52), which deal with process study, control and testing, fluidized-bed roasting, automatic control, statistical analysis and the observation of concentrating techniques performed mechanically on the stage of a microscope.

Despite the fact that many of these highly technical scripts were written in Russian, Japanese, Italian, German and French, the translation is admirable. The book is excellently produced and well indexed. It bears no signs of the problem which bedevils all important meetings today—so much to do, so little time. The subject-matter covered ranges over wide fields of research and production activity, and leaves me more conscious of what has been left out of his conspectus than of what he has crowded in. The book is essential for workers in the integrated field of mineral technology.

E. J. PRYOR

SHORT REVIEWS

Sir Isaac Newton

By Dr. H. D. Anthony. (Life of Science Library, Vol. 39.) Pp. 224+10 plates. (London: Abelard-Schuman, 1960.) 21*s*. net.

TO write a readable account of Newton's life and achievements is a task of very great difficulty, as all who have attempted it will agree. Although Newton's extension in time was considerable (85 years), his extension in space was restricted to the neighbourhood of Woolsthorpe, Cambridge and London. His achievements in astronomy and mechanics are not easy to explain, owing partly to the confusion surrounding his definition of 'mass', a definition which was wrongly translated by the exponents of his mathematical principles of natural philosophy from his day until the present. An exception was Henry Pemberton, one of the young men who received generous help from Newton, and who had the privilege of reading over, with him, the manuscript of his *View of Sir Isaac Newton's Philosophy* (1728). Newton defined mass as a 'measure'—not something which a body had, which 'could' be measured.

Dr. Anthony has succeeded in overcoming these difficulties by giving a more balanced account of Newton, including considerably more detail about his early life and contemporary background, his emergence into public life, his activities at the Mint and his theological writings, than has been usual in former biographies. The explanation of Newtonian dynamics is lightly but clearly outlined, with the exception of a paragraph on the tides, where we are unfortunately told that neap tides occur at full moon. Two minor errors are: "Sir" Robert Boyle (p. 102), and "the American airmen who made the first Trans-Atlantic flight" (p. 41). The author has forgotten two of Newton's countrymen who also voyaged "through strange seas . . . alone".

This is a well-produced book with excellent illustrations. An appendix containing interesting notes on the illustrations, a useful short bibliography and an explanatory list of words used with special meanings in British universities (for example, subsizar), all contribute to making this volume one that can be strongly recommended. G. BURNISTON BROWN

Science in Writing

A Selection of Passages from the Writings of Scientific Authors, with Notes and a Section on the Writing of Scientific Prose. By T. R. Henn. Pp. 248. (London: George G. Harrap and Co., Ltd., 1960.) 20*s*. net.

IT is a pity that so much scientific prose fails to be beautiful. Yet scientists in the past and of to-day have succeeded in writing with mastery. Mr. Henn has chosen examples from the works of several scientists ranging from Pliny to living authors.

These passages are not presented as models to be imitated, but they help to illustrate very cogent suggestions for good writing which Mr. Henn gives in the last part of his book. He feels that these authors have excelled because they have controlled their material so well. This control is derived from sheer knowledge of the facts and a persistent quest for what is relevant until "the whole assumes an ordered and organic pattern".

It is intriguing to see how devices of style are used (consciously or otherwise) and how the personality gleams through the prose. Newton's symmetry of thought, his dignified and authoritative tone are evident. Rayleigh's style, as complete in itself as a Mozart string quartet, would suffer loss were attempts made to amplify or compress it in any way. Darwin develops his argument simply and modestly yet evoking excitement. Allbutt's "sweeping brush-strokes" are a contrast to Duke-Elders's gradual unfolding of ideas. Punctuation is used by Whitehead, Max Born, Hinshelwood and Ricardo to give a limpid flow and abounding clarity. As in 'humane' prose, there can be scope for imagery and rhythm. Contrasted and associated rhythmic sequences can give a breathing quality to prose, so making it more pleasurable to digest. Imagery may provide analogies, may convey shades of meaning; sometimes frankly poetic as in Jacquetta Hawkes's writing.

The book should interest any scientist who wishes to write well. RUXTON VILLET

The Wildbooters

By Fritz Kern. Pp. xi+204. (Edinburgh and London: Oliver and Boyd, Ltd., 1960.) 21*s*. net.

THIS is an English translation, edited by K. J. Narr, of the late Prof. F. Kern's *Der Beginn der Weltgeschichte* (1953). Its thesis is the same as that put forward by Prof. W. J. Sollas in *Ancient Hunters* (1911), that archæology and ethnology can be combined to present "a picture of the early history of mankind" (p. 54). Wildbooters, Kern's word for hunters and collectors, replaces ancient hunters, protolithoid Lower Palæolithic, and instead of Australians (Palæolithic), Eskimo (Magdalenian) and Bushmen (Mesolithic), Ituri Pygmies, Chenchus and Yamanas are chosen to reveal the "essential traits" of man's "basic culture"; but the objections to this thesis remain the same. Archæology is a science, ethnology is not; the cultural stratigraphy of the ethnologist is one thing, that of the archæologist is another; and "reconstructive imagination" is no substitute for factual history. The first six chapters attempt to summarize and synthesize the prehistoric racial and archæological background, the seventh deals with the oldest cultural (ethnological) stratum, the rest describe the economy, religion and social organization of this stratum as deduced from ethnological accounts of their modern survivors.

There are no bibliography, maps or index. *The Wildbooters* provides a stimulating hypothesis of the life of early man, but archæologists and ethnologists will demand more detail, social anthropologists will wonder why so many of his sociological data are drawn from such little-known and inadequately studied tribes, while the general reader will be defeated by the morass of conflicting racial and archæological theories in the first few chapters. G. I. JONES

Archæological Investigations in British Guiana

By Clifford Evans and Betty J. Meggers. (Smithsonian Institution: Bureau of American Ethnology, Bulletin No. 177.) Pp. xxi+418+68 plates. (Washington, D.C.: Government Printing Office, 1960.) n.p.

THE authors, who are well known for their work at the mouth of the Amazon and other parts of lowland South America, spent the winter of 1952–53 in British Guiana. Their report can be assumed to have answered most of the questions of any importance which are likely to arise in the archæology. There is, however, one important problem, probably insoluble, to which they direct attention for the first time, namely, the possible existence of an early hunter horizon of the age of the first occupation of South America. The evidence consists of seven large stemmed stone points, four of which they illustrate, to which I may add three of rather different form in the University Museum of Archæology and Ethnology, Cambridge. All are casual finds, they are unlike anything known of later date, and the possibility of finding a site which puts them in their context is remote in the environment.

For the rest, the work covers the North-West District and two inland areas. The first produced a pre-ceramic shell-mound stage, with polished-stone tools and extremely crude percussion-flaked ones, with pottery and agriculture coming into its latest stages, perhaps from Venezuela. It is ascribed to the early centuries A.D. There follow three distinct ceramic phases, the first from Venezuela *c.* A.D. 500, the second from Trinidad *c.* 1100, and the third probably from the East *c.* 12–1300. All lasted until the arrival of the Europeans. The inland areas, the Rupununi Savanna and the Upper Essequibo, were chosen as likely ones for finding traces of people immigrating from the south. Traces were indeed found in the shape of distinctive pottery, but all belonged to people known to have come after 1670, some of them, the Wai-Wai, very recently indeed.

The sum of it all is that British Guiana was never a great centre of aboriginal development and had no great attraction for them. Various peoples entered it from time to time, most of them at a late period, and probably under pressure.

G. H. S. BUSHNELL

Natural Selection in Human Populations

Edited by D. F. Roberts and G. A. Harrison. (Symposia of the Society for the Study of Human Biology, Vol. 2.) Pp. viii+76. (London and New York: Pergamon Press. 1960.) 20*s*. net.

IN the first paper, "Natural Selection in Man: Some Basic Problems", Prof. L. S. Penrose discusses the particular genes recognizable in human populations in terms of their frequencies, precise effects, origins, degrees of stability, and quantitatively observable trends. The popular view that present-day cultural practices have abolished the effects of natural selection upon our species is rejected. Rather, the process has been transferred at certain points from one genotype to another, as may be observed in the role that medical control of infectious disease has exerted in reducing the advantage of those genes whose frequency in the gene pool was once maintained for fighting disease. Dr. Penrose regards the primary force of selection today to be directed towards the embryonic level, where most anomalies occur. Such selectivity does not harm the gene pool since its victims do not live to reproduce.

Dr. A. R. G. Owen's paper, "Mathematical Models for Selection", approaches selection in terms of its effects solely upon the genotypes themselves. His mathematical model is a representation of the polymorphism of multiple alleles which he suggests as a means of approach to the complex factors of heterozygosity.

"The Relative Fitness of Human Mutant Genotypes" by Dr. C. A. Clarke and "Natural Selection and Some Polymorphic Characters in Man" by Dr. P. M. Sheppard are clinical approaches to those gene complexes we identify as the units of human polymorphism. The fourth contribution to the Symposium, by Prof. T. Dobzhansky, "On Selection of Gene Systems in Natural Populations", emphasizes the frequently overlooked fact that natural selection works on organized gene systems rather than on single discrete genes which are the concern of some human geneticists embogged at the biochemical level.

The final paper, which is by Dr. Ashton, "The Rate of Change in Primate Evolution", is essentially a review of the primate fossil record and of studies of the St. Kitts green monkey population. Dr. Ashton regards the rate of morphological change for the primates as completely indeterminable.

This collection of researches should be useful to the physical anthropologist concerned with problems of human genetics and the intellectual history of this phase of the discipline. As a broad study of natural selection in human groups the book is only partially successful. Little attention is given to the cultural factors affecting the frequencies and distribution of human polytypes. An understanding of that relationship of social tradition to genetic complexes is the very crux of anthropological theory.

KENNETH A. R. KENNEDY

Mechanisms in Radiobiology

Edited by Maurice Errera and Arne Forssberg. Vol. 2: Multicellular Organisms. Pp. xiii+395. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 13 dollars.

THIS is not a text-book. The five chapters, all by different authors, show little sign of editorial integration and vary greatly in quality. "Gametes and Developing Embryo", by Roberts Rugh, is a useful literature source, but by no means easy reading. Rugh's contention that cells are more radiosensitive during differentiation than during division may help to loosen some of the present fixity of radiobiological thought. "The Adult Organism", by Clemedson and Nelson, is the least satisfactory chapter in the book. It is not only uncritical but seriously incomplete. For example, there exist six classical papers concerning radiation effects on the thymus, but only one of these is mentioned and that incorrectly. Structural changes in the retina, on which there is an important literature, are dismissed in five lines. Testis and ovary could have been omitted in view of their more complete coverage in Rugh's chapter.

"Immunology", by Hašek and Lengerová, is too short (sixteen pages, excluding chimeras) to do justice to this important topic. "Protective and Sensitizing Action", by Eldjarn and Phil, is a good and critical survey, despite its somewhat pro-SH bias.

The last chapter, "Recovery and Therapy", by van Bekkum, seems quite outstanding. Not only is it aptly critical and fully informed, but also the author has been able to digest his material and present it in a gracious literary form, and much wisdom withal: a model of science writing. O. A. TROWELL

Scolt Head Island

Edited by Prof. J. A. Steers. Revised edition. Pp. xi+269+29 plates. (Cambridge: W. Heffer and Sons, Ltd., 1960.) 50*s*. net.

SCOLT Head Island is a strip of marshland, shingle ridges and dunes approximately four miles long, lying off the north Norfolk coast between Burnham, Overy Staithe and Brancaster. Of the seventy-odd statutory Nature reserves which now exist throughout Great Britain, it can justly hold pride of place as one of the most intensively studied by botanists, zoologists and physiographers. The present volume provides ample testimony to the work of a great number of individuals during the past thirty-five years or so. As is to be expected, the physiography and evolution of the Island receive extensive treatment, evidence being provided of the structure of the various marshes, their rates of accretion and the influence of extraneous forces on them. The Island is evidently in a state of dynamic equilibrium, the beaches and dunes being built up at one time and eroded at another. Such circumstances demand constant adjustment and great adaptability by plant and animal colonists, and so provide wonderful opportunities for the ecologist. That these opportunities have been fully realized is evident from the accounts of pollen analysis in the peat and the detailed studies of flowering plant and bryophyte ecology. Of the animals, birds are treated in some detail with particular reference to terns, wildfowl and waders. There is a useful section on marine invertebrates and an interesting account of terrestrial molluscs in relation to the fluctuating variety of habitats that the Island has to offer. The insects of the Island appear to have been studied less than the other groups already mentioned, and the account of their ecology is rather fragmentary. Clearly, there are opportunities here for the investigation of micro-evolutionary changes for which they might provide admirable material.

The reappearance of this splendid symposium in revised and expanded form will be welcomed by anyone with a love of ecology and natural history. It provides an example of the ecological survey at its very best. W. H. DOWDESWELL

Schizophyzeen

Von Prof. Dr. Lothar Geitler. 2, umbearbeitete Auflage. (Handbuch der Pflanzenanatomie, Band VI, Teil 1. Zweite, völlig neubearbeitete Auflage herausgegeben von Prof. Dr. W. Zimmerman und Prof. Dr. P. Ozenda.) Pp. vii+131. (Berlin-Nikolassee: Gebrüder Borntraeger, 1960.) 57.50 D.M.

IT would be a mistake to assume that this volume, as might be suggested by the title of the series, deals only with the gross structure of the Schizophyceae—or, as they are better known in Great Britain, the Cyanophyta, Myxophyceae or blue-green algae. The author has interpreted anatomy in a broad sense, and almost every page has reference to other features of wider interest.

The morphological range in the group is adequately summarized, but in addition there is a particularly good account of modern work on the structure of the protoplast, a subject of considerable importance on account of the low level of cellular organization as revealed by the absence of differentiation into nucleus and cytoplasm. That botanical enigma, the heterocyst, also receives adequate treatment, but it would seem that there is still no satisfactory explanation as to the function, if any, of this peculiar type of cell.

The text is illustrated by many fine figures, while the printing and binding are all that could be wished for.

The Birds of the British Isles

By Dr. David Armitage Bannerman. Illustrated by George E. Lodge. Vol. 9: Scolopacidae (Part). Pp. xii+398+26 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1961.) 63*s*. net.

THIS ninth volume (with three still to come) is devoted entirely to the Scolopacidae and does not, indeed, completely cover that large family. Here we have the curlews, godwits, and an array of sandpipers; the phalaropes, separated by some authorities, are also included. So many of these species are not natives of the British Isles that Dr. Bannerman has had to be even more assiduous than usual in gathering extraneous material for full accounts of their life-histories. In doing this he has drawn on a wide knowledge of the literature and information supplied by correspondents, while other authors have contributed certain sections by invitation. In this way the birds are followed on one hand to breeding grounds in the high Arctic or in North America or Asia, and on the other to winter quarters by tropical coasts, rivers and lakes—a valuable compendium of evidence. Some of these 'waders' (or 'shore-birds' as the Americans have it) were among the best subjects of the late George Lodge's artistry. LANDSBOROUGH THOMSON

The Monarch Butterfly

By Dr. F. A. Urquhart. Pp. xxiv+361+12 plates. (Toronto: University of Toronto Press; London: Oxford University Press, 1960.) 52*s*. net.

IN a well-printed and well-produced book of 350 pages and twelve coloured plates, Dr. F. A. Urquhart, of the Royal Ontario Museum, has produced a very full account of the monarch butterfly, *Danaus plexippus* Linn., particularly from the point of view of its life-cycle, its habits—including its remarkable annual migrations—and its relation to other species in a 'mimicry' complex. The first part of the book, 96 pages, is a general survey: the second gives in more detail the investigations carried out by Urquhart and his collaborators in the field during many years.

Nearly all the book is of interest to the field naturalist, but its most outstanding contribution is undoubtedly the results of the author's work on marking of the butterflies on their autumn and spring migrations, and recoveries at great distances. It has been generally accepted for many years that the monarch in North America must migrate over long distances to the south in the autumn and back to the north in the spring, but absolute proof was lacking.

Marking experiments with insects have been tried in many countries in the past thirty years, but the number of recoveries at any long distance has been negligible. Urquhart has been much more successful. Out of many thousands of butterflies marked, chiefly in the autumn at the beginning of their southward movement, but some also in the spring, his list of recoveries includes 98 in the autumn and 13 in the spring at more than 13 miles from the place of marking. Of these the maximum distance for a spring marking was 600 miles; while for the autumn movement there were 25 recoveries at more than 350 miles, of which 8 were more than 1,000 miles. The most distant recovery was one marked in Ontario on August 18, 1957, and recovered on January 25, 1958, in San Luis Potosi, Mexico, a distance of 1,870 miles in a direct line. For these results alone the book should be in every library.

C. B. WILLIAMS

Solubilities

Inorganic and Metal-Organic Compounds—a Compilation of Solubility Data from the Periodical Literature. By Dr. Atherton Seidell. Vol. 1: A–Ir. Fourth edition by Dr. William F. Linke. Pp. iv+1487. (Princeton, N. J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1958.) 187*s.* 6*d.*

PROBABLY every scientific library contains at least one of the complete editions of this famous series *Solubilities*, first published by Dr. Atherton Seidell in 1907.

A regular revision of a compilation of data of this nature is absolutely imperative if the work is to provide the scientist with the most recent and up-to-date information. Even in the short period of a decade, numerous original investigations on organic and inorganic solution equilibria are published in the multitudinous modern scientific periodicals and journals, and all these require a critical periodical assessment and tabulation.

The volume under review is the first of the new fourth edition, revised and compiled by Dr. W. F. Linke, and is the result of a detailed, critical study of investigations recorded in the chemical abstracts up to the 1956–57 period. The data of various authors on particular substances have been compared and combined; unreliable observations have been omitted, whereas closely agreeing results are plotted and averaged.

As in the well-known earlier editions, the elements are listed alphabetically according to their chemical symbols, and the anions of compounds also fall into a similar alphabetical sequence. A welcome innovation to the present edition is the inclusion of a complete index of substances and compounds which renders the location of specific data a much more facile procedure. The usual author and reference index is extensive and comprehensive.

This new edition is to be published in three volumes. The first volume covers argon to iridium, and volume 2 will complete the inorganic compilation. Volume 3 is to be devoted to the solubilities of organic compounds in various solvents.

The new series comprises a valuable modernized edition of a reference work which has been of the greatest value to chemists for more than fifty years, and, like a good wine, it 'needs no bush'.

D. T. LEWIS

Advances in Clinical Chemistry

Vol. 3. Edited by Harry Sobotka and C. P. Stewart. Pp. xiii+400. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 12 dollars.

THE third volume of this useful annual, which has now established itself as an essential piece of equipment on the desk of every hospital biochemist and which can be read with profit by general biochemists and clinicians alike, maintains the high standards set by its two predecessors. Particularly valuable is the chapter by Brown on œstrogens, for it not only provides a useful survey of their metabolism and clinical significance but it also gives full experimental details for their estimation in blood and urine in a form which is not readily available elsewhere. Folic acid and vitamin B_{12} are discussed in twin chapters by Girdwood and Gräsbeck respectively, while the account of kernicterus by Arias includes a useful survey of present-day knowledge of bile pigment metabolism.

The chapter on flocculation tests in relation to liver disease by Reinhold is both exhaustive and critical in its treatment of a commonly employed procedure which is simple to carry out, but which has hitherto been difficult to explain in other than empirical terms. In striking contrast is the account of infra-red absorption analysis by Schwarz. While infra-red spectra are invaluable to the organic chemist in the identification of unknown compounds they have only recently come to be applied to the examination of tissue constituents and they are discussed here with particular reference to lipid analysis. Nevertheless, it is likely to be a long time before an infra-red spectrometer becomes a standard piece of equipment in every clinical chemistry laboratory, in Great Britain at least.

J. N. DAVIDSON

Advances in Biological and Medical Physics

Vol. 7. Edited by Cornelius A. Tobias and John H. Lawrence. Assisted by Thomas L. Hayes. Pp. ix+362. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 10 dollars.

THIS volume presents a further selection of seven topics from the wide field covered by this series.

First there is an excellent chapter by G. S. Stent and C. R. Fuerst on the genetic and physiological effects of the decay of phosphorus-32, following incorporation into bacterial viruses and bacteria. These effects are primarily the result either of the transmutation of phosphorus-32 to sulphur-32 or of the recoil of the nucleus and not to the ionization produced by the β-particle emission. There is a useful chapter on radiation effects on the nervous system by N. N. Livshits, covering much of the Russian literature in this field. Another chapter, by L. W. Law, on radiation carcinogenesis provides a wealth of data, but the discussion is confined almost exclusively to the mutational hypothesis for the induction of neoplasia. Confusion over dosage units leads to the incorrect statement that Wistar rats are 200 times more sensitive to phosphorus-32 than are CF_1 mice to strontium-90 for bone tumour induction. A chapter by D. Carlstrom deals with the technique of micro X-ray diffraction in biology, but only a few applications of the technique are described. There is a clear introduction to autoradiography with tritium-labelled compounds by J. H. Taylor; however, important results on the damage to cells by so-called 'tracer' doses in experimental animals have appeared in the Press since the article was written. L. E. Lipetz provides a chapter on mechanisms for the transfer of information from the light image to the optic nerve in the horseshoe crab and D. Gitlin and C. A. Janeway deal with isotopic studies on the distribution and metabolism of plasma proteins.

In general, the authors have presented their subjects clearly and concisely, and the volume should provide a useful introduction to the various topics covered. N. M. BLACKETT

Radioactive Wastes

Their Treatment and Disposal. General Editor: J. C. Collins. Pp. xxi+239. (London: E. and F. N. Spon, Ltd., 1960.) 55*s*.

MR. COLLINS has brought together ten chapters, each written by an expert in his own field, which, together under one cover, form a logical and thorough treatment of a relatively new subject. Introductory chapters on the nature of radioactivity and sources of radioactive waste put the general problem in perspective. R. F. Farr's contribution on the hazards of radiation is a masterly summary. It takes account of dose-rate, total dose, external and internal irradiation, biological effectiveness of different radiations, the concentration of ingested isotopes in specific organs, and then shows how these factors are allowed for quantitatively in fixing maximum permissible levels of radioactive species in the body, in air, in drinking water, and on contaminated surfaces.

The law about disposal of radioactivity in Britain is summarized. The last five chapters describe techniques for meeting in practice the stringent standards which have been set for safe disposal of gaseous, liquid and solid radioactive wastes.

This is a technical book, full of factual information, but its very full glossary and index to abbreviations, combined with its logical style, should make it attractive to a very wide readership. It will be valuable to all those people who have responsibilities for public health, the husbanding of natural resources, nuclear power programmes and industrial use of radioactive materials. One feels that the book will be enjoyed also by those who read *Nature*, because they enjoy dipping into branches of science other than their own, and one hopes that through them this book may lead to well-informed discussion of a subject of importance to the general public.

K. D. B. JOHNSON

Digest of Literature on Dielectrics

Vol. 23, 1959. Edited by Louis J. Frisco and Thomas D. Callinan. (Publication No. 799.) Pp. xiv+423. (Washington, D.C.: National Academy of Sciences—National Research Council, 1960.) 8 dollars.

THE latest volume of this series comes up to the expected high level. The number of publications on the subject within a year is surprisingly large. There are 2,214 abstracts, and the origin of the papers is truly international.

In the measurement field, frequencies greater than 100 Mc./s. are coming into the forefront, and at the highest frequencies optical techniques attract increasing attention. A good deal of work is reported on dielectric properties and molecular structure. The chapter on conduction contains experimental results which are interpreted in terms of band theory, but conduction in good dielectrics still remains a difficult subject.

Breakdown in solids, liquids and gases has been reviewed systematically this year. At present there is interest in pre-breakdown currents, and relatively much work is reported on non-uniform fields.

In ferro-electricity and piezo-electricity one is impressed with the wide range of techniques used and the large Russian effort on the subject. The so-called relaxation dielectrics are attracting attention. The digest includes an impressive chapter on magnetism, with 528 references. Developments in this subject include new theoretical work on exchange forces and statistical mechanics and experimental work on thin films and devices.

The last third of the book is devoted to the technological application of insulating materials, and contains references to the patent literature. There is much interest in insulation for high temperatures.

In conclusion, the digest contains a complete bibliography of the subject, and the authors succeed in giving a fair critical appraisal of the papers abstracted. VERA DANIEL

Atlas of γ-Ray Spectra from Radiative Capture of Thermal Neutrons

By L. V. Groshev, V. N. Lutsenko, A. M. Demidov and V. I. Pelekhov. Translated from the Russian by J. B. Sykes. (International Series of Monographs on Nuclear Energy. Division 4: Isotopes and Radiation, Vol. 1.) Pp. iv+198. (London and New York: Pergamon Press, 1959.) 140*s*. net.

THE *Atlas*, which was first published in English in 1959, was the first attempt at an exhaustive compilation of information on thermal neutron capture γ-rays to be published in book form, though a rather similar compilation, not quite so comprehensive, was published as a Chalk River Report, *AECL*.669, by Bartholomew and Higgs in 1958. The work of the Russian group led by Groshev, in the field of neutron capture γ-rays, is too well known to require comment, and the *Atlas* contains much of their own work and that of the Chalk River Group, together with the relevant work published elsewhere up to and including 1958. The bibliography contains 97 entries.

The information presented begins with tables of stable isotopes giving isotopic abundances, thermal neutron capture cross-sections, spins and parities of target and product nuclei, and binding energies and half-lives of the product nuclei. The tables contain references to the original literature. Following the tables are the capture γ-ray spectra, together with further tables of intensities as measured by various authors, assignment, where possible, of the γ-rays to the appropriate compound nuclei, and also, where possible, energy-level diagrams.

This impressive compilation is invaluable both to the nuclear physicist and to the reactor engineer who is concerned with shielding problems. The only criticism of the book which I feel is justified concerns the technical matter of the reproduction of the figures. This appears to have been done rather hurriedly so that some of the curves are distorted and occasionally the lettering is difficult to read. Such technical defects seem out of place in a book costing £7. E. R. RAE

The Space Encyclopædia

A Guide to Astronomy and Space Research. Second, revised edition. Pp. 288. (Horsham: The Artemis Press, 1960.) 35*s*.

THE first edition of this fascinating work was published just before the launching of *Sputnik* 1. Much that was then mere speculation is now accomplished fact, and the many alterations in the new edition reflect the present rapid advance in space research. The section on "Artificial Satellites" has been completely re-written, with details of the 25 satellites and space probes launched prior to April 1960. The forty-odd new sections, ranging from "*Black Knight*" to "Van Allen Belts", have been added at the expense of certain minor topics and the condensation of others. In particular, several missiles hitherto treated separately are now collected under the new heading "Missile".

The appearance of the book is much improved by the addition of eight colour plates, one of which shows the beautiful "Veil Nebula in Cygnus". The famous Russian "Far side of the Moon" is one of several new photographs.

Certain misprints persist. In the Table of Planets, p. 230, the masses of Mars to Neptune are incorrect and on p. 155 the period of Phobos is still given as 7d. 39m. A curious mistake also occurs in the diagram on p. 28, where the track of *Sputnik* 1 is shown starting off south-east instead of north-east. These errors apart, the *Space Encyclopædia* will provide a useful reference to space enthusiasts for the next year or two, when we shall look forward to a further revised edition. R. H. MERSON

Principles of Electricity and Magnetism

By Y. Rocard. Translated by G. F. Herrenden Harker. Pp. x+779. (London: Sir Isaac Pitman and Sons, Ltd., 1959.) 70*s*. net.

A NEW book in this field would need to be outstanding to make the task of translating worth while. The book is very up to date, and the many examples of the practical uses of the topics discussed bring new life into what is often regarded as a dull, text-book subject. Although the author advocates the use of rationalized M.K.S. units he in fact uses e.s.u., e.m.u., and practical units. The scope of the book is much wider than is implied by the title. Some sections have become so condensed that they have little value. The section on X-rays, which seems out of place in this volume, contains a number of mistakes (for example, it is suggested that a Laue photograph is obtained with a stationary crystal and *monochromatic* radiation, and, later, a powder diagram is described as a Laue photograph).

The Grenoble electrostatic generator is described in considerable detail, but no information is given about its performance. It is encouraging to see the permalloy magnetometers included in this type of book. The chapters on polyphase currents and d.c. machines are more complete than one expects in a book aimed at physicists rather than engineers. The notation throughout the book is not uniform. In Chapter 13 the Langevin formula is given and the symbols used are defined. In Chapter 31 the detailed theory is given using a different set of symbols. It is a pity, in a book designed for undergraduates, to find no problems for the students to solve.

E. STANLEY

Proceedings of the Institute of Petroleum Hydrocarbon Research Group Conference on Molecular Spectroscopy

Held in London, 27–28 February, 1958. Edited by E. Thornton and Dr. H. W. Thompson. Pp. vii+352. (London and New York: Pergamon Press, 1959.) 70*s*. net.

THIS report gives the twenty-two papers presented together with the relevant discussion at the second two-day conference on molecular spectroscopy held by the Hydrocarbon Research group. The contributors are from both industrial and academic laboratories in several countries, and are leaders in fields ranging from nuclear magnetic resonance through infra-red to ultra-violet spectroscopy, able to discuss with authority new ideas and techniques.

The range of topics presented is far from being confined to the petroleum field and will be of interest to every physical and organic chemist. The extent to which the conference is topical is demonstrated by the high proportion of space devoted to nuclear magnetic resonance spectroscopy and other new fields, for example, the applications of polarized infra-red radiation, shock-tube excitation and free-radical spectra. A range of papers shows advances in the infra-red region, including new instrumentation, theories of band intensities and the study of intermolecular forces in compressed gases, in surface adsorption, in solution and in the formation of hydrogen bonds.

The organizers of the conference are to be complimented on bringing this interesting diversity of speakers together. However, it does seem that the fields covered by the heading of "Molecular Spectroscopy" are rapidly becoming too numerous and too specialized to be covered adequately in a two-day meeting. A. KNOWLES

A Mathematical Tool-Kit for Engineers

By H. A. Webb and Dr. D. G. Ashwell. Second edition. Pp. vii+116. (London: Longmans, Green and Co., Ltd., 1959.) 15*s*.

A CONCISE account of the more important mathematical topics dealt with in the final year of engineering undergraduate courses is published, in second edition, under the intriguing title *A Mathematical Tool Kit for Engineers*. This title is truly descriptive of the book. Within the compass of little more than one hundred pages, eleven chapters deal with complex numbers, differential equations, Fourier series and Bessel functions, together with the calculus of variations and the theory of errors. A chapter on Macaulay methods discusses a little-known technique for discontinuous differential equations, and the Laplace transform is associated, through the integral inversion formula, with a chapter on contour integration. The most important basic formulæ, in relation to engineering application, are derived as simply and directly as possible, without, of course, any attempt at mathematical rigour, and techniques of application are suitably illustrated. That arguments presented with such conciseness and economy can still carry conviction is a tribute to the skill and ingenuity with which the derivations have been devised and developed. The selection of material is excellent. The little book should be of great value to engineering undergraduates and, as a work of occasional ready reference, to practising engineers. J. GREIG

(*Continued from page* 40)

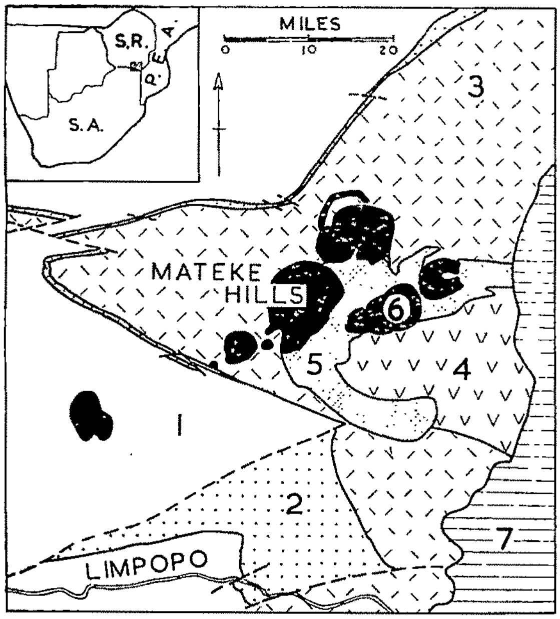

Fig. 1. Geological map of the Mateke Hills area in south-eastern Southern Rhodesia. Inset map: S.R., Southern Rhodesia: S.A., South Africa; P.E.A., Portuguese East Africa.

7.	Sediments	Cretaceous System
6.	Intrusive complexes	Karroo System
5.	Main Granophyre	
4.	Rhyolites	
3.	Basalts	
2.	Sediments	
1.	Gneisses, etc.	Basement Complex

Limpopo River. In contrast, neighbouring areas to the north and south are characterized by a very thin sequence of Karroo sediments which in places is absent altogether. Strong normal faulting developed along the sedimentary trough during the period of Karroo sedimentation.

A very much thicker volcanic sequence overlies the sediments and comprises a lower group of olivine basalts and tholeiites with associated picritic minor intrusives, and an upper group of ignimbrites of rhyolitic composition. A thick and extensive sill of granophyre, here termed the Main Granophyre, intrudes the volcanics near the base of the ignimbrite group.

The volcanic activity culminated with the development of eight intrusive complexes closely comparable with those found in Damaraland, South West Africa[2]. Six of the complexes lie in the vicinity of the Mateke Hills, Nuanetsi District, Southern Rhodesia, and have been mapped in detail. The alignment of most of the complexes along the Limpopo orogenic belt is a further demonstration of the importance of the belt in controlling Karroo geology.

Three of the complexes, of which the largest is the Northern Ring, consist of early gabbroic and later acid phases, the latter commonly being ring-intrusions. In the Northern Ring[3], an extensive layered gabbro series is exposed. The gabbroic intrusions are older than the Main Granophyre, while the acid ring-intrusions are younger. Most of the remaining complexes consist only of acid rocks, several phases of granitic and granophyric intrusion being present in each.

In the eastern part of the area, Cretaceous marine strata unconformably overlie the Karroo rocks, including the Main Granophyre. This is a strong indication that all the intrusive activity can be assigned to the late Karroo period.

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METALLURGY

Occurrence of an Ordered Phase in an As-Cast Titanium-Aluminium Alloy

Phase equilibria in the titanium-rich region of the titanium–aluminium constitution diagram have not been elucidated despite many experimental investigations. The results of earlier work[1] indicated an extensive region of hexagonal close-packed α solid solutions. At a temperature of 800° C. it was reported that 36·5 atomic per cent aluminium was soluble in the low-temperature modification of titanium. It was also concluded that the first intermediate phase on this side of the diagram was γ, which exists over a region of homogeneity and resulted from a pertectic reaction involving a β solid-solution and the melt. A study of the variation in lattice parameters as a function of composition in the proposed α phase region has been made[2]. Several papers have presented X-ray powder diffraction evidence for an intermediate phase in this same region of the diagram. This crystallographic species, the δ phase, has been observed in heat-treated specimens containing 25 and $33\frac{1}{3}$ atomic per cent aluminium[3,4]. Experiments performed with an electron microscope[5] have demonstrated the epitaxial relationship of α and δ. A constitution diagram has been proposed[6] in which two intermediate phases exist between the terminal α phase and γ.

During an X-ray diffraction investigation of this region of the constitution diagram the δ phase has been observed in an as-cast alloy containing 37·1 atomic per cent aluminium. A fragment of an arc-melted ingot was examined and revealed Laue symmetry 6/*mmm*. Weissenberg photographs of the zero and first layer lines were obtained with copper and molybdenum radiation. The diffraction maxima were sharp and doublets well resolved. Multiple-film techniques have been employed to obtain the structure factors. The unit cell content was placed in the following special positions of space-group D_{6h}^4: 2 aluminium atoms in (*c*), and a statistical distribution of titanium and aluminium atoms in the six-fold position (*h*). The compositional parameter associated with this configuration was assumed to be constant in the trial structure, and it was derived from the ingot composition. This assumption seems reasonable in view of the lattice parameter versus composition data[2] and the variation of structure factor with composition. Agreement of intensity measurements for strong and weak reflexions indicates a single crystal in object space. Previous papers reporting evidence for the δ phase derived their data from heat-treated specimens. The fact that the δ phase has been found in an as-cast alloy would suggest that the proposed phase equilibria for this constitution diagram be given careful consideration.

A paper published by previous investigators[4] requires a comment. I established the similarity between X-ray powder diffraction photographs of Ti_3Sn and those of a titanium–aluminium alloy and a titanium–aluminium–vanadium alloy (Jaffee, L. D., to Margolin, H., private communication, September 29, 1955). The latter films had been obtained by other research workers in previous investigations at the California Institute of Technology Jet Propulsion Laboratory. These existing data were evaluated by me without prior knowledge that either a unit cell or indexing of maxima had been proposed for the δ phase. Furthermore, the necessity for consideration of composition and position parameters in the crystal structure determination of δ has been discussed in a private communication by myself to H. Margolin, October 25, 1955. I have neither demonstrated nor asserted that Ti_3Sn and Ti_3Al are isomorphous, as has been stated in the literature[4].

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CHEMISTRY

Oxidation of Galena to Lead Sulphate

Beveridge and Denbigh[1] have directed attention to the development of a concentric shell of zinc oxide when a compressed sphere of zinc sulphide powder was oxidized in air at 1,250° C. This shell was presumed to arise from the reaction between zinc and sulphur diffusing outwards from the surface of the zinc sulphide and oxygen diffusing inwards. The distance between the core and the shell increased with increasing temperature, and the diameter of the shell was greater than the diameter of the original core. During the oxidation of a crystal of galena (lead sulphide) to lead sulphate we have observed the formation of an approximately spherical product layer detached from and concentric with the galena crystal. The mechanism by which this product layer is formed appears to differ from that proposed for zinc oxide[1].

Using a thermobalance we have examined the rate of oxidation of discrete crystals of galena in an atmosphere containing oxygen, sulphur dioxide and nitrogen at temperatures from 700° to 800° C. Depending on the respective partial pressures of oxygen and sulphur dioxide, the oxidation product may be lead, lead oxide, mono-, di-, or tetra-basic lead sulphate, or lead sulphate[2]. In particular we have followed the formation of lead sulphate in oxidizing atmospheres containing 1–50 per cent oxygen and 1–50 per cent sulphur dioxide, the balance being nitrogen. The rate of oxidation has been calculated from the increase in weight of the sample observed over measured time-intervals. The galena crystals were not preheated in an inert gas before oxidation on account of the loss in weight from vaporization and oxidation to lead by traces of oxygen in the inert gas. Our results show that the oxidation proceeds as follows: Initially an adherent layer of lead sulphate builds up on the surface of the galena crystal; later this layer of lead sulphate becomes detached from the surface of the galena (leaving it free from oxidation products) and forms a brittle spherical envelope the dimensions of which approximate those of the original galena crystal; finally, cracks occur in the envelope as it progressively thickens and expands, and lead sulphate is again formed on the surface of the galena crystal. The composition of the product is confirmed by X-ray analysis.

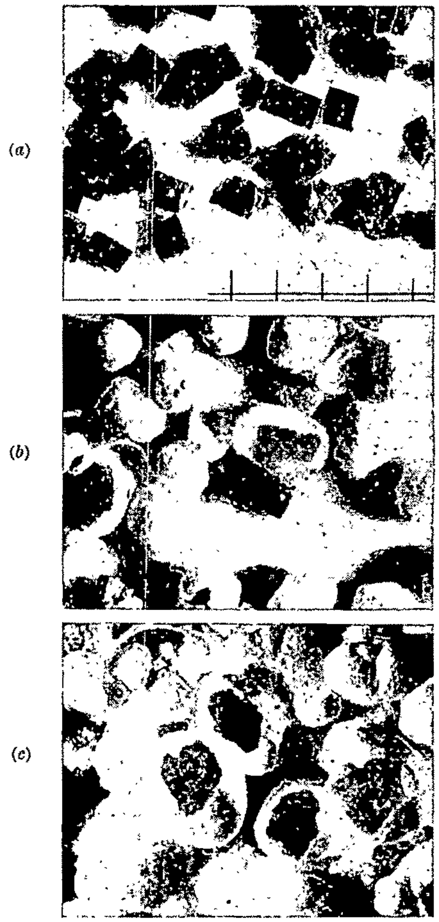

Fig. 1. − 36 + 44 mesh galena crystals. *a*, Unoxidized; *b*, oxidized for 1 hr.; *c*, oxidized for 2 hr. Scale: 1 division = 0·5 mm.

Fig. 1*b* and *c* shows galena crystals which have been oxidized at 750° C. in an atmosphere containing 10 per cent oxygen, 15 per cent sulphur dioxide, and 75 per cent nitrogen. Several of the lead sulphate envelopes have been broken to reveal the partly reacted galena crystals.

In accounting for the development of the spherical envelope, we suggest that there is a gradual diminution in the permeability of the adherent layer of lead sulphate to the oxidizing atmosphere. Then following the vaporization of galena—with some dissociation into lead and sulphur[3]—a stage is reached where oxidation occurs predominantly by diffusion of lead and sulphur ions through the envelope to react with the oxidizing atmosphere outside. Thus the envelope thickens, and, as the volume ratio of lead sulphate to lead sulphide is greater than unity, the resulting stresses cause the envelope to expand.

The rate of formation of the adherent layer of lead sulphate has been found to depend on the temperature and the sulphur dioxide content of the atmosphere; variations in the oxygen content, however, do not markedly affect the reaction-rate.

We acknowledge the support of the Australian Atomic Energy Commission for this work.

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Sodium Chloride Whiskers

It is generally believed that the fast and unidirectional growth of the major part of whiskers may be partly due to the winding up of one or more screw dislocations along the whisker axis[1]. S. Amelinckx demonstrated this dislocation mechanism in sodium chloride whiskers by the decoration technique[2]. He observed on whiskers grown by a simple method also the broadening of hollow, tubo-like pyramids probably developed from spiral terraces which are thought to originate from axial screw dislocations[3].

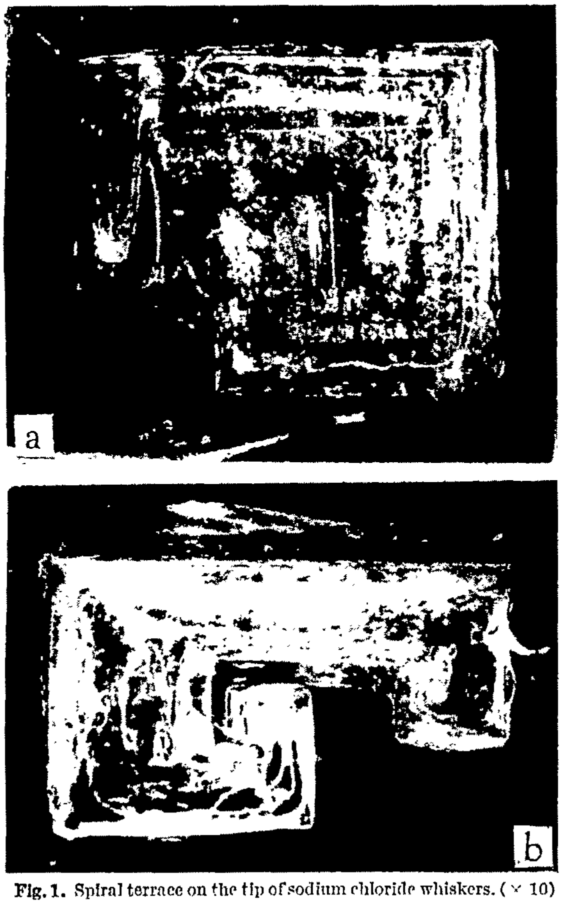

Fig. 1. Spiral terrace on the tip of sodium chloride whiskers. (× 10)

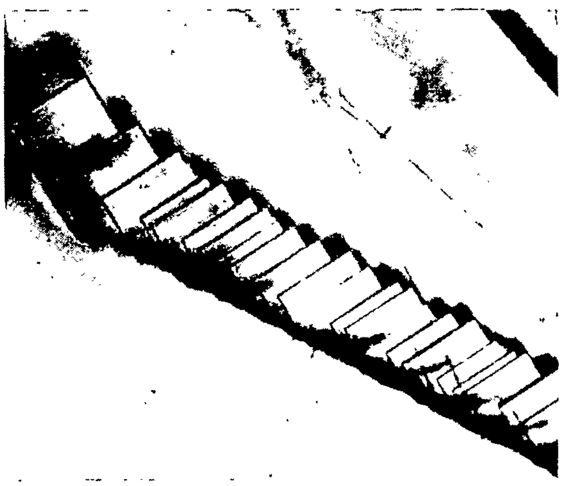

Fig. 2. A scale-like sodium chloride whisker. (× 150)

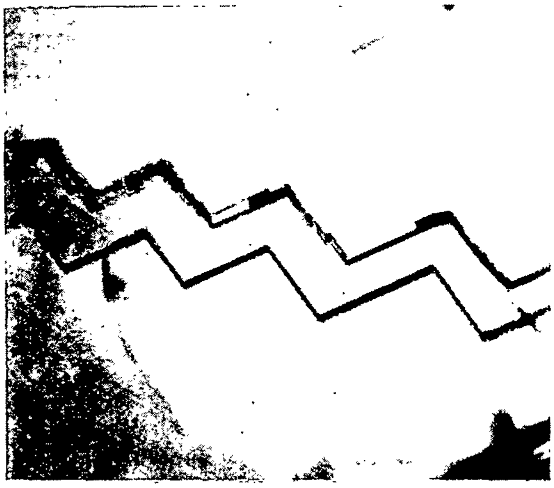

Fig. 3. A two-dimensionally twisted sodium chloride whisker (× 150)

The pyramid growth pattern is known. V. D. Kusnezov suggested that its presence in crystals grown from solution may be related to the small dependence on temperature of the solubility of rock-salt[4]. The observations of Z. Gyulai refer to the growth of such pyramids from vapour[5]. We, too, found such 'troughs', not only on the tip of whiskers grown from vapour but also on sodium chloride plates exposed for some time to vapours of sodium chloride[6]. Hexagonal spiral-terrace patterns of N. A. Shamba and N. N. Sheftal on silicon crystals frozen from cooled melt, and observed easily with the optical microscope, are further examples[7].

We wish to report some interesting patterns obtained by growing sodium chloride whiskers from vapour. The pyramidal tip of the whiskers in Fig. 1(*a*) shows undoubtedly a spiral construction. The linear length of the pattern was 8 mm. Fig. 1(*b*) shows a similar whisker tip after the other parts of the pyramid were removed. The linear measurement of the spiral was in this case 6 mm.

Fig. 2 shows another example: it is suggested that this whisker with a scale-like construction refers to spirals[8].

The zig-zag pattern in Fig. 3 indicates that the two-dimensional explanation of S. Amelinckx[9] of the helically twisted silver whiskers observed by Brenner[10] refers to a two-dimensionally twisted whisker shape which actually exists.

We wish to thank Miss F. Jost for her help.

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Effect of Strong Electric Fields on the Boiling Points of some Alcohols

WHILE investigating the molecular theory of liquids and liquid mixtures we were led to examine the effect of strong electric fields on the saturation vapour pressures (boiling points) of liquids. Results of those investigations for methyl, ethyl and *iso*propyl alcohols are reported here.

The liquid is in a cylindrical tube about 20 cm. long which is immersed in a transformer oil bath. A condenser, fitted to the side of the tube through a ground-glass joint, keeps the boiling liquid in contact with the outside atmosphere while at the same time vapours condense and flow back into the liquid without escaping out of the apparatus. A Beckmann thermometer, graduated in hundredths of a degree centigrade, is inserted from the upper end of the tube and held vertical with its bulb just above the surface of the boiling liquid. When the liquid is boiling the bulb remains completely covered with a continuous film of the liquid formed by the condensing vapours which trickle back into the liquid in the form of drops. A built-in condenser is provided near the upper end of the tube to prevent any possible leakage of vapours through the upper portion. A regulated supply of heat to the oil bath is given by an electric heater controlled by a 'Variac' transformer. The temperature of the oil bath is maintained a few degrees above the boiling point of the liquid. Under these conditions the boiling point could be maintained constant to 0·01 deg. C. for fairly long intervals.

The tube containing the boiling liquid and its vapours is held between two parallel metal plates to which an alternating voltage of about 20 kV. can be applied through a transformer.

Samples of pure specimens used in the present investigations were Merck's analytical reagents from freshly opened bottles which were further purified by the usual methods until their boiling points and densities agreed with those quoted in the literature. The liquid was allowed to boil first in the absence of the field, and after the temperature remained steady for about ½ hr. the field was switched on. The temperature starts falling almost immediately and becomes steady after some time. On switching off the field the boiling points rose to their original values.

The depression in the boiling points, ΔT, for fields E, varying from 0 to 5 kV./cm., were determined for methanol, ethanol and *iso*propanol.

Observations were also made with three different tubes A, B and C of diameters 3·65 cm., 3·00 cm. and 2·50 cm. respectively. These are shown in Table 1.

Table 1. DEPRESSIONS IN THE BOILING POINTS OF METHANOL ETHANOL AND *ISO*PROPANOL IN ELECTRIC FIELDS

Applied voltage (kV./cm.)	ΔT (°C.)								
	Methanol			Ethanol			*Iso*propanol		
	A	B	C	A	B	C	A	B	C
0·00	0	0	0	0	0	0	0	0	0
2·36	0·68	0·70	0·80	—	—	—	—	—	—
2·89	1·05	1·08	1·10	0·32	0·34	0·35	—	—	—
3·42	1·25	1·29	1·40	0·40	0·43	0·45	0·20	0·20	0·23
4·00	1·80	1·87	1·90	0·60	0·65	0·64	0·25	0·26	0·26
5·00	2·00	2·00	2·10	0·70	0·72	0·76	0·40	0·42	0·42

However, when the field was applied only to the liquid region and not to the vapour phase, a rise of 0·1 deg. C. was observed in the case of methanol at 5 kV./cm.

Observations with other polar as well as non-polar liquids are in progress.

We wish to thank Prof. D. S. Kothari for suggesting the problem and helpful discussions. One of us (M. M. C.) is grateful to the Council for Scientific and Industrial Research for financial assistance.

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Thermo-Reversible Denaturation of Eucollagen in Solution

A SUBSTANTIAL proportion of mature collagenous tissue will dissolve in weakly acidic media after a cold pre-treatment in alkali. The resulting solutions of soluble eucollagen show qualitatively many of the characteristics of solutions of acid-soluble collagens[1]. In particular, a very dilute solution in 0·15 M citrate (pH 3·7) possesses a high specific viscosity (η_{sp}/C about 10 dl./gm. at $C = 0{\cdot}02$ gm./100 ml.) over the temperature range 5–25° C., and a very much lower value (η_{sp}/C about 0·4 dl./gm.) at 35° C. and higher temperatures. The high viscosity at low temperatures suggests that eucollagen, like collagen, has a rigid, rod-like form in solution, while the relatively low viscosity at 35° C. and higher temperatures reflects the very flexible nature of the gelatin molecules which are formed rapidly and irreversibly under such conditions from the eucollagen solution.

At intermediate temperatures (for example, 30° C.) the initially high viscosity falls with time, and on re-cooling to 20° C., the solution shows a marked and rapid rise in viscosity. Examples are given in Fig. 1. Boedtker and Doty[2] have stated that solutions of soluble collagen, after partial denaturation by heating, show an increase in viscosity on re-cooling, and have suggested that this is due to aggregation of the gelatin molecules previously formed

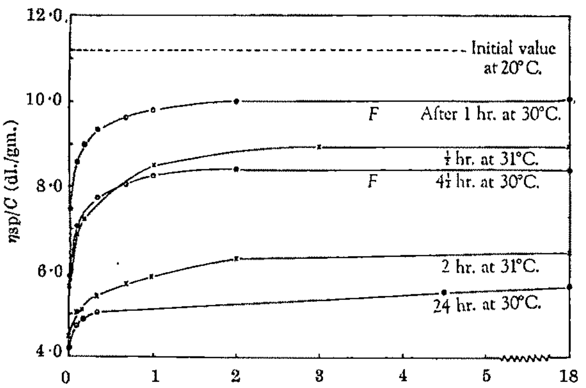

Fig. 1. The time dependence of η_{sp}/C at 20° C. for eucollagen solutions which have been heated at 30° or 31° C. for the times stated. Concentration, $C = 0{\cdot}05$ gm. eucollagen per 100 ml. 0·15 *M* citrate (*p*H 3·7). Eucollagen prepared from demineralized bone

at the higher temperature. However, if this were the explanation of the present results, the viscosity should increase with time only slowly in the initial stages, at 20° C., more rapid increases following as larger and larger aggregates are built. Fig. 1 shows clearly that the rate of rise decreases with time. Moreover, the maximum possible contribution to the viscosity from aggregation of derived gelatin is far smaller than the observed changes in the viscosity of soluble eucollagen at 20° C. after a period at 30° C. This is confirmed (Fig. 2) by observing the aggregation in eucollagen solutions which have been completely denatured by heating at 60° C. for 10 min. Thus the recovery in viscosity at 20° C., after partial denaturation at 30° or 31° C., can be due to gelatin aggregation only to a minor extent.

It is therefore presumed that the structural disorganization on heating eucollagen in solution at 30 or 31° C. proceeds in two steps, the first of which is, at least, partially reversible by cooling, while the second gives rise irreversibly to gelatin. The partially disorganized, thermo-reversible states for dissolved eucollagen have considerable stability at 30° C. (Fig. 1). At higher temperatures, however, the life-time of any intermediate state is short, so that the conversion of eucollagen to gelatin proceeds in effect in only one step.

Exploratory experiments have shown that fibrils are formed from incompletely denatured eucollagen solutions re-cooled to 20° C. and dialysed against 1 per cent sodium chloride. (The solutions used are marked *F* in Fig. 1.) Such fibrils have a shrinkage temperature 5–10 deg. C. less than the value for fibrils prepared from a sample of the original, undenatured solution. The formation of fibrils from the denatured solutions could arise either from eucollagen particles which were unaffected by the higher temperature, or from particles re-organized from intermediate states formed on heating. The observed fall in shrinkage temperature suggests that the latter particles are an important constituent.

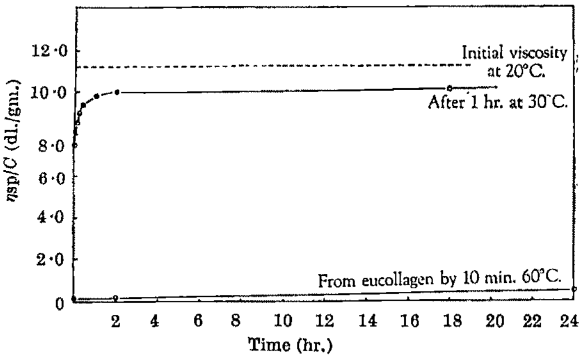

Fig. 2. The time dependence of η_{sp}/C at 20° C. for a partially and a completely denatured eucollagen solution, in 0·15 *M* citrate (*p*H 3·7)

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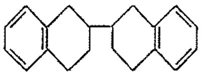
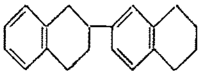
Reductive Dimerization of Naphthalene by Sodium–Ethylamine

Benkeser *et al.* have reported[1] that naphthalene is reduced to octalin and decalin by lithium–ethylamine; however, sodium–ethylamine was said to convert naphthalene to unidentified polymeric materials. Because of our interest in the reduction of organic compounds and of coal by metal–amine systems[2], we have re-investigated the naphthalene–sodium–ethylamine reaction. The so-called polymeric material has been identified as a stereoisomer of 1,2,3,4,1′,2′,3′,4′-octahydro-2,2′-dinaphthyl, (I).


(I)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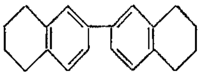
(III)

A mixture of 6·40 gm. of naphthalene, 10·35 gm. of sodium, and 200 gm. of ethylamine was refluxed for 5½ hr. Ether was added, the solution decanted from a large amount of unreacted sodium, and the product washed, dried and distilled. There was obtained 3·73 gm. of material, b.p. 180–200° (2–3 mm.), which slowly crystallized. The low-voltage mass spectrum showed mass 132 (tetralin), 260 (hexahydrodinaphthyl) and 262 (octahydrodinaphthyl). Three recrystallizations from ethanol gave 1·70 gm. of white crystals, m.p. 84·0–85·0°, mass 262. Anal. calcd. for $C_{20}H_{22}$: C, 91·55; H, 8·45; found: C, 91·38; H, 8·43. This purified material was used to calibrate the mass spectrometer, and the assumption was made that the instrument would have the same sensitivity for the hexahydrodinaphthyl as for the octahydrodinaphthyl. On this basis the original reaction product contained: tetralin, 9 per cent; hexahydrodinaphthyl, 19 per cent; octahydrodinaphthyl, 72 per cent. Dehydrogenation of the octahydrodinaphthyl (palladium-on-charcoal, 330–355°) gave 2,2′-dinaphthyl, identified by melting point and infra-red spectrum.

Only three structures (I, II, III) can be written for an octahydro-2,2′-dinaphthyl if it is assumed that each nucleus contains a benzene ring. The ultraviolet absorption spectrum (peaks at 2732 Å., $E = 1417$ and at 2660 Å., $E = 1241$; shoulder at 2615 Å., $E = 893$) of the hydrocarbon described here eliminates the structure (III), which would have a spectrum similar to that of diphenyl[3]. The structure (II) is eliminated because it contains a 1,2,4-substituted benzene ring, which would be expected to have the long wave-length band at about 2790 Å. (ref. 4). Since (I) consists of two tetralin nuclei it should have peaks at positions almost identical with those of tetralin, with about twice the intensity of the tetralin peaks. For comparison, values for tetralin are 2734 Å., $E = 625$; 2662 Å., $E = 537$; 2618 Å., $E = 370$.

The structure (I) was substantiated by dichromate oxidation of the hydrocarbon, which gave only phthalic acid; by nuclear magnetic resonance spectroscopy (aliphatic H/aromatic H, calcd., 1·75; found, 1·8; aliphatic H_α/aliphatic H_β, calcd., 1·33; found, 1·4); and by the infra-red spectrum (absorption maxima at 5·15, 5·22, 5·30, 5·45, 5·53, 5·57, 12·25, 13·36 and 13·52μ) which is consistent only with (I) (ref. 5).

The structure (I) could exist in both a meso and a racemic form, but only one compound was actually isolated; hence some step in the reaction sequence which fixes the configuration of the product must be stereospecific.

The reaction is under investigation to find other systems which will give dimeric products. Anthracene and phenanthrene have been reduced, but no dinuclear materials have been found. It is of interest that electrolytic reduction of phenanthrene yields 9,10,9′,10′-tetrahydro-9,9′-diphenanthryl, but no dinuclear product is obtained by electrolytic reduction of naphthalene[6]. On the other hand, we have found (Sternberg, H. W., Delle Donne, C. L., Reggel, L., and Wender, I., unpublished work) that lithium–ethylenediamine reduction of naphthalene at 15° gives about 30 per cent of dimeric reduction products, the structures of which have not yet been determined.

We wish to thank Dr. R. A. Friedel for the spectroscopic studies.

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Ionization Potential and Molecular Complex of Benzothiazol with Iodine

THE three aromatic molecules benzophenone, indol and benzothiazol should act as electron donors and, as such, should favour the formation of molecular complexes with electron acceptors such as the halogens[1]. A spectroscopic investigation of such a possible effect between these molecules and iodine was therefore undertaken and we briefly report the results here.

For benzophenone and indol, no changes in the spectra of solutions of these compounds were noticed when adding iodine (solvents were *n*-hexane for indol, and carbon tetrachloride for benzophenone), and neither any colour modification of the iodine solution nor new absorption in the ultra-violet region was observed. No complex thus seems to be formed between benzophenone or indol and iodine; at least none is detectable under the conditions of our experiments.

On the contrary, in the case of a solution of benzothiazol in carbon tetrachloride, a slight colour modification of the iodine solution was noticed when properly chosen proportions of benzothiazol and iodine were used (about 30 times more benzothiazol than iodine). The 517-mμ absorption maximum characteristic of iodine is practically not shifted (new maximum at 516 mμ), but a new absorption occurs in the near ultra-violet region around 300 mμ, as observed with a Zeiss spectrophotometer. Using a Beckman automatic *DK* II spectrophotometer, a new and intense ultra-violet absorption band characteristic of a new compound was observed at 282 mμ. Its intensity is about that of the usual high ultra-violet absorption of molecular complexes, and may perhaps be ascribed to a charge-transfer phenomenon.

It is well known that charge-transfer maxima are closely related to ionization potentials and a simple expression for this relation has been proposed[2]. A curve relating ionization potentials and ultra-violet charge-transfer maxima was published recently[3]; from this it is possible to deduce an ionization potential for benzothiazol.

It is found in this way that the 282 mμ maximum corresponds to an ionization potential of 8·65 V. for benzothiazol. This is considerably lower than the ionization potential of benzene[4], but closer to the ionization potentials of pyrrole[5] and thiophene[5], and might indicate that it corresponds to the removal of a non-bonding electron from either the nitrogen or sulphur atom, rather than a π-electron of the benzenic system.

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BIOCHEMISTRY

In vivo Formation of Normorphine in the Rat as a Metabolite of Tritium Nuclear-labelled Morphine

EVIDENCE for the N-demethylation of morphine has been provided by the demonstration *in vivo* of the pulmonary excretion[1,2] of carbon-14 labelled carbon dioxide from morphine-N-methyl-^{14}C and *in vitro*[3,4] by the enzymatic liberation of formaldehyde. The formation of normorphine *in vivo* has not been demonstrated directly, principally because of the low sensitivity and poor specificity of procedures used. The recent preparation of tritium nuclear-labelled morphine[5] has provided the means by which the *in vivo* formation of normorphine from morphine could be observed in a sensitive and specific manner using selective paper chromatography.

Male rats (Sprague–Dawley strain) were injected subcutaneously with tritium nuclear-labelled morphine sulphate (10 mgm./kgm., as free base) and the urine was cage-collected for 48 hr. under toluene. The urine was autoclaved with 1/10 volume of concentrated hydrochloric acid for 25 min. at 18 lb. pressure, cooled, neutralized with sodium hydroxide solution to *p*H 9, and buffered with phosphate buffer, *p*H 10·3. An appropriate amount of cold normorphine was added as carrier, and the solution saturated with solid sodium chloride and extracted by shaking for 45 min. with ethylene dichloride containing 30 per cent *n*-amyl alcohol. The organic layer was separated and the aqueous phase re-extracted with fresh organic solvent. The residue, obtained by evaporation of pooled organic solvent extracts, was dissolved in absolute methanol and the solution chromatographed (descending) on a large Whatman No. 3 *MM* sheet using *n*-butanol, acetic acid and water (4 : 1 : 2, by volume) as the solvent system. A narrow guide strip from the chromatogram was scanned for radioactivity by elution of 1-cm. strips with dilute hydrochloric acid, the eluates being transferred to individual counting vials and evaporated to dryness. The residues were dissolved in absolute methanol, phosphor solution was added, and the samples were counted in a liquid scintillation Packard 'Tri-Carb' spectrometer. The scan revealed a single sharp peak of radioactivity (R_F 0·57). The area under this peak was cut from the rest of the chromatogram, eluted repeatedly using hot absolute methanol, and the eluate concentrated to a small volume and chromatographed on Whatman 3 *MM* paper strips, buffered with 0·2 *M* phosphate buffer (*p*H 8) using *tert.*-amyl alcohol, *n*-butyl ether and water (80 : 7 : 13, by volume) as a solvent system. The scanning of a guide-strip resolved the single peak of the previous chromatogram into two peaks of radioactivity, one of R_F 0·76, due to tritium-labelled morphine, and the other of R_F 0·20.

The area under the peak of R_F 0·20 was eluted with hot absolute methanol and chromatographed on Whatman 3 *MM* paper strips with *n*-butanol, acetic acid and water (4 : 1 : 2). Scanning the chromatogram revealed a major sharp peak of radioactivity at R_F 0·57 coinciding with cold normorphine on co-chromatography. A small broad peak with R_F 0·39–0·51 which produced a diazoblue colour reaction (50 mgm. tetrazotized diorthoanisidine dissolved in 15 ml. of 0·2 *M* phosphate buffer *p*H 9 ; purchased from Dajac Laboratories as Naphthanil Blue *B*) and another very small peak at R_F 0·82 also appeared in this chromatogram. The identity of the compound giving the peak of radioactivity at R_F 0·57 was further confirmed by co-chromatography with cold normorphine on Whatman 3 *MM* paper strips buffered at *p*H 8·9. The R_F of the radioactive peak was 0·18, and identical with the R_F obtained by spraying with diazoblue reagent. Some radioactivity also appeared at the origin of this chromatogram and in a small broad band at R_F 0·37–0·49.

Adequate chromatographic controls ensured the absence of normorphine in injected drug and the possibility of chemical artefacts resulting from the extraction procedures.

Direct chromatography of urine from a second group of rats, injected as described previously, on large Whatman 3 *MM* sheets using *n*-butanol, acetic acid and water (4 : 1 : 2) showed two areas of radioactivity, one having broad-band R_F 0·027–0·19 (probably due to conjugated morphine and conjugated metabolites) and another with R_F 0·67–0·72 (probably due to free morphine and free metabolites). The two areas were manipulated separately. Acid eluate from the area having R_F 0·67–0·72 was extracted as before, using cold normorphine as carrier. The residue obtained by the evaporation of the organic phase was dissolved in absolute methanol, chromatographed as described on Whatman 3 *MM* paper strips buffered at *p*H 8·0 and 9·3. Known cold normorphine gave identical R_F values.

Similarly, the area having R_F 0·027–0·19 was eluted, the eluate autoclaved and treated as described to give similar results. Evidence was obtained that normorphine was also present in conjugated form as a metabolite. A detailed report will appear elsewhere.

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Denaturation of Catalase by Freezing and Thawing

THE effects of freezing and thawing on the structural and functional integrity of various biological materials, for example tissue, red blood cells[1], spermatozoa[2] and mitochondria[3], have received increasing attention recently, especially from the point of view of low-temperature storage. At the level of isolated enzyme proteins, investigations[4,5] have been qualitative in nature.

This communication deals with the fundamental aspects of the effects of freezing and thawing on the biochemical activities of fresh ox liver catalase. (Catalase became completely resistant against freezing and thawing denaturation after several months storage in solution (*p*H 7·0) or crystalline states in a refrigerator.) In our experiments, 0·5-ml. samples of *M*/100-buffered catalase solution (0·8 μgm./ml.) in test-tubes (1·0 cm. × 10·0 cm.) were frozen by immersion in a bath set to each required low temperature. After a given time the solution was removed and thawed rapidly in a bath at 25° C. After suitable dilution the remaining activity was determined in *M*/75 phosphate buffer, *p*H 7·0, at 0° C.

Partial inactivation of catalase occurred within 10 min. after immersion and then its activity remained constant during the next few hours. The extent of this inactivation depended on various experimental conditions, such as freezing temperature, enzyme concentration, and *p*H of the enzyme

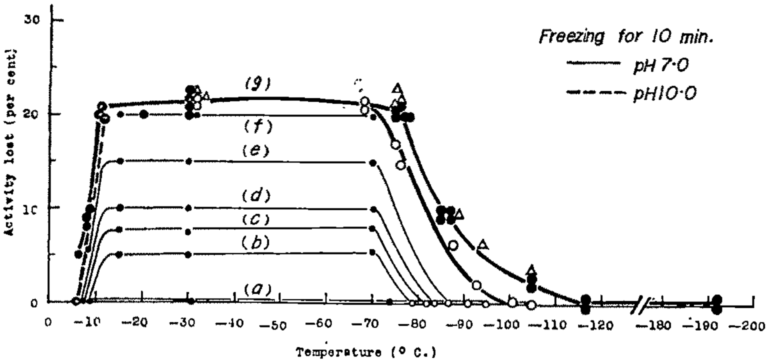

Fig. 1. Activity lost when catalase solutions containing various solutes are frozen. In all experiments the enzyme was frozen in *M*/100 phosphate buffer at *p*H 7·0 (except for *p*H 10·0 indicated by a broken line), for 10 min. by immersion at the temperature indicated, directly (●), or transferred from pre-freezing at −192° C. (○), or by an air jacket (△). Catalase concentration: $8·4 \times 10^{-3}$ mgm. dry weight/ml.
Curve (*a*) illustrates the protective action of 0·01 per cent gelatin; (*b*) 0·5–1·0 *M* glycerol; (*c*) 1 *M* glucose, 0·1 *M* sodium acetate, ammonium sulphate, sodium butylate, sodium caprylate; (*d*) 5×10^{-4} *M* sodium dodecyl sulphate; (*e*) 1 *M* sucrose; 2·5 *M* ethylene glycol; (*f*) 0·01 per cent ovalbumin; (*g*) no additive

solution. Concentrated enzyme solution was more resistant against freezing inactivation. The activity lost – freezing temperature curve (Fig. 1) indicates that there is a temperature-range in which catalase is denatured during freezing and thawing. The inactivation of catalase by freezing commenced at about − 6° C., then reached the 20 per cent level at − 12° C., remained at this level to near − 75° C., and at last was reduced gradually between −75° C. and −120° C.; and over the range from −120° C. to −192° C., catalase solutions retained their activities completely against freezing and thawing.

The upper boundary of this critical region depends on the initial *p*H of freezing solutions. At −6·5° C. no damage occurred at *p*H 10·0, but the increasing damage occurred on either side[6]. At temperatures below −12° C., however, no dependence on *p*H was observed (*p*H range 5–11). The lower boundary, on the other hand, seems to be connected with the cooling-rate. Some shifting of this inactivation curve has been demonstrated under three conditions (Fig. 1); first, test-tubes were immersed directly into low-temperature baths; secondly, they were immersed with air jacket by placing them in larger tubes; and thirdly, they were frozen rapidly to −192° C., and then transferred to the required low-temperature bath.

It is interesting that even if catalase solutions are transferred to −30° C. after pre-freezing at −6·5° C. (*p*H 10·0) or at −192° C. without inactivation, they both suffer 20 per cent inactivation of activity within 10 min., which completely coincides with the level of inactivation by freezing directly in −30° C. bath for 10 min. This strongly suggests that the denaturation of the enzyme by freezing and thawing may be caused by the transition processes of the ice-crystal state responsible for the critical region of temperature, whether from the liquid state or from the frozen state outside the above range.

It was of interest to investigate whether glycerol and ethylene glycol, which have attracted increasing attention recently as 'antifreezer'[1,2], provided protective action against denaturation of catalase by freezing and thawing. In addition, some sugars, inorganic electrolytes, fatty acids (which have been known to inhibit the denaturation of some proteins) and other substances of interest were also tested (Fig. 1).

The results of these observations show the considerable protective action of 1 *M* glycerol, 1 *M* glucose, 0·1 *M* sodium acetate, 0·1 *M* ammonium sulphate, 0·1 *M* sodium butyrate, 0·1 *M* sodium caprylate, and also 5×10^{-4} *M* sodium dodecyl sulphate. Gelatin gave complete protective action over the whole temperature region in a low concentration of 0·01 per cent, but the same concentration of ovalbumin had little effect.

Our present knowledge is insufficient to give an experimental explanation for the existence of our critical region of temperature. However, it is interesting to compare these results with the analogous phenomena which Lovelock[1] reported on the hæmolysis of human red blood cells by freezing and thawing.

We wish to thank Prof. K. Aoki, of this University, for his interest and encouragement.

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Assay of Pyridine Nucleotide Coenzymes and Associated Dehydrogenases with Phenazine Methosulphate

SINCE the original application of phenazine methosulphate to the assay of soluble succinic dehydrogenase by Singer and Kearney[1], this useful reagent has been increasingly employed as a carrier in a number of histochemical techniques[2,3].

Recent announcements of the use of the dye colorimetrically, coupled to 2 : 6-dichlorophenol indophenol, for the assay of certain soluble pyridine nucleotide-linked enzymes[4], and for the oxidative decarboxylation of pyruvate[5], prompts the publication of a manometric assay method, which has been in use in this laboratory for some time, employing phenazine methosulphate to measure di- and triphosphopyridine nucleotide-linked dehydrogenases in connective tissue. As the method described can be used for particulate as well as for soluble dehydrogenases a short account of its use is given here. Full details of theoretical and practical considerations will be reported elsewhere.

It has been found that the aerobic oxidation of reduced pyridine nucleotide coenzymes can be made to proceed stoichiometrically via the autoxidation of phenazine methosulphate in the Warburg apparatus. This is illustrated for the case of reduced

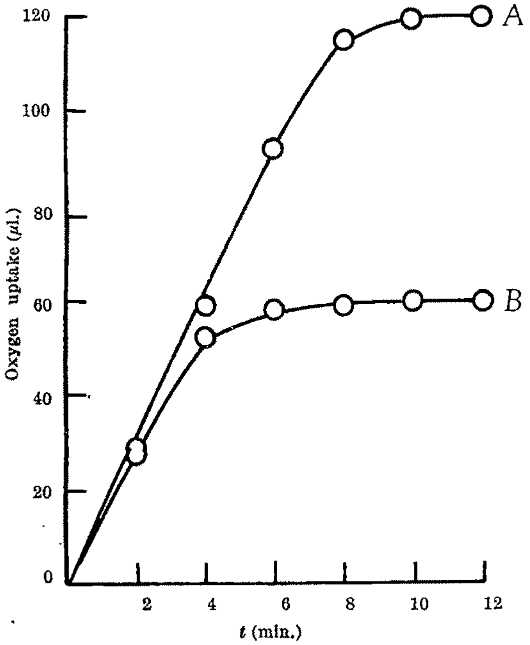

Fig. 1. Oxidation of DPNH by phenazine methosulphate. Warburg flasks contained: 2·45 ml. phosphate buffer, 0·1 *M*, *p*H 7·3; 0·1 ml. phenazine methosulphate, 10 mgm./ml.; 0·25 ml. DPNH (83 per cent by optical assay), in side-arm. *A*, 7 μmoles; *B*, 3·5 μmoles; water to 3·0 ml. Flasks tipped at zero time. Temperature, 20°. Gas phase air

diphosphopyridine nucleotide (DPNH) in Fig. 1. Experimentally, it applies equally to reduced triphosphopyridine nucleotide (TPNH). The amount of reduced coenzyme originally present in a test sample can be ascertained from the total oxygen consumption, and the fact that one mole of reduced coenzyme per mole of oxygen participates in the reaction. Added diaphorase is without effect on either the rate or extent of the oxygen uptake.

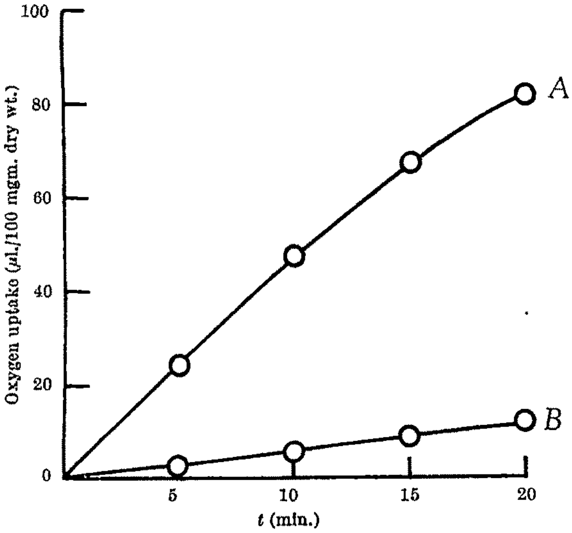

Fig. 2. Glucose 6-phosphate dehydrogenase activity in experimentally treated rabbit synovial tissue pulp. Warburg flasks contained: 1·7 ml. phosphate buffer, 0·1 *M*, *p*H 7·3; 0·1 ml. phenazine methosulphate, 10g mm./ml.; 0·2 ml. D-glucose 6-phosphate (25 m*M*); 0·2 ml. TPN, 15 mgm./ml.; 0·1 ml. cyanide (0·1 *M*); 0·2 ml. 10 per cent potassium hydroxide (in centre well); 0·5 ml. tissue. *A*, Complete system; *B*, minus D-glucose 6-phosphate. Substrate, TPN and phenazine methosulphate in side-arm; tipped after equilibration. Temperature, 37°. Gas phase air

From these and other observations it appears that, in a similar fashion, reduction of pyridine nucleotide coenzyme also can be followed. Dehydrogenase activity of a chosen tissue in the presence of suitable substrate and coenzyme can be compared with the endogenous activity of the same tissue with or without addition of inhibitors. Lately, the dehydrogenases of both pulped and intact connective tissue preparations from the region of joints have been studied in this manner and, to choose one example, Fig. 2 shows the D-glucose 6-phosphate dehydrogenase activity of a pulp-preparation of synovial tissue from experimentally treated rabbits. Cytochrome oxidase and catalase, if present, are inhibited by use of cyanide in the incubation mixture[6]. This extension of the Singer and Kearney method for succinic dehydrogenase to pyridine nucleotide-linked enzymes may therefore find application in situations where, by the nature of the tissue, optical or colorimetric assays are precluded.

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Hormonal Induction of Alkaline Phosphatase in Human Cells in Tissue Culture

During an investigation of differences between established lines of human cells in culture, the alkaline phosphatase content was found to vary from high activity in certain epithelial lines to trace or no detectable activity in other epithelial lines. In a series of six HeLa lines the variation in alkaline phosphatase was as great as among cell lines derived from other tissues and from different species. Among the six lines of uncloned HeLa cells four showed marked 'constitutive' alkaline phosphatase activity and two showed trace or no activity (Table 1).

The human origin of the HeLa lines was established by their poliovirus susceptibility, immunological properties and chromosomal morphology[1].

Table 1. Phosphatase Activity of Six HeLa Cell Lines

HeLa cell line	Alkaline phos-phatase*	Acid phos-phatase*	Source of cultures
MBA	0·50	0·06	Microbiological Associates, Bethesda, Md.†
A_{75}	0·58	0·10	Merck, Sharpe and Dohme, West Point, Pa.
K	0·12	0·08	Dr. M. Kline, Philadelphia, Pa.†
G	0·41	0·09	Dr. H. Ginsberg, Philadelphia, Pa.
Henle	< 0·01	0·10	Dr. W. Henle, Philadelphia, Pa.
NIH	< 0·01	0·08	Miss M. Levy, Bethesda, Maryland†

* Mgm. of phenolphthalein released by a deoxycholate lysate containing two million cells. Incubation with substrate was for 2 hr. (ref. 5) at 37° C.

† Furnished by Dr. L. Coriell, Southern New Jersey Research Institute, Camden, N.J., who had propagated these lines in his laboratory for more than a year.

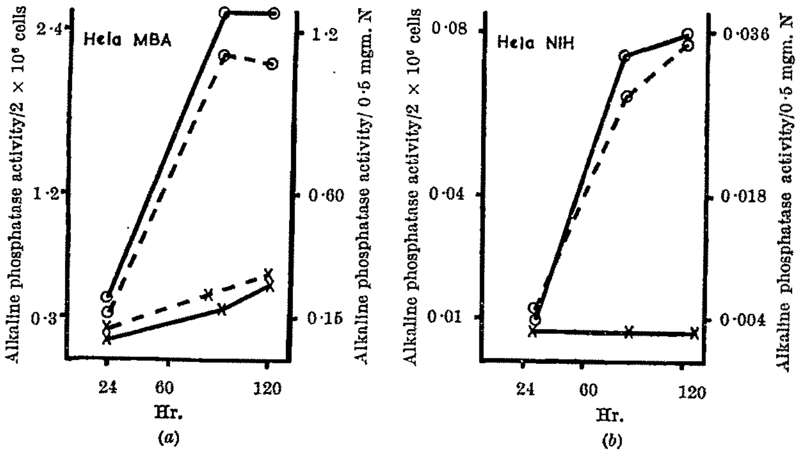

Fig. 1. Alkaline phosphatase activity of cultures grown with and without prednisolone 0·5 γ/ml.: (*a*), HeLa MBA, an example of a strain with 'constitutive' activity rising about 8-fold with prednisolone induction; (*b*), HeLa NIH, an example of a strain without detectable 'constitutive' alkaline phosphatase which develops activity by prednisolone induction. ×, without prednisolone; O, with prednisolone. Solid line, alkaline phosphatase activity per 2 million cells; broken line, alkaline phosphatase activity per 0·5 mgm. nitrogen

Alkaline phosphatase synthesis is known to be of the inducible type in micro-organisms[2], in explants of avian and mammalian embryonic tissue[3], and in human leucocytes *in vivo*[4]. It is of particular interest that adrenal glucocorticoids induce alkaline phosphatase activity in embryonic intestinal epithelium of chicks and mice earlier than it normally appears during development.

Two HeLa lines (Henle and NIH) contain trace or no detectable alkaline phosphatase unless grown in medium containing prednisolone (Δ′ hydrocortisone) 0·5 γ/ml. In the presence of prednisolone these otherwise 'negative' lines developed as much as one-fourth the alkaline phosphatase activity usual in the other four lines. Either prednisolone hemisuccinate or prednisolone phosphate was equally effective as inducer. Most but not all epithelial cell lines with high constitutive alkaline phosphatase show an increase in enzyme when cultivated in medium with added prednisolone. We are preparing a report on the effects of prednisolone on different cell lines.

In addition to inducing alkaline phosphatase, prednisolone has a number of other effects on human epithelial cell lines. It reduces cell multiplication by about 40 per cent; it causes an increase in epithelial cell cytoplasm with almost a doubling of cell volume and a 30–50 per cent increase in cell protein. In epithelial cells grown in the presence of prednisolone, acid phosphatase activity remains constant per unit of cell nitrogen. On the other hand, alkaline phosphatase activity of inducible lines increases markedly.

Table 2. Increase in Alkaline Phosphatase Activity of Cells grown with Prednisolone added

HeLa cell lines	No. of experiments	Cells (2 × 10⁶) grown without prednisolone			Cells (2 × 10⁶) grown with prednisolone		
		Alkaline phosphatase*	Acid phosphatase*	Protein N†	Alkaline phosphatase*	Acid phosphatase*	Protein N†
MBA	7	0·40	0·04	0·10	1·50	0·05	0·13
Henle	6	< 0·01	0·12	0·10	0·05	0·22	0·15
NIH	7	< 0·01	0·08	0·10	0·06	0·15	0·15

* Mgm. of phenolphthalein released by a deoxycholate lysate containing 2 × 10⁶ cells. Incubation was at 37° C. for 2 hr. (ref. 5).

† Total nitrogen in deoxycholate lysates of two million cells. Determined by micro-Kjeldahl.

Within each experiment the cultures grown with and without prednisolone were replicate. Cells grown in medium 199 with 10 per cent human serum.

Cells were free of contamination with pleuropneumonia-like organisms and no latent viruses could be demonstrated.

Table 2 presents the result of experiments on three HeLa lines. One of these, MBA, has constitutive alkaline phosphatase and the two others, Henle and NIH, have trace or no detectable activity unless grown in medium with added prednisolone. Within each experiment all cultures were replicate and were handled similarly except for the addition of prednisolone 0·5 γ/ml. to the experimental cultures. As shown in Table 2, alkaline phosphatase activity is increased in cultures grown in medium with prednisolone whereas acid phosphatase is not significantly altered when expressed on the basis of cell nitrogen.

Fig. 1*a* shows the results of a representative experiment with HeLa MBA, which possesses constitutive alkaline phosphatase. Alkaline phosphatase activity is expressed on the basis of two million cells (solid line and left-hand ordinate) and also on the basis of cell nitrogen (broken line and right-hand ordinate). The cells grown in medium containing prednisolone 0·5 γ/ml. have marked elevation of alkaline phosphatase activity when compared to a replicate culture grown in the absence of the hormone. Fig. 1*b* shows an experiment with HeLa NIH, a cell line with no detectable alkaline phosphatase when grown in the absence of prednisolone. In fact, alkaline phosphatase activity of lysates prepared from six times the usual concentration of cells was below the sensitivity of our method[5] (0·01 mgm. of phenolphthalein released in 2 hr. at 37° C.). When grown in medium containing prednisolone alkaline phosphatase activity is easily detected in Hela NIH.

Unlike bacterial alkaline phosphatase[2] the mammalian cell enzyme is independent of the phosphate ion concentration in the culture medium. Reduction of the phosphate medium to 0·04 m*M* does not enhance the alkaline phosphatase content in cell lines that contain the enzyme nor does it induce alkaline phosphatase in the prednisolone inducible 'negative' HeLa cells. Increase of phosphate ion concentration of the medium to 5·0 m*M* does not repress prednisolone induction of alkaline phosphatase nor does it alter the enzyme activity of cells with constitutive alkaline phosphatase. Under conditions of de-adaptation, the alkaline phosphatase activity of prednisolone-induced HeLa cells is diluted by cell multiplication.

Alkaline phosphatase may be induced also in certain cultured cell lines by high concentrations of organic monophosphates (greater than 200 γ/ml.[6]).

In summary, a hydrocortisone analogue, prednisolone, can induce alkaline phosphatase activity in certain epithelial cell lines in culture. Acid phosphatase is not significantly altered. Induction is independent of phosphate ion concentration. Under conditions of de-adaptation the induced enzyme appears stable and is diluted by cell multiplication.

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Intracellular Free Amino-Acids of Certain Dermatophytes

THE identification and classification of the dermatophytes is dependent on macroscopic and microscopic morphological characteristics developed during growth on standard media. The formulation of biochemical tests to assist in identification would be of considerable value. The work of Mattick *et al.*[1], which showed that extracting cells of various species of *Lactobacilli* with 10 per cent acetic acid removed a mixture of amino-acids and peptides from the cells which was characteristic for each species and constant for strains within a species, suggested the application of a similar technique for the identification of dermatophyte species.

This communication records the findings for representative strains of *Trichophyton*, *Epidermophyton* and *Microsporum* species. The results show the presence in such extracts of a pattern of free amino-acids which is very similar for all organisms examined and the absence of any distinctive peptide components. It was the latter that Mattick *et al.* found of greatest taxonomic value in the *Lactobacilli*. Thus the results appear to show the pattern of cytoplasmic amino-acids in these fungi, which is borne out by the fact that other extractants, for example, 75 per cent ethanol, dimethylformamide and boiling water, known to extract the cytoplasmic contents from bacteria and fungi[2-5], have given extracts containing the same amino-acids.

It is well recognized that the patterns of the free amino-acids present in the cytoplasm of closely related micro-organisms show considerable similarity. Thus the technique is not of value in identification of the organisms; but the results are recorded as affording an interesting comparison with similar findings in *Aspergillus oryzae*[6], *Aspergillus flavus*[7], *Penicillium chrysogenum*[8] and *Neurospora crassa*[9], and as a preliminary to a more detailed examination of the amino-acid metabolism of these fungi.

The organisms were grown on a synthetic medium with DL-glutamic acid as nitrogen source. Strains of *Microsporum canis* were grown for 8–10 days at room temperature, whereas those of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton verrucosum* var. *discoides* and *Epidermophyton floccosum* were grown for 11–14 days at 30° C. The mycelium was collected on a sintered glass funnel and washed three times with a large volume of distilled water. The last washing was concentrated and examined by paper chromatography to ensure that carry-over of glutamic acid from the medium was minimal. The mycelium was dried so far as possible by suction, weighed and then extracted by immersion in 10 per cent (v/v) acetic acid (5 ml./gm. mycelium) for 4 hr. at room temperature. The extracts were examined for amino-acids by two-dimensional paper chromatography based on the method of Levy and Chung[10]. Whatman No. 4 chromatography paper (22 × 22 in.) was irrigated in one direction by the organic phase of butanol–acetic acid–water (4 : 1 : 5 by volume), then dried and, except for the strip containing the partially separated amino-acids, sprayed with a borate buffer of *p*H 8·3 (prepared from 300 ml. of 0·1 *M* boric acid and 60 ml. of 0·1 *N* sodium hydroxide) and again dried. The solvent system for the second dimension was prepared by mixing 30 gm. of *m*-cresol, 15 gm. phenol and 7·5 ml. of the borate buffer. The amino-acids were detected by spraying the dried chromatograms with the modified ninhydrin spray of Mattick *et al.*, drying at 40° C. for about 15 min. and then heating at 105° C. for 5 min. Hydrolysis of extracts was effected by heating with 6 *N* hydrochloric acid for 18 hr. at 120° C.

The principal amino-acids found and the frequency of their occurrence in the extracts are given in Table 1. In addition to the amino-acids shown the following were occasionally detected as faint spots on the chromatograms: cystine, lysine, asparagine, valine/methionine, glycine, leucine/*iso*leucine and threonine.

Table 1. INTRACELLULAR FREE AMINO-ACIDS OF DERMATOPHYTES (Figures refer to the number of times each amino-acid occurred)

	E. floccosum	*M. canis*	*T. rubrum*	*T. mentagrophytes*	*T. verrucosum* var. *discoides*
No. of strains examined	7	6	6	1	1
Glutamic acid	7	6	6	1	1
Glutamine	7	6	6	1	1
Aspartic acid	7	6	6	—	1
Alanine	7	4	6	1	1
Serine	4	6	6	—	1
Proline	5	6	5	—	1

Growth medium: 130 mgm. DL-glutamic acid, 36 mgm. potassium dihydrogen phosphate, 20 mgm. dipotassium hydrogen phosphate, 200 mgm. D-glucose, 20 mgm. crystalline magnesium sulphate, 140 μgm. crystalline zinc sulphate, 100 μgm. crystalline calcium chloride, 1 mgm. thiamine hydrochloride, 1 mgm. *i*-inositol and 100 μgm. biotin dissolved in 20 ml. glass-distilled water and brought to *p*H 6·8. The inoculum was 1·0 ml. of a homogenate of mycelium grown on Sabouraud's broth.

Hydrolysis eliminated the glutamine spot and led to the intensification of a number of spots by the hydrolysis of peptides which did not move in the solvent systems used; however, no amino-acids were found on hydrolysis which were not also present in the free state in the original extract.

These results show a similarity to those found in the other fungi cited here in that the major components of the intracellular free amino-acids are aspartic and glutamic acids, glutamine, alanine, serine and proline (except in *N. crassa*). A major difference appears to be that the basic amino-acids are absent or only present in minor amounts in the dermatophytes, whereas one or more of them are present as major components in the other fungi listed. Glycine was also present in *A. flavus*, *A. oryzae* and *P. chrysogenvm* but was only seen occasionally in the dermatophyte extracts.

We wish to thank Dr. Jaqueline Walker and Dr. C. J. La Touche for strains of the organisms used in this work.

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A New Fatty Acid from Royal Jelly

IT has been shown by Butler, Callow and Johnston[1] that queen honey-bees (*Apis mellifera*) secrete material which affects bees in two ways, inhibiting the development of ovaries in workers and influencing their behaviour by inhibiting queen rearing. Callow and Johnston[2] have determined that the active principle in the material is 9-oxodec-2-enoic acid. It is referred to as 'queen substance'.

The close structural relationship between this 'queen substance' and 'royal jelly acid', 10-hydroxydec-2-enoic, found abundantly in royal jelly, would lead one to assume that the latter is a precursor of the former. Moreover, it would seem reasonable to predict that 9-hydroxydec-2-enoic acid might be an intermediate, and possibly occur in small amounts in royal jelly.

We observed that the carboxylic acid mixture, isolated from royal jelly by the procedure described by Brown and Freure[3], gives a weak iodoform reaction but no test for the carbonyl function with 2,4-dinitrophenylhydrazine. Oxidation of this acid mixture with aqueous chromic acid gave a product which, on treatment with aqueous 2,4-dinitrophenylhydrazine reagent, yielded a small amount of a 2,4-dinitrophenylhydrazone. This derivative has an infra-red absorption spectrum identical with that of the 2,4-dinitrophenylhydrazone of 9-oxodec-2-enoic acid.

Thus it appears that 9-hydroxydec-2-enoic acid is present in royal jelly in minute amounts. A more detailed account of the investigation will be published later.

Authentic samples of 9-oxodec-2-enoic acid and its 2,4-dinitrophenylhydrazone were supplied by Dr. R. K. Callow and Miss Norah C. Johnston, for which we are grateful.

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PHYSIOLOGY

Oscillatory Flow in a Fluid-filled Elastic System

THERE is a pronounced tendency for a fluid-filled elastic tube to oscillate when the fluid is caused to flow by a small head of pressure. This may be demonstrated with a model (Fig. 1) which stems from a new theory of the action of the heart, published by me[1].

Water flows from the tap into a reservoir where a constant level is maintained, then down a rigid tube connected at the lower end to a rubber tube lying horizontally on a small platform. The distal end of the rubber tube is connected to a short length of rigid tubing, through the other end of which fluid is allowed to escape freely into the sink. Provision is made to vary the height of the reservoir and of the outlet.

Arranged parallel to the platform is a metal bar on which three springs are mounted which can be moved at will. These are of mild steel, twisted around the bar and secured at one end by a clamp. The other end of each is drawn out into a straight extension that presses against the rubber tube, with a force equivalent to a weight of 50 gm. approximately, the tension being adjusted by twisting the spiral part of the spring with the clamp.

Latex tubing is employed (0·7 cm. in diameter) with a volume pressure curve almost linear up to a pressure of 150 cm. of water. A suitable length, to start with, is 97 cm., the reservoir being set 1 m. above, and the outflow 4 cm. below, the level of the platform.

The position and pressure of the springs are adjusted until maximum oscillation occurs, when each is found to be at a critical distance from the distal end of the tube. A water hammer now develops, associated with momentary interruption and even regurgitation of flow. A sharp sound is heard at each pulse,

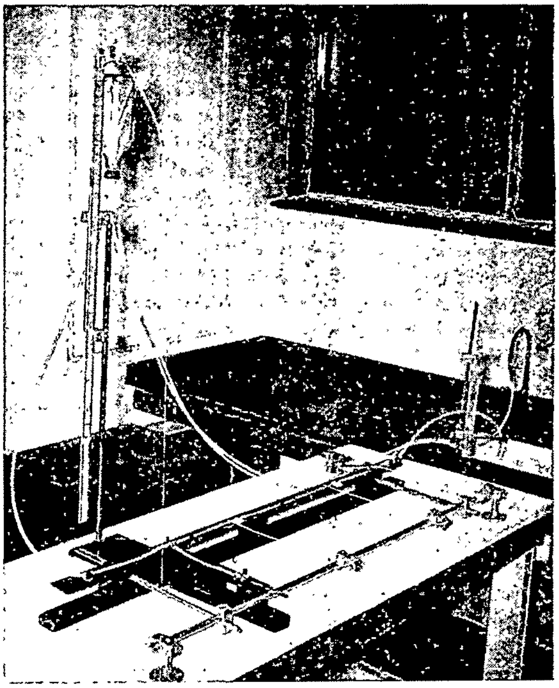

Fig. 1. The oscillator

audible at a distance of half a metre when the springs are correctly arranged. To facilitate the adjustment, the sound is detected with the aid of a microphone which is seen to the right of the model, at the back. Sounds are generated at the site of each spring but the loudest occur at the site of the distal end.

Oscillation is explained as follows: Viscous drag of the fluid in contact with the elastic wall produces waves which are reflected from the distal end of the tube: flow being slowed by the distal spring, stationary waves develop upstream where the flow velocity corresponds to the wave-length.

The stationary waves are of two types, long of some 75 cm. and short of about 1·5 cm., corresponding to gravity and capillary waves respectively. Current and pressure become 90 deg. out of phase in a manner similar to that of the current and voltage when stationary waves arise in an electrical transmission line, as a result of reflexion from a short-circuited end. The two proximal springs locate adjacent antinodes of the system of longer waves, giving the half wave-length. The corresponding quarter wave-length is given by the distance between the middle and distal springs; but, owing to the development of short waves at the distal end, the distal spring determines the site of a pseudo-stationary node about 1·5 cm. from the end of the tube.

When stationary wave motion is established, either of the two proximal springs, by altering the cross-sectional area of the tube, produces velocity modulation of the stream. The distal spring affects density modulation and, resonating, gains energy from the stream. Part of the energy is fed back as a wave which drives the proximal spring.

By altering the height of the reservoir and that of the outlet, differing frequencies of oscillation are obtained, as also by regulation of the pressure of the individual springs. In the case of the distal spring the closer the point of application to the end of the tube, the higher the frequency and conversely. Harmonics occur, a variety of modes of oscillation and occasionally a mode jump.

A 'T'-piece is introduced into the proximal segment of the elastic tube at the site of a node, the side branch communicating with a further piece of rubber tubing 20 cm. in length, the other end of which is closed with a piston. Adjustment of the position of the piston enables the device to be used as a quarter-wave matching stub, to suppress stationary waves at the inflow which otherwise interfere with the motion of the proximal spring.

The model, which will oscillate when filled with blood instead of water, demonstrates that stationary waves, in the centimetre range, readily arise in fluid-filled elastic tubes at physiological pressure. That oscillation is obtained from a stream maintained in flow by a pressure, initially constant, raises the question as to whether the peripheral pulse is developed from the static pressure of the blood in the great vessels and not from the heart wave.

Further work on this is proceeding.

Thanks are due to the Postgraduate Medical School of London, in particular to Prof. Ian Aird and Mr. W. J. Dempster, for research facilities in the Experimental Surgical Unit, also to Dr. D. G. Melrose for loan of equipment.

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Response of Thymus to Increase of Intake of Sodium Chloride

It is a well-known fact that the metabolism of the body fluids and electrolytes is closely connected with all metabolic processes in tissues. I have examined the significance of the changes in sodium balance for the activity of the lymphatic tissue of the thymus.

Adrenalectomized rats were used (Wistar, males. average weight of each group 190–222 gm.): (1) treated with deoxycorticosterone acetate (DCA), 2 mgm. intramuscularly per rat daily, and supplied with tap water; (2) treated with the same doses of DCA and supplied with isotonic saline solution. Treatment with DCA started immediately after adrenalectomy and later after 24 and 48 hr. respectively. 72 hr. after adrenalectomy the rats were killed by decapitation. The thymus was weighed and the lymphocytes of thymus were counted. At the same time a part of muscle (m. quadriceps femoris) was removed for electrolyte analysis. The specimens of the muscle were dried, defatted, and the potassium and sodium content was determined by flame photometry and expressed in milleqnivalents of ions per 100 gm. of fat-free dried material.

The analysis of the electrolyte concentration of the muscle has shown that the animals of the group treated with DCA and supplied with tap water maintain a relative balance in electrolytic proportions (intact control rats: potassium, 46·0 m.equiv./100 gm.; sodium, 10·5 m.equiv./100 gm.). Animals treated with DCA and saline have shown an increased retention of sodium which is demonstrated by a lower concentration of potassium and a corresponding increase of sodium in muscle. It is a well-known fact that sodium chloride stimulates the excretion of potassium in animals treated with DCA (ref. 1).

Table 1 shows that the animals treated with DCA and saline have an enlarged thymus. An increase in the average weight of the thymus is conditioned by an increase in the number of cells. The mean weight of the thymus of animals treated only with DCA does not differ from the mean weight of the thymus of intact control animals of the same body-weight (*n*-13, mean thymus weight: 180 mgm.).

Table 1

Treatment	Mean body-weight (gm.)	Mean thymus weight (mgm.)*	No. of cells $\times 10^6$*	Muscle electrolytes (m.equiv./100 gm.) Na	K	Na/K*
DCA	192 (10)†	177	576	12·1	44·9	0·26
DCA + NaCl	199 (21)	241	872	13·3	41·0	0·31

* Statistical significant differences between both groups ($P < 0·01$).
† In parentheses, number of rats used.

The experiments have shown that the thymus of adrenalectomized animals reacts to stimuli produced by an imbalance in the electrolytes of the internal environment (increase of sodium intake) by intensified activity. The mechanism of this action is not clear. We may assume both direct and indirect influence. It is known that the maintenance of ion concentration gradients requires metabolic energy. The hyperfunction of the thymus tissue may perhaps be an expression of increased metabolic efforts to maintain the homeostasis of the internal environment. My results indicate that the electrolyte metabolism has an important role in the regulation of the growth of thymolymphatic tissue.

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Determination of Physiological Rhythms of Unrestrained Animals by Radio Telemetry

The results of 24-hr. rhythm measurements are frequently influenced by perturbations due to the presence of an observer and the measuring equipment. These perturbations can be minimized if the desired physiological data are obtained by radio telemetry[1,2]. The transmitter carried by the animal should be characterized by small size, long battery life, and sufficient range for unrestrained movements. Several transmitters with these design goals were constructed successfully and used to relay temperature and heart-rate information to a distant observer.

The transmitters contain a basic oscillator which is frequency-modulated by a voltage proportional to the phenomenon measured. For a description of the design, see ref. 3. The frequency of each temperature transmitter is controlled in part by the intrinsic dependence on temperature of the transistor in the oscillator and also by a voltage derived from a thermistor half-bridge circuit. The transmitter is built around the antenna, which is a ferrite core. The life of the 75 MAH mercury cell in this circuit exceeds 200 days. The frequency of the E.K.G. transmitter is controlled by amplified potentials derived from the heart. Additional circuitry is needed for amplification ; this increases power consumption and reduces battery life to about forty days. Units with more batteries have a longer life. Both the temperature and the E.K.G. units are suitable for surgical implantation provided a non-toxic covering is used. In these experiments temperature transmitters were hermetically sealed in cases machined from nylon rod. The temperature-sensitive elements were covered with the tip from a finger of a latex surgical glove, although they could be designed to protrude into a portion of a nylon shell. Seals between the latex finger tip and the nylon case were made successfully by wrapping and tying with nylon suture. No toxic reactions were noted when a unit prepared in this manner was implanted in a dog. Ref. 3 describes the pretreatment required before implantation.

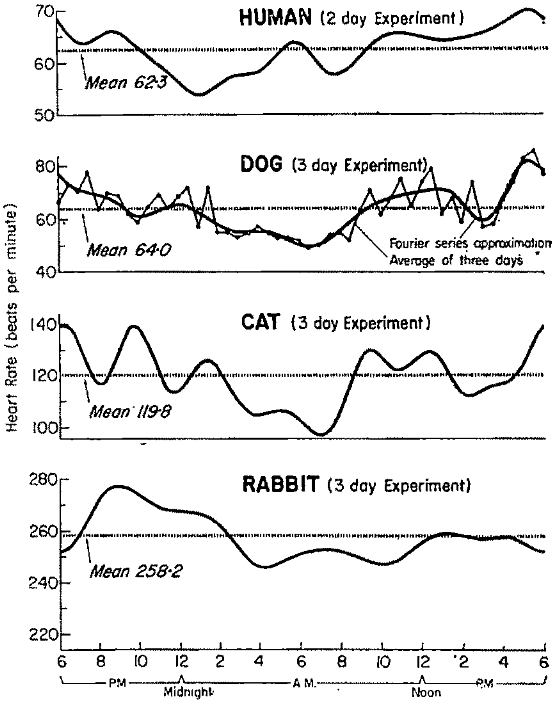

Fig. 1. 24-hr. periodicity in heart-rates of man and three laboratory animals. A Fourier series approximation was used for curve smoothing. Original data for the dog are included

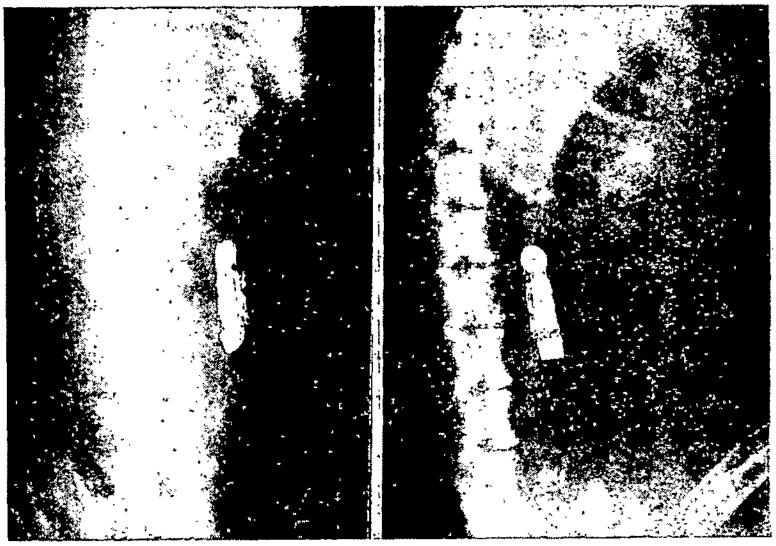

Fig. 2. Dorsal-ventral (left) and right to left lateral (right) roentgenographs showing position of the temperature transmitter in the dog

In a series of short experiments 24-hr. rhythms (circadian rhythms) of heart-rates for a dog, a cat and a rabbit were obtained. In each case the animal was trained to wear a harness on which the transmitter was mounted. The input leads to the transmitter were connected to tantalum electrodes which were implanted surgically weeks in advance. The electrodes were put in subdermally over the right scapula and on the dorsal mid-line near the first lumbar vertebra, a position favouring the detection of heart-potentials. Fourier analysis of the data was made in order that the 24-hr. periodicity could be expressed quantitatively in the form :

$$F(t) = C_0 [1 + C_1 \sin(15t + \theta_1) + C_2 \sin(30t + \theta_2) + \\ \ldots + C_n \sin(15t + \theta_n)]$$

One may think of this Fourier series as a curve of best fit for the given frequency components. The Fourier series approximation, which is uniquely applicable to repetitive phenomena, is analogous to the *n*th order polynomial of best fit frequently used in statistical analysis. The 3-day average heart-rate of a dog is shown in addition to the Fourier series approximation (Fig. 1). In each analysis the average value (C_0) plus eight frequency components were used. Note that the centre of the period of lowest heart-rates for man occurs at 3 a.m., and this time progresses to 5 a.m. for the dog, 6 a.m. for the cat, and 8 a.m. for the rabbit. These experiments were conducted when the human subject and the experimental animals were isolated in an insulated, quiet chamber with a 12-hr. light cycle (6 a.m.–6 p.m.). Data were recorded outside the chamber from signals picked up by an antenna.

Simultaneous body temperatures and heart-rates of the dog were also recorded to study the interdependence of these variables. The technique for measuring heart-rates in this experiment is as described, but with the animal wearing a harness. A temperature transmitter was put in retroperitoneally immediately caudal to the right kidney (Fig. 2). A 12-day recovery period was allowed before the experiment was begun. The resulting data (Fig. 3) indicate a close correlation between the two variables.

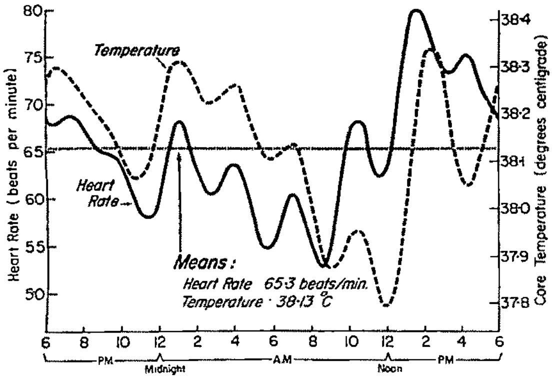

Fig. 3. Fourier series approximation of 24-hr. periodicity in core temperatures and heart-rates of a dog. The original data were obtained during a 24-hr. experiment

The data presented here support previous concepts of the day–night heart-rate characteristics of human beings, dogs, cats and rabbits. In addition, a very close correlation has been shown to exist between heart-rate and core temperature in the dog. These pilot experiments, which tested the radio telemetry technique for measuring temperature and heart-rate over extended periods of time, justify additional experiments of longer duration involving more animals.

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Effect of Dexamethasone on the Phagocytic Activity of the Reticulo-Endothelial System

It has been reported previously[1] that cortisone, prednisone and prednisolone are strong depressants of the reticulo-endothelial system. The mechanism by which cortisone lowers the body defences has been shown by Nicol *et al.*[2] to be due to depression of the phagocytic activity of the reticulo-endothelial system, the total and differential white cell counts in the blood, and the gamma globulin-level in the serum. It has also been shown that after cortisone treatment is stopped, the depressant action of the cortisone can be reversed[3] and converted into one of moderate stimulation by diethylstilbœstrol. However, if cortisone and diethylstilbœstrol are administered separately, but at the same time, the depressant action of cortisone is prevented and considerable stimulation of phagocytic activity occurs.

The present research was carried out to ascertain the effect of dexamethasone phosphate on the phagocytic activity of the reticulo-endothelial system and compare it with the effects of cortisone acetate, prednisone and prednisolone previously reported[1].

The present experiments were made on 20 male white mice (*T.O.* Swiss strain) of 20–25 gm. body-weight. Ten of the animals each received 0·5 mgm. dexamethasone phosphate in aqueous solution subcutaneously, once daily, for 6 days. On the eighth day after the commencement of the treatment, the phagocytic activity was measured by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood[4]; the procedure which we used is that described in previous communications[1,3] and the total body phagocytic activity or phagocytic index is denoted by the symbol *K*. Ten control animals received no treatment and in these the phagocytic activity was also measured by the carbon method.

After the assessment of the phagocytic activity, blood counts were made in all the animals, which were then killed and the weights of the liver and spleen determined.

The results show that in the mouse the phagocytic index in the control animals ($K = 20$) is depressed by dexamethasone ($K = 9$), compared with cortisone ($K = 7$), prednisone ($K = 8$) and prednisolone ($K = 8$)[1]. In the animals treated with dexamethasone, no change was found in the weights of the liver or spleen; and in the blood, the total red cell count was normal, the circulating lymphocytes were reduced from a control-level of 5,000 to 1,900 per cu. mm., the polymorphs increased from 1,400 to 2,500 per cu. mm. and the monocytes remained unaltered in number. Reduction in the number of lymphocytes was also found in guinea pigs treated with cortisone[5], but during the first week of treatment the polymorphs became reduced in number, then increased during the second week.

The therapeutic dose of dexamethasone is stated to be 5–7 times less than that of prednisone or prednisolone; and clinically, it is 28 times more potent than hydrocortisone and about 35 times more potent than cortisone.

The present results show that dexamethasone phosphate, like cortisone acetate, prednisone and prednisolone, is a strong depressant of phagocytic activity, but, although it is much more potent clinically than cortisone, its depressant effect on the reticulo-endothelial system is of no greater magnitude than that of the other corticosteroids at present in use.

In these researches we gratefully acknowledge a gift of dexamethasone from Messrs. Merck, Sharp and Dohme and financial assistance from the Medical Research Council.

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Effect of Hæmophagin on the Phagocytic Activity of the Reticulo-Endothelial System

Hæmophagin is produced at the Austrian Institute for Hæmoderivatives, Vienna, by a special catalytic method from the blood of normal donors. It is stated to increase antibody formation against various bacterial antigens and also to cause increased phagocytosis; and it is given intramuscularly or intravenously when there is danger of infection after operation or injury.

It is generally acknowledged that the reticulo-endothelial system is an important defence of the body against infection, and extensive researches on the functions of the reticulo-endothelial system have recently been reported. We have previously shown[1-3] that the active œstrogens are strong stimulants of the reticulo-endothelial phagocytes; and unpublished results in our laboratory also show that when the reticulo-endothelial system of the guinea pig is stimulated the serum gamma-globulin is raised to about twice the normal level, and, in addition, both mice and guinea pigs are protected against virulent infections. It therefore seems rational that when antibacterial substances are being used to combat infection the reticulo-endothelial system should at the same time be stimulated to raise the body defence. The œstrogens may be used for this purpose but most of them have undesirable side-effects. In view of this, extensive research is being conducted to find, if possible, a strong reticulo-endothelial stimulant without œstrogenic effects. In these circumstances, we have been interested in testing the claimed action of hæmophagin by our experimental methods.

Fifty male white mice (*T.O.* Swiss strain) of 20–25 gm. body-weight were used for this investigation. Ten of the animals each received 1/40 of the human dose of hæmophagin once daily intramuscularly for 6 days and a second group of ten received half the dose for the same period. On the eighth day the phagocytic activity was measured in each animal by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood[4], the procedure used being that described in previous communications[2,3] and the total body phagocytic activity or phagocytic index being denoted by the symbol *K*. Thirty of the animals were used as controls. Ten control animals received no treatment; ten received diethylstilbœstrol 0·1 mgm. once daily subcutaneously for 6 days; and ten received tri-*p*-anisylchloroethylene 1·0 mgm. once daily orally for 6 days. The phagocytic activity was also measured in the control groups by the carbon method, which was carried out on the eighth day in those treated with diethylstilbœstrol and tri-*p*-anisylchloroethylene. After the assessment of phagocytic activity blood counts were made in all the animals; they were then killed and the weights of the liver and spleen determined.

The results were as follows: In the untreated control animals the total phagocytic index (*K* value) was 16 ± 2, while in the control groups given diethylstilbœstrol and tri-*p*-anisylchloroethylene the *K* values were 75 and 83 respectively. In the animals which received a daily dose of 1/40 of the human dose of hæmophagin the *K* value was 19 ± 2 and in those which received a daily dose of 1/80 of the human dose the *K* value was 13 ± 1.

In the blood of the hæmophagin-treated animals no change was found in the total red cell count. When the dose of hæmophagin was 1/40 of the human dose, the total white cell count increased from a control level of 8,000 to 11,000 per cu.mm., the increase being due to increase in the number of circulating lymphocytes from 6,000 to 9,000 per cu.mm.; when the dose of hæmophagin was 1/80 of the human dose the total white cell count remained within normal limits.

The above results suggest that hæmophagin has little or no effect on the phagocytic activity of the reticulo-endothelial system of mice and that its action may be related to its influence on the white cells of the blood.

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Incorporation of Palmitic-1-^{14}C Acid into Blood Cells

It has been shown that Ehrlich mouse ascites tumour cells *in vitro* readily absorb palmitic acid and use it as an energy source and for synthesis of other lipids[1]. Although this has not been demonstrated for blood cells, it has been reported by several investigators that these cells are able to synthesize lipids from acetate[2-5].

The present investigation concerns the incorporation of palmitic acid-1-^{14}C into the lipids of blood cells during incubation.

To 150 ml. of fresh human blood was added 37·5 ml. of A.C.D. solution (1·32 per cent sodium citrate, 0·48 per cent hydrous citric acid, 1·47 per cent dextrose). After centrifugation at 900*g* for 15 min., 50 ml. of the plasma was removed and shaken for 5 min. with 9·5 ml. of potassium palmitate-1-^{14}C solution. (This solution contained 3 mgm. of palmitic acid-1-^{14}C dissolved in 9·5 ml. of 0·01 *M* potassium hydroxide with a total radioactivity of 35,112 c.p.s.) The plasma-palmitate mixture was added to the blood cells and remainder of the plasma. Fifteen-ml. samples of this suspension were then incubated with slight shaking at 37° C. in an atmosphere of 5 per cent carbon dioxide–air and in the presence of 1,000 units per sample of both penicillin and streptomycin. Some of the incubations were carried out in the presence of 72γ of œstrone or 200γ of ethylenediamine tetraacetic acid (added as the sodium salt) per ml. of suspension. After 8 hr. the cells were separated and freed from the plasma and the lipids of both were extracted by the method of Delsal[6].

Total radioactivity was measured in a flow counter (Nuclear Instruments and Chemical Corporation *D*-47 Micromil Flow Counter). Total lipids were separated by silicic acid chromatography into six fractions (cholesterol esters, triglycerides, cholesterol, diglycerides, monoglycerides and phospholipids) using a modification (1, 5, 10 and 25 per cent ether in petroleum ether, ether and methanol) of the method described previously[1-7].

The first 3 fractions were purified by passage through a short alumina column to remove free fatty acids[7] and the radioactivity of each fraction was measured by the direct plating technique of Entenman *et al.*[8].

Under the conditions of the present experiment, at least 10 per cent of the total radioactivity of added palmitic acid was recovered in the blood cells. The radioactivity was found largely in the triglyceride, cholesterol and phospholipid fractions.

In general the glyceride fractions had the highest specific activity although the greatest percentage (more than one-half) of the total activity was in the phospholipid fraction. No significant amount of radioactivity was found in the cholesterol ester fractions. Some evidence was obtained indicating that in the presence of ethylenediamine tetraacetic acid and œstrone the distribution of the radioactivity among the different lipids was altered.

These results will be discussed in greater detail in a subsequent communication.

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Effect of Castration on Experimental Liver Lesion in *Iguana iguana*

To gain an impression of the development of the hormones produced by the ovary in the liver of *Iguana iguana* in 30 castrated and 30 mature females a liver lesion was effected by means of the carbon tetrachloride method of Kaufmann. Afterwards the animals were observed for 70 days, the deaths occurring during this period being recorded. Just as in the rat[1,2], deaths began immediately in the mature group, most animals succumbing in the third week.

In the group of the castrated animals no losses were suffered in the first few weeks. Deaths occurred not before the fourth week with a maximum in the fifth week. In a period of 70 days, 24 of the 30 mature animals died and only 10 of the 30 castrated animals. This significant difference between the death-rates of the two groups suggests that castration is capable of exerting a protective action in the experimental liver lesion and that the hormones provided by the ovary reach development more poorly in the mature than in the castrated animals. This is also suggested by observations that in the case of an equal degree of liver lesion the castrated animals tolerate better the load of experimentally administered ovarian extract and evidently the injected animals develop more rapidly than the mature animals.

These results are in good agreement with the data obtained in the rat[1,2], and therefore have a certain general significance. Obviously, the no longer normal liver is seriously damaged by the hormones produced by the ovary, so that the mature animals perish more rapidly and in larger numbers after liver lesions. When the source of these hormones is eliminated by castration, the liver can to a considerable extent be relieved, through which the progress of the disease is prevented.

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HÆMATOLOGY

Absence of Abnormal Hæmoglobins in Colombian Tunebo Indians

AN examination by paper electrophoresis has been carried out of hæmoglobin from 100 Tunebo Indians from the Santa Librada Mission located at San Luis de Chuscal (Department of Santander, Colombia). This tribe, whom historical and anthropological references consider to be a branch of the large Chibcha Indian group, consists of approximately 2,000 inhabitants spread over 22 small communities along the Rivers Suisiga, Cobaría and Cobugón, tributaries of the River Arauca, situated on the eastern slopes of the Sierra Nevada de Cocuy, between lat. 6° 15′ and 7° 10′ N., and long. 71° 50′ and 72° 20′ W. All the individuals studied were adults, apparently normal. Both sexes were proportionally represented and the blood was drawn only from subjects not closely related.

The hæmoglobin solution was prepared by Drabkin's method[1] without the use of aluminium chloride. Paper electrophoresis was carried out in a tank permitting the use of two sheets of 46·5 cm. × 21·0 cm. filter paper, resting on two glass rods, on an inclined plane at 45° with the anode facing the higher end. The size of the sheets facilitated comparing the relative migration of seven different samples. This method is somewhat similar to that used by Gordon *et al.*[2] for serum proteins.

All the samples examined showed a single spot with the same mobility as hæmoglobin A, indicating that no abnormal hæmoglobin could be detected by this method in the Tunebo Indians.

This work, which is apparently the first of its kind ever conducted on a Colombian population, confirms previous assumptions[3] that either American Indians lack abnormal hæmoglobins, or the frequency of occurrence of such hæmoglobins is very low. In spite of the relatively few subjects examined, who represent but 5 per cent of the whole tribe, the results obtained seem to indicate that either the stimuli which in other continents are presumed to have led to the production of abnormal hæmoglobins have not been operative in this Mongoloid branch on the American continent, or the whole Indian stock has enough 'resistance' not to undergo these mutations.

It is interesting to note, in this respect, that the ecological conditions in which these Indians live still permit a high incidence of malaria. By combining the results obtained in the present work with results found for Venezuelan Indians inhabiting malarial zones[4], it may be concluded that although the sickle-cell trait confers a certain resistance to malaria, as shown by Allison, the mutation leading to the production of hæmoglobin S is apparently not produced by malaria. As a matter of fact, this phenomenon has not yet been observed in pure South American Indians.

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Bohr Effect in the Hæmoglobins of Marine Mammals

VERTEBRATE blood hæmoglobins discharge protons on oxygenation. This is part of the Bohr effect[1]. Recent work[2,3] has shown that in hæmoglobins from a variety of terrestrial mammals ranging in size from mouse to elephant the smaller the animal the greater is the sensitivity of its hæmoglobin to changes in pH. Thus elephant hæmoglobin discharges only about 1·5 protons during oxygenation while mouse hæmoglobin discharges about 3·8. An approximate inverse linear relation exists between the number of protons discharged by the hæmoglobin during oxygenation and the logarithm of the body-weight of the animal from which the hæmoglobin comes: one additional proton discharged corresponds to a decrease in body-weight by a factor of about 300. The extrapolation of this relationship to the weights of large whales suggested that whales might possess hæmoglobins with either a zero or small Bohr effect.

I have now examined the hæmoglobins of the humpback whale (*Megaptera nodosa*), pilot whale (*Globicephala scommonie*), and harbour porpoise (*Phocaena phocaena*), and find that the relation between the Bohr effect and body-weight existing among terrestrial mammals does not appear, so far, to extend to marine forms.

All blood samples were treated immediately with 0·1 vol. of 0·5 per cent sodium citrate and placed on ice. The preparation of the hæmoglobins was identical with that previously described[3]. The washed cells were lysed with distilled water and the hæmolysate then dialysed versus glass-distilled water for 15 hr. Cellular debris was removed by high-speed centrifugation. The oxygen equilibrium was determined as previously described in 0·1 M potassium phosphate buffer at various pH values between 7·0 and 7·6 at 35° C.[3]. The hæmoglobin concentrations were $1·4 \times 10^{-3}$ M (whale), $0·9 \times 10^{-3}$ M (porpoise), and $0·6 \times 10^{-3}$ M (pilot whale) on the basis of oxygen capacity measurement and assuming 4 molecules of oxygen per mole hæmoglobin. All measurements were made within 72 hr. of the original drawing of the blood.

The results of these measurements are given in Table 1. The porpoise and pilot whale have hæmoglobins with identical Bohr effects, about 0·52, expressed as $\frac{\Delta \log P_{50}}{\Delta p\text{H}}$, although porpoise hæmoglobin has an over-all oxygen affinity 38 per cent lower than pilot whale hæmoglobin. In contrast, humpback whale hæmoglobin possesses a large Bohr effect, $\frac{\Delta \log P_{50}}{\Delta p\text{H}}$ being about 0·82. Both the Bohr effect and the oxygen affinity are almost identical with those previously found for guinea pig hæmoglobin[3].

Large whales are known to be able to remain submerged for at least 1 hr.[4]. It appears that the large Bohr effect may be a homeostatic molecular adaptation facilitating diving for long periods. It would augment the physiological adaptations already known: the tolerance of high carbon dioxide concentration, the large blood volume, the high hæmoglobin concentration, the high concentration of myoglobin in the muscles, and low lung volume[4,5]. A large Bohr effect would enable the blood to absorb more carbon dioxide with a smaller drop in blood pH than would otherwise be possible. Similarly, a large Bohr effect should facilitate the discharge of the accumulated carbon dioxide when the animal surfaces.

The lower Bohr effect in pilot whale and porpoise hæmoglobins presumably reflects their predominantly surface behaviour. Although the humpback whale is also a surface feeder, it can dive for much

Table 1. ALL EXPERIMENTS PERFORMED AT 35° C. IN 0·1 M PHOSPHATE BUFFER

Animal	pH	log P_{50} (in mm. mercury PO_2)	$\frac{\Delta \log P_{50}}{\Delta p\text{H}}$	Estimated weight (kgm.)
Porpoise	7·03 7·32	1·382 1·231	0·52	90
Pilot whale	7·08 7·39	1·220 1·058	0·52	680
Humpback whale	7·08 7·42	1·395 1·115	0·82	41,000

longer periods than either the porpoise or the pilot whale. Observations by Dr. Kenneth S. Norris (private communication) indicate that the average diving time of pilot whales is less than 3 min., and seldom more than 5 min. Similarly, the harbour porpoise seldom dives for more than a few minutes.

In summary, the humpback whale, known to be capable of long dives, has a hæmoglobin with a large Bohr effect, while the pilot whale and harbour porpoise apparently do not dive for as long periods and have blood hæmoglobins with much smaller identical Bohr effects.

Humpback whale hæmoglobin was obtained from an adult male, estimated weight 41,000 kgm., within 12 hr. after the death of the animal. Pilot whale blood was obtained by syringe from the counter-current vessels of the flukes of a 12-ft. male weighing about 680 kgm. Porpoise blood was obtained by syringe immediately after catching a harpooned animal weighing about 90 kgm.

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Leptospira-agglutinating Factors in Turtle Sera

IN our epidemiological research on the carriership of pathogenic *Leptospira* among wild animals in Israel, 58 swamp turtles (*Clemmys caspica*) of various ages originating from twelve different regions of the country were investigated for the presence of agglutinins. Serum tests were carried out against 13 serotypes of *Leptospira* (*L. icterohaemorrhagiae, canicola, grippotyphosa, pomona, hebdomadis, sejroe, australis A, autumnalis Rachmat, bataviae, hyos, ballum, poi* and *mini Szwajizak*). With 11 of these strains no reaction was observed. However, all sera showed highly significant titres in the agglutination test with *L. ballum*, both the 'complete' (Castellon) strain and the 'incomplete' (*S* 102) strain. (Three sera reacted with titre 250, two with titre 500, seven with titre 1,000, fourteen with titre 2,000, fourteen with titre 4,000, twelve with titre 8,000, three with titre 16,000 and four with titre 32,000.) Besides agglutinins, lysins were also demonstrated. In fifteen sera low titres were also found for *L. hyos* (eight up to 50, five up to 100, three up to 250), the remaining sera showing no agglutination with this serotype. Negative results were obtained too with *L. icterohaemorragiae* 'incompleta' and *L. sarmini*, which have some antigenical relationship with *L. ballum*.

None of the turtle sera agglutinated four out of five saprophytic water *Leptospira* strains (*L. biflexa*, provided by Dr. C. D. Cox, Vermillion, S.D.), and *L. Sao Paulo, L. Patoc I, L. Wa-Z* and *L. Wa-Leiden*, all provided by Prof. J. W. Wolff, Amsterdam).

A fifth strain of water *Leptospira, L. biflexa-Gent* (from Prof. Wolff), however, was agglutinated to approximately the same titres as *L. ballum* by all *Clemmys* sera examined (one in dilution 1 : 250, five 1 : 500, four 1 : 1,000, seven 1 : 2,000, six 1 : 4,000, four 1 : 8,000, six 1 : 16,000 and two 1 : 32,000).

Leptospira could not be isolated in direct culture from the liver, spleen and kidneys of the turtles, nor from the organs of hamsters intraperitoneally injected with a mixture of drained *Clemmys* organs.

It is remarkable that without exception the sera of *Clemmys* (of very different ages and at least partly from widely removed regions of the country) showed practically the same high agglutination-level with one of the strains of water *Leptospira* (*L. biflexa-Gent*) as with *L. ballum*, although these serotypes have no antigen in common. This aroused some doubt as to whether the serum reactions of the turtles depended on the classical antigen–antibody phenomenon.

The following investigations were intended to throw more light on the active agent in the sera of turtles compared with agglutinating antibodies provoked as a result of active immunization with the serotype strains concerned.

The globulins were separated from our *Clemmys caspica* sera, by ammonium sulphate precipitation, then washed and dialysed. Quantitatively the total amount of the agglutinating factor was contained in the globulin fraction of the sera.

A culture of *L. ballum* failed to be agglutinated in the presence of *Clemmys* sera and of sera of rabbits immunized against *L. ballum*, when the antigen was washed and finally suspended and the sera were diluted in distilled water. Consecutive addition, to the negative tests, of saline to a final concentration of 0·9 per cent brought about immediate reaction up to the original agglutination-level of the sera. This result indicated the necessity of the presence of electrolyte for this reaction.

The influence of high temperature on the agglutinating agent was investigated in twelve sera of *Clemmys caspica* and three sera of rabbits immunized against *L. ballum*. The critical temperature at which the turtle and rabbit sera lost their agglutinating property was 68°–69° C.

After freezing and consecutive thawing of turtle sera, the concentration of the agglutinating factor on the bottom of the tubes was about 20-fold that of the supernatant layer.

The 'agglutinins' were totally absorbed from *Clemmys* sera by addition of an adequate amount of *Leptospira* of the ballum serotype. The 'incomplete' serotype (*S* 102) deprived turtle sera of their agglutinating factor for the 'complete' (Castellon) strain as well as for the incomplete serotype. Hence the active factor in turtle sera reacts as an agglutinin of the 'incomplete' *ballum* serotype.

Turtle sera with low *hyos*-titre, absorbed by *L. ballum*, lost their agglutinating power for both serotypes. Absorption by *L. hyos*, however, did not change the titre for *L. ballum*.

Preliminary investigations have been carried out in order to determine whether there are other properties in addition to agglutinating and lytic factors which prevent infection by *L. ballum* in *Clemmys* sera.

Intraperitoneal infection in white mice with a mixture of two cultures of *L. ballum* resulted in prolonged presence of the organism in the kidneys, and its excretion in the urine of all the animals. No *Leptospira* were, however, found in mice that had been injected with the same culture to which a small amount of *Clemmys* serum was added.

These results strongly suggest that *Clemmys* sera contain immune bodies against the *Leptospira* mentioned. It appears that the capability of *Clemmys caspica* to produce *Leptospira*-agglutinating antibodies is very limited. Two turtles showed a titre as low as 50, and a third one reached only 1 : 100 after repeated injections of large quantities of *L. grippotyphosa* culture into its body cavity.

It seems desirable to investigate a possible relationship between the agglutination of *L. ballum* and *L. biflexa-Gent* in turtle sera.

After absorption with *L. ballum* (strain Castellon or *S* 102), the 'agglutinins' disappeared, not only for the *ballum* strains but also for the heterologous *biflexa-Gent* strain. Absorption with *L. biflexa-Gent* similarly eliminated the agglutinating power for both strains. This unexpected result is remarkable in the light of our experience with sera of rabbits, guinea pigs and land tortoises (*Testudo graeca*), which were immunized separately by repeated injection with *L. ballum* or *L. biflexa-Gent*. These immune sera contained agglutinins only for the strains injected, and absorption with the heterologous strain did not significantly affect the serum titre for the homologous serotype.

Sera of hedgehogs naturally infected with *L. ballum* and showing significant titres with this serotype revealed no agglutinins for *L. biflexa-Gent*.

It would thus seem that the agglutinating factor in *Clemmys* sera is based on a principle different from that of the immune antibodies. The origin and character of this factor and the mutual relationship between the *ballum* and *biflexa-Gent* agglutination are as yet obscure. Contrary to the positive results with the sera of *Clemmys caspica*, no agglutination was observed in blood samples of local land tortoises (*Testudo graeca*), soft water turtles (*Trionyx triunguis*) and water snakes (*Natrix tessellata*).

Similar results have been recorded recently by Combiesco *et al.*[1]. Six land tortoises did not reveal *Leptospira* antibodies, but ten out of eleven swamp turtles (*Emys orbicularis*) reacted strongly with *L. mitis* (probably syn. *L. hyos*). *Leptospira* were not isolated from the reacting animals. Injection of a virulent *L. icterohaemorrhagiae* culture produced only little agglutinin (titre 80–320).

Further investigation is necessary to determine the origin and nature of the agglutinating and protecting factors present in certain water turtles. As a working hypothesis, we assume that saprophytic water *Leptospira* play a part in the genesis of these factors.

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PATHOLOGY

Detection of Serum Proteins within the Renal Collecting Tubule Cells of the Potassium-deficient Rat

In potassium-deficient rats characteristic granules appear within the cytoplasm of the collecting tubule cells; the origin and nature of the granules have not been established. By labelling the serum proteins with the dye *T*-1824 it has been examined whether or not they might be made up, at least in part, of serum protein entering from the tubular lumen.

Solutions of *T*-1824, kindly presented by the Warner-Chilcott Laboratories, New York, were injected into the tail veins of twelve 200-gm. male Wistar rats (5 mgm./100 gm. body-weight of the rat) that had been made potassium-deficient by feeding for three weeks on a diet deficient in potassium. Twelve control rats on a similar diet adequately supplemented with potassium were also injected with dye. Rats from the experimental and control groups were killed 1, 2, 8, and 14 days after injection, and unstained frozen sections of the kidneys were examined microscopically. In the potassium-deficient rats killed 8 and 14 days after dye injection the collecting tubule cells contained blue granules ; no such coloured granules were seen in this location in either the control animals or in the potassium-deficient rats killed 1 and 2 days after injection.

T-1824 is firmly bound to serum proteins under many varying conditions[1-4], and the blue granules in the collecting tubules are presumed to contain dye-tagged serum protein. Since the granules containing the dye were mostly at the side of the tubular cells nearest the lumen it seems probable that the dye-protein complex entered the cell from that direction. This conclusion is supported by the absence of coloration in the granules in rats killed 1 and 2 days after dye injection, as more prompt coloration might be expected to occur after *T*-1824 injection were the peritubular capillaries the source of the dye-protein complex.

It is known that when *T*-1824 is injected into normal rats it accumulates in droplet form within the proximal convoluted tubule cells of the kidney[1,2] ; this has been related to the normal process of reabsorption of serum protein by the convoluted tubule cells[2,3]. There was a diminution in the number and intensity of *T*-1824 stained material in the convoluted tubules of all the potassium-deficient animals examined when compared with the controls ; this suggests that the normal process of protein reabsorption by the convoluted tubule cells may be decreased in potassium-deficient rats. The attractiveness of this explanation is enhanced by the appearance of blue-coloured protein in the urines of the potassium-deficient animals injected with *T*-1824, while the urinary protein of the injected controls was uncoloured. In addition, in a separate experiment, it was demonstrated that potassium-deficient rats had a greater proteinuria than normal rats. This was done by measuring the urinary protein, precipitated by the method of Shevky and Stafford[5], in four male Wistar rats before and after potassium depletion ; a two- to three-fold increase was found in the rate of protein excretion after the deficiency was established (Table 1).

It is concluded that potassium-deficient rats possess collecting tubule cells so altered by the deficiency as to permit the movement of serum protein from the

Table 1. URINARY PROTEIN EXCRETION-RATE OF RATS BEFORE AND AFTER POTASSIUM DEFICIENCY

	During control period (mgm./hr.)*		During potassium deficiency (mgm./hr.)*		
	Day *A*	Day *B*	Day 13	Day 20	Day 27
Rat 1	0·29	0·42	1·70	1·00	0·88
Rat 2	0·09	0·22	0·54	0·99	0·45
Rat 3	0·33	0·43	1·13	0·83	0·70
Rat 4	0·59	0·32	1·20	0·83	0·73
Means	0·33	0·35	1·14	0·91	0·69

* All urine specimens analysed were 24-hr. collections.

tubular lumen into the cells, where it appears in the form of granules in the cytoplasm. This work supports the view[6,7] that the cytoplasmic granulations of the collecting tubules in potassium-deficient rats are not simply distorted mitochondria as has been claimed[8,9]. Potassium-deficiency also leads to a greater degree of proteinuria in the rat. This may result from a decrease in the proximal convoluted tubule reabsorption of protein, from changes in the glomerular filtration-rate, or from changes in the integrity of the glomerular filter.

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Variant of Parotid Tumour (Polyoma) Virus showing a Change in Oncogenic Properties

THE striking differences, as reported by several laboratories, in the types and frequencies of lesions and neoplasms produced in the same inbred strains of mice by parotid tumour virus indicate the existence of different variants. Close antigenic relationships, however, have been established for many of the different 'strains' of virus.

In our laboratory a thymotropic strain of virus was isolated from a *C3Hf/Bi* mouse with a tumour. This strain has been propagated serially by passage in a tissue-culture cell-line (*P*–388 *D*1) derived from a lymphocytic neoplasm of *DBA/*2 origin. The origins of the tissue culture cell line and of the virus have been described[1,2]. The biological properties of the virus-strain have been reported in detail[2–4]. The tumour-inducing capacity of the virus, propagated by passage in *P*–388 *D*1, has been evaluated through the fifteenth passage material. This passage represents a total effective theoretical dilution from the starting material of approximately 1×10^{-45}. Throughout this period, this strain of virus has been strikingly virulent in its oncogenic properties, and it induced in *C3Hf/Bi*.$(C3H \times AKR)F_1$ and $(C3H \times AKR)F_2$ mice nearly 100 per cent thymic epithelial tumours as early as sixteen days after neonatal injection. Many other primary sites besides the thymus have been involved in tumour induction. Parotid, submaxillary and sub-lingual salivary glands have been affected, as well as the lacrimal and thyroid glands and glands within the submucosal tissues of the trachea, oropharynx and nares. In addition, a lesion of the renal cortex, producing an alteration of the tubular epithelium[5], regularly results from the inoculation of this strain of virus[3].

At the time this polyoma virus strain was first introduced into *P*–388 *D*1 cell cultures grown in a medium containing 40 per cent human serum[3], the virus was also introduced into *P*–388 *D*1 cultures which had been adapted to grow in a serum-free medium containing non-fat milk[6]. Cytopathic effects in tissue culture, and induction of multiple sarcomatous and angiomatous lesions in hamsters and *Mastomys*, have been described for this virus strain after multiple passages in milk medium[7–9].

The tumour-producing capacity of polyoma virus passaged in *P*–388 *D*1 cells in a serum medium was compared with tumour-inducing capacity of virus after passage in the serum-free milk medium. Virus was collected from the combined fourteenth and fifteenth consecutive passages of each tissue-culture cell-line. Subcutaneous inoculations of 0·05 ml. into $(C3H \times AKR)F_1$ and $(C3H \times AKR)F_2$ mice less than 72 hr. of age were performed. Recipient mice were born of mothers having hæmagglutination-inhibiting antibody titres of < 100. Under these conditions antibodies[4] would not interfere with tumour-inducing capacities.

Polyoma passaged in the 30–40 per cent human serum medium has consistently shown a low titre as measured by hæmagglutination, tissue-culture infectivity and mouse-antibody production[10] determinations, but has maintained its high tumour-inducing capacity. The amount of $TCID_{50}$/ml. of the fourteenth-fifteenth passaged pool of *S* virus was $1 \times 10^{4\cdot9}$ and of the *M* virus, $1 \times 10^{6\cdot1}$; infectivity tests were run in parallel using *P*–388 *D*1 cells in a medium supplemented with 5 per cent calf serum. The number of hæmagglutination units/ml. for serum and serum-free milk media was 100 and 2,500, respectively.

The comparative tumour-inducing effects are shown in Table 1 in mice observed over a 10–14-month period. In addition to the striking reduction in frequency of tumours induced by virus propagated in *P*–388 *D*1 cells grown in the milk medium, attenuation of tumour-inducing capacity was also indicated by the decreased number of primary tissue sites involved in tumour formation. Of the 2 mice which developed pleomorphic parotid gland tumours, one had a small thymic epithelial tumour; the other had parotid gland involvement only. In only one of the three mice with thymic epithelial tumours were any other primary sites of tumour formation found. In this mouse, small areas in the parotid gland tissue were altered. Of 14 mice which have come to autopsy in milk the medium group, only two showed definite renal lesion. There appeared to be, however, no attenuation of the oncogenic activity for hamsters of the polyoma virus passaged in *P*–388 *D*1 cells in milk medium. Four separate pools of virus, ranging in $TCID_{50}$/ml. from $10^{5\cdot2}$ to $10^{7\cdot2}$, induced characteristic tumours in hamsters as follows: 16 in 16, 5 in 6, 5 in 6 and 4 in 10 animals inoculated

Table 1. TUMOUR-INDUCING CAPACITY IN MICE OF PAROTID TUMOUR (POLYOMA) VIRUS OF TWO STRAINS HAVING A COMMON ORIGIN

Virus in medium	Dose of virus/mouse $TCID_{50}$	No. of litters from hæmag. inhib.-negative mothers	No. of mice	No. of tumours*		Latent period in months (range)
				Thymus	Parotid gland	
Serum (14th–15th passages)	$1 \times 10^{3\cdot 7}$	5	43	28	8	3·3 (1½–6)
				(83·7 per cent)		
Milk (14th–15th passages)	$1 \times 10^{4\cdot 0}$	12	72	3	2	4·8 (1½–6)
				(6·9 per cent)		

* Sites of first appearance of tumours, grossly.

shortly after birth and subjected to autopsy afterwards.

The results reported here in which polyoma virus showed attenuation of tumour-inducing capacity for mice without obvious changes in tumour-inducing capacity for hamsters or infectivity for tissue culture and mouse tissues (antibody-production tests) are in contrast with the results reported by Sachs and Medina[11]. These authors observed attenuation of a strain of polyoma virus in its tumour-inducing capacities for both hamsters and mice without loss of cytopathic effect in tissue cultures.

Our findings may indicate selection of a genetic variant with little oncogenic activity in mice. They could also be explained on the basis of some change in protein coat of the virus. A third possible explanation is that a large amount of non-oncogenic virus is produced in the infected cells in milk-medium in association with a small amount of virus with oncogenic properties similar to those of virus grown in serum-containing medium. Inoculation of newly born mice with such a mixture might produce a rapid immune response and hence interfere with induction of tumours.

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RADIOBIOLOGY

Changes in Density of Blood Serum after Total-Body Irradiation

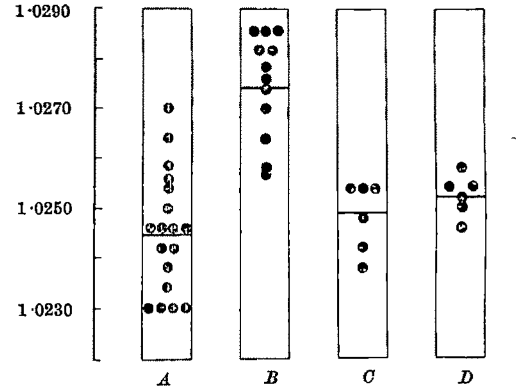

Fig. 1. Abscissa, density of blood serum; ordinate, col. *A*, control animals; col. *B*, animals irradiated by 600 r.; col. *C*, scorched rats; col. *D*, rats deprived of drink

IT is known that the ionizing radiation causes changes in some physical–chemical properties in living organisms[1,2]. It is assumed that changes of the physical-chemical properties have an important bearing on the development of the post-irradiation syndrome. We have followed the changes in the density of blood serum.

The blood serum was obtained by decapitating rats and centrifuging their blood. Serum density was determined by measuring 0·25 ml. volume by means of a microburette manipulated with a micrometric screw and by weighing on an analytical balance. The microburette was kept at 20° C. The volume of the serum pressed out from the microburette was always calibrated by redistilled water.

The rats were irradiated by X-ray therapeutic Meta apparatus at 200 kV., 10 m.amp., 4 mm. aluminium filter, from a distance of 75 cm. in a rotating low wooden cylinder in which the animals were placed in individual boxes. The average radiation beam was 13·5 r./min.

Scorching was effected by putting a 4-cm.^{2} hot metal sheet on the back of the animals for 15 sec.

We examined the blood serum density in groups of six white laboratory rats, Wistar strain, of both sexes. The animals were fed on standard diet.

The results obtained in three control groups are indicated in Fig. 1, col. *A*. In Fig. 1, col. *B*, are the results obtained in two groups of rats irradiated with 600 r. The rats were killed 6 hr. after irradiation. In col. *C* are the results of the scorched-rat groups 6 hr. after scorching and in col. *D* representing the group of animals that were refused drink for 48 hr. before killing. In these experiments the density of the serum was deducted after previous 24 hr. incubation, at 4° C.

The convenient period of incubation was verified by measurement of the serum density after different

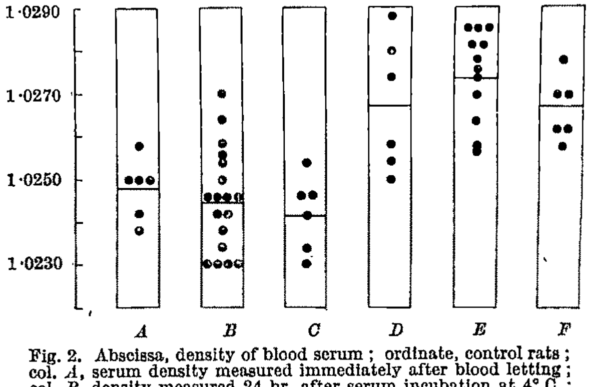

Fig. 2. Abscissa, density of blood serum; ordinate, control rats; col. *A*, serum density measured immediately after blood letting; col. *B*, density measured 24 hr. after serum incubation at 4° C.; col. *C*, density measured 48 hr. after serum incubation at 4° C.; irradiated rats dosed 600 r.

periods of storing, at different temperatures. While the stored serum in a thermostat, at 37° C., appeared to be inconvenient, incubation at 4° C. always yielded statistically significant results between the controlled and the irradiated animals.

In Fig. 2 are indicated the values of serum densities in three control groups after immediate decapitation of the animals (col. *A*), after 24-hr. incubation of the serum at 4° C. (col. *B*), and after 48 hr. incubation at 4° C. (col. *C*). In cols. *D, E, F* are values obtained in respect of three groups of rats after 600 r. irradiation, where the measurements were carried out under the same conditions as those of the control groups.

From these experiments it follows that after the total-body irradiation the blood serum increases in density. In the irradiated rats, in contradistinction to the controlled, as well as to the stressed animals, the differences are statistically significant. On the other hand the difference between the controlled and the stressed groups is not statistically significant.

We are examining the cause affecting post-irradiation increase in blood serum density. We believe that the cause may be due to the post-irradiation of nucleic acid katabolites because it is well known that these, especially deoxyribonucleic acid, are highly radiosensitive and they decrease in organs after irradiation[3,4]. Nucleosides and their precursors are represented in blood even under physiological conditions[5]. After irradiation an agglomeration of some of them occurs in a more considerable measure in blood and in urine excreted as in the case with deoxycitidine[6].

It is also possible to imagine that there is a correlation between sudden decrease of leucocytes after irradiation and the increase in serum density.

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Hæmagglutinin Production in Pre-immunized Mice exposed to Continuous γ-Radiation

Benjamin and Sluka[1] first showed that X-radiation in sub-lethal doses could depress precipitin titres in rabbits when the radiation was given just before administration of the antigen, but that when the radiation was given after the antigen the precipitin titres were not depressed to the same extent. Since that time many workers have confirmed these observations (for review, see Taliaferro and Taliaferro[2]). With smaller doses of acute whole-body radiation, the results have been more conflicting, some workers claiming that antibody production could even be enhanced by small doses of X-rays. Very few, if any, observations have been made on the effect of continuous, as opposed to intermittent, radiation on antibody production.

In this preliminary report of some observations on the effect of continuous γ-radiation on pre-immunized mice it is shown that, despite the accumulation of a large dose of radiation, hæmagglutinin titres are maintained at normal levels during the period of radiation and at higher levels for a period of time after radiation.

Thirty-two Strong *A* male mice 3–4 months old were divided into 2 groups of 16 mice each and each group further subdivided into 4 boxes of 4 mice each. All 32 mice were then given a single intraperitoneal injection of 0·1 ml. of packed, washed sheep red cells as a primary immunizing dose of antigen. Fourteen days later, all the mice were weighed and then bled from the tail to a maximum of 0·25 ml. of blood.

The serum was separated from each individual sample and hæmagglutinin titration of the serum was carried out in 'Perspex' trays with doubling dilutions of the serum in saline against equal volumes of a 3 per cent suspension of washed sheep red cells. After 60 min. incubation at 37·5° C. the end points were read under the low power of the microscope. Ten days after the first bleeding, the 16 mice to be irradiated were then placed in aluminium boxes in a special unit containing a central source of 6·7 c. cæsium-137 so that they were receiving a continuous daily radiation dose of 84 rad/day. A short description of this unit has been published elsewhere[3].

At weekly intervals, 2 mice were removed at random from the unit and, after weighing, were bled from the tail under anæsthesia for serum and then killed. Two control mice were bled in the same way each time a pair of radiated mice was removed from the unit.

After 31 days in the unit, during which time the remaining mice had accumulated a total of 2,604 rads, these mice were then removed. Pairs of mice from this group together with pairs of control mice were bled from the tail and killed at intervals for a further month.

Fig. 1 shows the change with time of the hæmagglutinin titres for both the irradiated and control groups. In all cases the change is expressed as the difference in logarithms of the titre at the time of killing and that determined for the same animal 14 days after the primary antigenic stimulus of sheep red cells and prior to the start of continuous radiation (in the case of the irradiated group).

During the period of radiation, although the mean increase in the titres of the irradiated group is higher than that of the control group, this difference is not statistically significant. However, in the post-irradia-

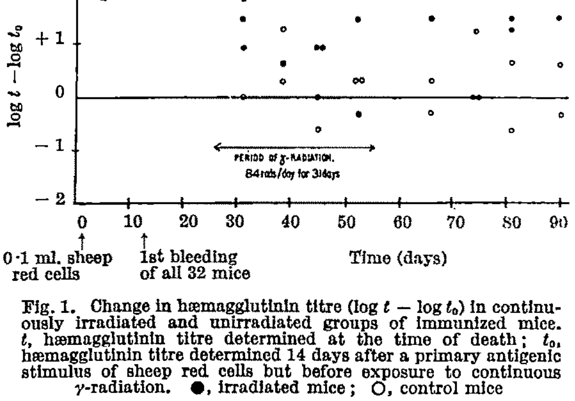

Fig. 1. Change in hæmagglutinin titre (log t − log t_0) in continuously irradiated and unirradiated groups of immunized mice. t, hæmagglutinin titre determined at the time of death; t_0, hæmagglutinin titre determined 14 days after a primary antigenic stimulus of sheep red cells but before exposure to continuous γ-radiation. ●, irradiated mice; ○, control mice

tion period there is quite clearly a statistically significant difference at the 5 per cent level between the two groups.

From the results of the present experiment, the time of onset of this difference between the two groups is not readily apparent. However, the results of a small preliminary experiment in which mice were immunized with sheep red cells and exposed to radiation in the same way, but in which the sera were obtained from consecutive bleedings of the same animal, indicated that this relative increase in hæmagglutinin titre in the irradiated group could be demonstrated towards the end of the period of radiation and persisted well into the post-irradiation period.

The maintenance of normal hæmagglutinin titres during continuous irradiation indicates that antibody-producing mechanisms are not seriously disturbed despite the accumulation of relatively large doses of radiation, and the fact that antibody titres are sometimes higher than before radiation suggests that active antibody production is still in progress and that it is not merely preformed antibody-levels which are being measured.

It is unlikely that the maintenance of antibody-levels is merely a reflexion of the destruction of large numbers of lymphoid cells, with the consequent liberation of antibody as was suggested by Dougherty, Chase and White[4], following the injection of pituitary adrenotrophic hormone, since during continuous irradiation the peripheral white blood count falls to low levels within a few days at this dose-rate, and remains low throughout the period of radiation and for some time afterwards. Moreover, Marshall and White[5] failed to detect increases in circulating antibody following the destruction of lymphocytes by nitrogen mustard.

It seems likely, therefore, that some other relatively radioresistant cell belonging to the reticulo-endothelial system is responsible for antibody production.

Hill[6] has described the development of increasing pyrinophilia of the reticular cells of lymphoid tissue and the development of immature plasma cells following doses of 500 r. whole-body radiation. Nossal[7] has also observed marked plasma cell proliferation in rats following single doses of 500 r. whole-body radiation and considers that the plasma cell is more radioresistant than the lymphocyte. Preliminary histological studies of the spleen and lymph nodes in the present experiment suggest that plasma cell proliferation is also taking place in these continuously irradiated mice. These observations and their relationship to antibody production will be reported in more detail elsewhere.

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BIOLOGY

Incorporation of Tritium-labelled Thymidine in *Bufo* ♀ × *Rana temporaria* ♂ Hybrid Embryos

IT is well known that cross-fertilization can often be performed in amphibians; development, in anurans, often does not proceed further than the late blastula or early gastrula stage. The reasons why this block in development occurs are not yet well known. I hoped that an autoradiographic study of the incorporation of nucleic acid and protein precursors into lethal hybrids might help in elucidating the problem. The precursor used in this work is tritium-labelled thymidine. B. C. Moore[1] has shown that this precursor, if injected into female *Rana pipiens* before ovulation, is incorporated, at the morula stage, in the nuclei of normal *Rana pipiens* and of androgenetic *pipiens* embryos. Thymidine is also incorporated into the deoxyribonucleic acid of the lethal diploid hybrids *pipiens* ♀ × *sylvatica* ♂.

The incorporation of labelled precursors into amphibian embryos is limited by the presence of the surface coat which renders the embryo nearly impermeable to a large number of substances. In order to rule out this permeability problem, we have worked on embryos the coat of which had been disintegrated in a medium devoid of calcium ions.

The embryos at the 2-cells stage were incubated in the following medium: sodium chloride (17 per cent) 20 ml.; potassium chloride (0·5 per cent) 10 ml.; water 966 ml.; *tris* 560 mgm.; *N* hydrochloric acid 4 ml.; sodium citrate 5 gm. Tritium-labelled thymidine was added at a concentration of 5×10^{-6} *M* (2·5 mc.). After a 17-hr. incubation, the embryos were fixed by freeze-substitution[2] and the incorporation was studied by the autoradiography technique of A. Ficq[3].

In the lethal combination studied in the present work, the embryos stopped development at the late blastula stage. The incorporation was investigated at the time of the arrest of development and 24 hr. later. The controls were at the gastrula and at the neurula stage respectively. In these experiments, I have followed Barth's[4] directions concerning the dissociation of the cells and the culture medium. The embryos, deprived of the vitelline membrane, were first incubated in the dissociating medium; after dissociation, they were placed in the culture medium containing labelled thymidine. The cells were fixed, after a 90-min. or a 24-hr. incubation, with a mixture of acetic acid and alcohol (10/90 v/v) and put through the autoradiography procedure.

Thymidine was found to be incorporated into the nuclei, and nearly all the radioactivity could be removed by deoxyribonuclease (0·1 mgm./ml. in *tris* buffer *p*H 7·5 containing Mg^{2+} *M*/300—1 hr. at 37°); on the other hand, there was no diminution of radioactivity following a ribonuclease treatment (0·2 mgm./ml. in *tris* buffer *p*H 7·5 containing Mg^{2+} *M*/300 —1 hr. at 37°). In the arrested blastulæ, we have observed the cytological abnormalities already described by Brachet[5]; in several nuclei the chromatin sticks to the nuclear membrane, where it forms a thin layer; the radioactivity is associated with this area. Sometimes, labelled chromatin is found in the cytoplasm. Finally, the nuclei often consist of two hemikaryons which can be distinguished by their staining properties towards methyl green and pyronine ; in this case, there is a good correspondence between methyl green staining and the number of silver grains in the emulsion.

Labelling of deoxyribonucleic acid could thus be observed in morula as well as in blastula cells of the lethal hybrids. Moreover, 24 hr. after development stopped, tritium-labelled thymidine was still incorporated into deoxyribonucleic acid, a fact which suggests that the block in development is not due to the arrest of deoxyribonucleic acid synthesis. Such a conclusion has already been reached by Løvtrup and Gregg[6], for the similar *Rana pipiens* ♀ × *Rana sylvatica* ♂ combination, on the basis of biochemical measurements of deoxyribonucleic acid synthesis.

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Chloride-secreting Cells in the Gills of European Eels

In a recent communication Parry, Holliday and Blaxter[1] reopened the question of the chloride-secreting cells of the gills of fish and, in agreement with Bevelander's[2] arguments, they brought new indirect evidence against Keys and Willmer's theory[3] and Copeland's[4] conclusions.

Eels provide an excellent material for direct investigation of this point : Keys[5] demonstrated that branchial chloride is excreted by animals adapted to sea-water ; whether chloride absorption occurs is doubtful[6]. Moreover, silver eels migrate from fresh water to the sea and this behaviour should be related to a change in osmo-regulative capacities.

Gills of yellow and silver eels, adapted to fresh and sea-water, have been studied with Leschke's histochemical test for chloride. Although Leschke's method lacks specificity and is reproducible only within rather wide limits (cf. Lison[7]), a comparison of results obtained with identical tests in standard conditions from different animals can give reliable information.

The acidophil cells of yellow eels living both in fresh and in sea-water gave a positive chloride test, although slight and somewhat variable. With silver eels, the chloride test was strongly positive (Fig. 1) for animals adapted to sea-water, but clearly negative for those maintained in fresh water (Fig. 2).

These results confirm Keys and Willmer's theory and Copeland's data (on *Fundulus*) of the role of the acidophil cells of the gills of some fishes in chloride secretion, and demonstrate a different osmo-regulation between yellow and silver eels.

Yellow eels appear to be more euryaline and capable of secreting and absorbing chloride through the gills, but this conclusion should be confirmed by physiological experiments on eels of a definite stage of development. Silver eels seem to be able to secrete but not to absorb chloride by the gills.

These differences in osmotic function between yellow and silver eels explain the migration of silver eels from fresh water to the sea more clearly than the 'demineralization' theory postulated by Fontaine[8].

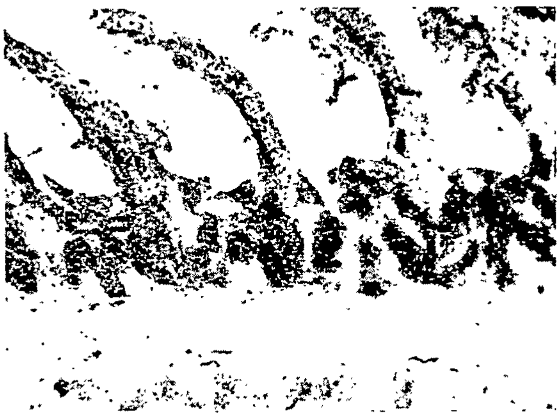

Fig. 1. Longitudinal section of gill filament of a silver eel adapted to sea-water. The acidophil cells at the base of lamellæ show a positive test for chloride, with Leschke's method. (× 300)

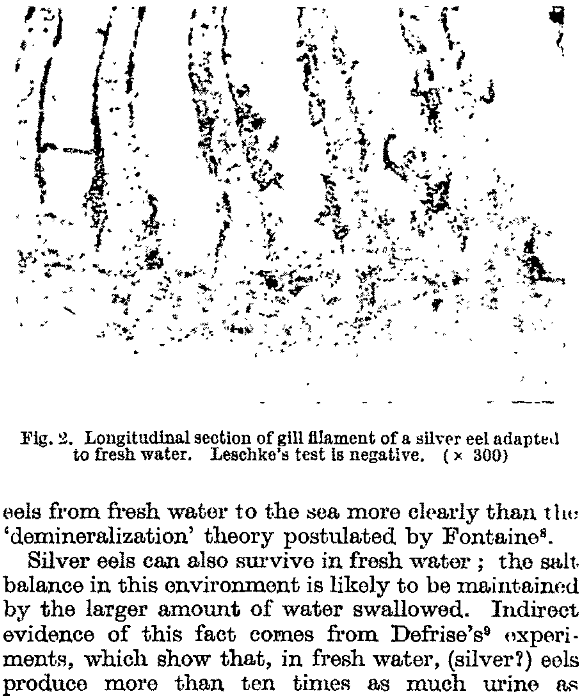

Fig. 2. Longitudinal section of gill filament of a silver eel adapted to fresh water. Leschke's test is negative. (× 300)

Silver eels can also survive in fresh water ; the salt balance in this environment is likely to be maintained by the larger amount of water swallowed. Indirect evidence of this fact comes from Defrise's[9] experiments, which show that, in fresh water, (silver?) eels produce more than ten times as much urine as sea-adapted eels.

A full account of this research is in the press[10].

I am indebted to Prof. U. D'Ancona for suggestions and criticisms.

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Photoperiodicity of Seasonal Rhythm of Wool Growth in Sheep

The existence of a seasonal rhythm of growth of wool of sheep on a uniform diet was first shown in 1949 by Ferguson, Carter and Hardy[1]. These authors and Wodzicka[2] present indirect evidence which suggests that this rhythm, with maximum growth in summer and minimum in winter, is induced mainly by seasonal changes in temperature. However, certain photoperiodic responses in wool growth reported by Coop and Hart[3] and by Hart[4], together with the proof by Yeates[5,6] that the seasonal breeding-cycle in sheep is photoperiodically controlled, suggested that seasonal changes in length of day should be investigated in some detail as a possible cause of the seasonal wool growth cycle. The following experiment was therefore carried out.

Three groups of mature non-pregnant Romney Marsh ewes were fed a uniform ration of lucerne chaff which was sufficient to maintain a slight steady increase in body-weight. The control group was kept under environmental conditions (sub-tropical) normal to Brisbane. The second group remained under normal environmental temperature conditions but was subjected to an accentuated reversed rhythm of seasonal changes in length of day as used by Yeates[6] and illustrated in Fig. 1. The third group received normal hours of daylight but was exposed to a somewhat reversed seasonal temperature-cycle as shown in Fig. 2. It was not possible to arrange an ideal reversal of seasonal changes in temperature for this third group, owing to other projects using the controlled-temperature rooms at the same time.

The experiment started with 8 sheep in each of the three groups, but during the 2 years of the experiment a total of 6 sheep died, and during the second year two suffered a marked decrease in body-weight and were therefore excluded from the experiment. This left 16 sheep (4 controls, 7 in the reversed day-length group and 5 in the reversed temperature group) which remained in good condition throughout the experiment, and from which the wool growth curves in Figs. 1 and 2 were compiled. It was observed that each of the eight sheep finally excluded because of death or loss of weight closely followed the wool growth rhythm of its respective group while it remained in good condition.

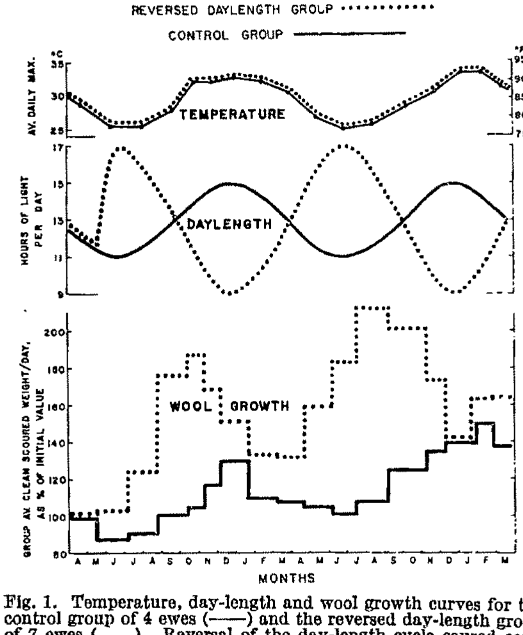

Fig. 1. Temperature, day-length and wool growth curves for the control group of 4 ewes (——) and the reversed day-length group of 7 ewes (....). Reversal of the day-length cycle caused complete reversal of the wool growth-cycle

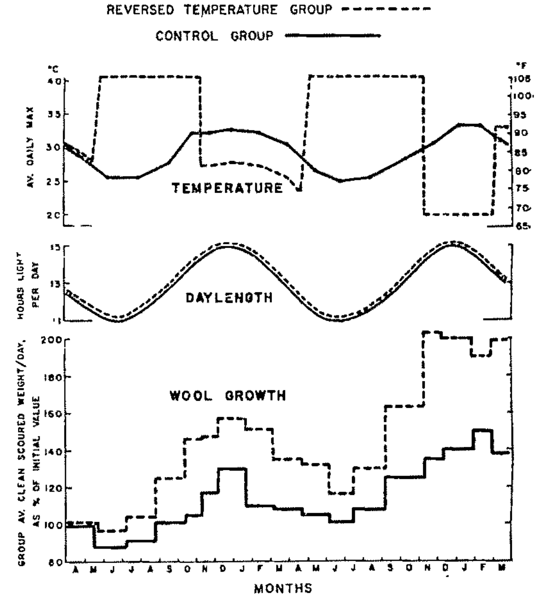

Fig. 2. Temperature, day-length and wool growth curves for the control group of 4 ewes (——) and the reversed temperature group of 5 ewes (– – –). An ideal reversal of the seasonal temperature cycle was not possible, but it can be seen that heating the latter group in winter and cooling them in summer had no obvious effect on the wool growth-cycle

Wool samples were clipped at approximately 6-week intervals from a 100 sq. cm. tattooed area on the mid-side of each sheep. The group averages of clean scoured weight/day for each growth period are shown in Figs. 1 and 2 as percentages of the initial average for the respective group. Fibre-diameter and staple-length were measured on the samples at selected periods during the experiment.

The following results were obtained :

(*a*) The control ewes showed a minimum wool growth-rate in winter and maximum in summer, as found by other workers[1–4].

(*b*) The ewes subjected to the reversed seasonal day-length rhythm developed a reversed seasonal wool growth rhythm with maximum wool growth in winter and minimum in summer. This reversal of wool growth rhythm occurred gradually, and took two years to complete.

(*c*) The reversal of the clean scoured weight rhythm resulted from a reversal of the rhythms of both fibre-diameter and staple-length.

(*d*) Upsetting the normal seasonal temperature cycle in the third group had no obvious effect on the wool growth-cycle of this group.

(*e*) The seasonal variation in wool growth was greatest in the reversed day-length group, a result which can reasonably be attributed to the greater seasonal variation in day-length experienced by this group.

(*f*) The overall increase in wool growth-rate over the 2 years was greater in the reversed day-length and temperature groups than in the control group. It is not known whether this is a direct result of the experimental treatments, the result of some other factor such as the more secluded environment in the reversed day-length pen and the controlled temperature rooms, or simply a result of chance.

The most significant of the above findings is the reversal of the seasonal rhythm of wool-growth by a reversal of the seasonal rhythm of day-length. This means that progressively decreasing day-lengths decrease wool growth and increasing day-lengths increase it, regardless of temperature changes. It is concluded therefore that the seasonal wool growth-cycle is induced primarily by seasonal day-length changes and not by seasonal temperature changes. Further evidence supporting this conclusion is provided by the temperature experiment illustrated by Fig. 2, in which the natural wool growth-cycle was not obviously altered by heating the sheep in winter and cooling them in summer.

The fact that 2 years of exposure to a greatly accentuated reversed day-length rhythm was required to reverse completely the wool growth rhythm shows that the light-induced wool growth rhythm is difficult to alter, and this is probably why Wodzicka[2] was not able to eliminate the natural seasonal wool growth-cycle simply by subjecting sheep to a uniform day-length régime for 12 months. The effect of temperature on wool growth-rate should be studied in some detail, and with due regard to the persistence of the light-induced rhythm, since it is clear from the results reported here that the temperature effect, if one exists, is not very marked.

The suggestion that a high level of adrenocortical activity in winter might cause the natural decline of wool growth during this season (Lindner and Ferguson[7]) now seems unlikely, since the seasonal cycle of wool growth is light-induced whereas the seasonal cycle of adrenocortical activity seems to be induced by temperature and not by light (Robinson and Morris[8]).

This work will be published in more detail at a later date.

I sincerely thank the Wool Research Trust Fund (Australia) for financing this project.

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Elminius modestus on the South-East Coast of Scotland

Elminius modestus Darwin was first recorded in Britain in 1947 by Bishop[1], who found it to be well established in Chichester Harbour. Since that date numerous reports have been published giving the spread of this immigrant barnacle afterwards around the coasts of the British Isles.

Table 1. Population Densities of *Elminius modestus* at Various Stations in the Forth and Tay Areas

Forth Area

	South shore	Adults	Juveniles		North shore	Adults	Juveniles
				12	Kincardine Bridge	*A*	—
11	Blackness	*A*	*A*	13	Charlestown	*A*	*A*
10	South Queensferry	*A*	*B*	14	North Queensferry	*B*	*B*
9	Cramond	*B*	*B*	15	Aberdour	*C*	*D*
8	Portobello	*B*	*A*	16	Kinghorn	*B*	*B*
7	Gosford Bay	*B*	*B*	17	Kirkcaldy	*B*	*B*
6	Gullane Bay	*B*	*B*—*C*	18	East Wemyss	*B*	*C*
5	North Berwick	*C*	—	19	Elie	*D*	—
4	Belhaven	*D*	—	20	Anstruther Easter	—	—
3	Dunbar	*D*	—				
2	Reed Cove	—	—	21	Crail	—	—
1	Eyemouth	—	—				

Tay and St. Andrews Area

		Adults	Juveniles
22	St. Andrews	*D*	—
23	Tayport	*D*	—
24	Newport-on-Tay	—	—

Grades. *A*, Found with ease, population estimated as more than 10 per cent; *B*, found without difficulty, population estimated as more than 1 per cent; *C*, found fairly easily, either as isolated specimens or small groups fairly widely spaced; *D*, found only with difficulty after some minutes searching, usually isolated specimens; —, none found after searching for about 10 min. at each of three or four suitable places. In each grade the numbers of juveniles are slightly higher than those of the adults.

This spread was relatively rapid in most parts of England and Wales but in the north-east, about 1951, it ceased abruptly at the mouth of the Humber[2]. A recent report by Crisp[3] states, however, that *Elminius* has now been found as far north as the mouth of the River Tees.

In October 1960 I found numerous mature specimens of *Elminius* and a fairly recent but heavy settlement of spat at Portobello, on the south shore of the Firth of Forth. Dr. Crisp, in a private communication, has since confirmed the occurrence of the barnacle in this area, settlement having occurred on test panels immersed at Rosyth between August 24 and September 7; and October 3 and 27, 1960.

Therefore, in October and November 1960 I made an extensive but fairly rapid survey of the Forth estuary to determine the local limits of distribution of *Elminius* and to estimate roughly the proportion that it forms of the total barnacle population. The results of this survey are shown in Table 1 and Fig. 1.

A clear distribution pattern appeared on both shores of the Firth of Forth. The highest proportion of *Elminius* was found to the west of the Forth Bridge, at Queensferry, diminishing steadily towards the eastern, open end of the estuary. *Elminius*

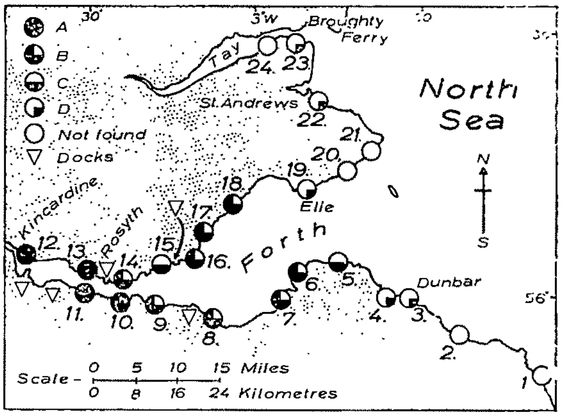

Fig. 1. Distribution of *Elminius modestus* Darwin in south-east Scotland. For key to station numbers and symbols see Table 1

extended slightly farther east on the south coast (Dunbar) than it did on the north coast (Elie). In addition, this barnacle was found in small numbers at St. Andrews and at Tayport, at the south-east end of the Tay estuary.

The distribution of the most recently settled larvæ followed a similar pattern to that of the adults but was less extensive. The young stages were more numerous on the south shore of the Forth, but a comparison of their sizes suggests that settlement had probably occurred earlier and persisted over a longer period on the north shore.

It seems likely that the focal point of the colonization of the Forth area developed as a result of remote dispersal[2] by ships, since it appears to have been centred on the inshore western end. Even if the settlement in the Tees area[3] were not of recent date, the distance and the nature of the intervening coast make it unlikely that marginal (that is, coastal) dispersal could have been responsible, since the maximum possible distance across which the animal can be dispersed by such means appears to be not more than about thirty miles[4].

The exact locality of the original Forth settlement must remain a matter for conjecture as there are numerous docks which take shipping from warmer waters (Fig. 1); perhaps the most likely possibility is the naval dockyard at Rosyth. In addition, there is a number of smaller fishing and sailing harbours which could have assisted in the subsequent local spread.

It is difficult, also, to estimate the probable date of the arrival of the initial colony or colonies. In 1956 Dr. Crisp failed to find *Elminius* at Portobello and Queensferry, where it is now common, but the present density and extent of the populations suggest that at least two, or possibly three, seasons have passed since the barnacle first became established. The rate of colonization may have been influenced in the early stages by the size of the original settlement, or settlements, and possibly by later introductions; but an initial period of build-up is necessary in order to produce the large numbers of larvæ requisite for dispersal and settlement in the area. It is likely that growth and reproduction will be slower in the colder latitude of the Forth than in the southern parts of Britain, where Crisp[2] suggested that the rate of colonization by marginal dispersal along an open coast is about 20–30 km. per annum. On consideration of these facts, therefore, it seems probable that the initial settlement occurred in 1958; a consolidation of the settlement and an extension in the enclosed waters to the west and the immediate east of the Forth Bridge may have occurred during the warmer summer in 1959; and the extension in quantity to the open part of the Firth in 1960.

The earliest record of *Elminius* on the east coast of Scotland comes from Dr. G. A. Horridge, of the Gatty Marine Laboratory, who, in a private communication, reports that it was noted at St. Andrews in 1958. Dr. Crisp, however, was unable to find the species at Broughty Ferry, Arbroath or Montrose, all north of the Tay, in February 1960.

The past history of the *Elminius* populations of this area is therefore far from clear, but it seems likely that the species will become established over a wider area of the east coast of Scotland with its numerous small harbours and sheltered coves. Although only recently established, the population in the Forth area has already become more numerous than the former dominant species, *Balanus balanoides* L., at some points, namely Blackness Point, Kincardine Bridge and Charlestown.

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Bathynella and Other Interstitial Crustacea in Southern England

The report of a specimen of *Bathynella* from near Oxford[1] was a reminder that in Great Britain zoologists have still neglected to study the fauna inhabiting the interstitial water of strata below ground, though the potential interest of this habitat was clearly pointed out by A. G. Nicholls as long ago as 1946[2].

More recent work on the Continent, as, for example, that of Husmann[3] in Germany, is beginning to throw light on the nature of this fauna and how its members are distributed, at least in the more accessible sites. It has been realized for some time that several small 'hypogean' animals that occur sporadically in wells or cave water are really generally spread in interstitial water of strata bathed by a permanent water-table, and indeed primarily belong to this habitat. The crustacean family Bathynellidae provides perhaps the most striking example. About thirty species, classified in five genera, are now known from various parts of the world, including Eurasia, Africa, South America and Madagascar. Few of them greatly exceed 1 mm. in length and being very narrow are well suited for moving through not only gravels but also coarser sands. Throughout central Europe, eastward to Roumania, one species[4]—*Bathynella natans* Vejdovsky (= *chappuisii* Delachoux)—has been found in interstitial ground water in many places where it has been sought. Its status in Britain might be presumed to be the same, but evidence has been lacking.

Among other crustacean genera known from underground interstitial water in central or southern Europe are three (*Microcharon*, *Ingolfiella* and *Bogidiella*) that are represented in marine gravel on the bed of the English Channel[5]. This find raises such questions as whether these genera may not also be represented in sub-surface fresh water in Britain, and, more generally, whether there is not a transition zone where sub-surface fresh water grades into that held in strata below the sea bed, making possible a gradual exchange of fauna between the two.

More information is needed from the landward side, and, in pointing out this neglected field, and urging that fresh water biologists might profitably turn attention to it, I can report the results of some tentative steps I have myself been taking in this direction.

Attempts to sample ground-water have been made in three ways: (1) by filtering water delivered by an electric pump through a pipe driven into water-bearing gravel; (2) by washing stones and gravel at the point of issue of hill-slope springs; and (3) by digging shallow pits in gravel where the water-table is high, such as in the ox-bow of a river. Each of these methods, simple though they are, produced encouraging results at the first attempt, particularly with regard to the crustaceans collected. Three species in particular deserve mention.

Bathynella natans Vejd. Examples have been collected by all three techniques. (1) At Whitchurch (Oxon), in the Thames valley opposite Pangbourne (Berks), through an opportunity kindly presented by Dr. I. H. H. Yarrow (to whom and to whose family I am extremely indebted for help), an estimated volume of 3,400 gallons of water raised from the alluvial gravel, on April 2 and 3, 1960, and filtered through a fine-meshed plankton tow-net, produced ten specimens. Here a 1-in. metal pipe had been driven 14 ft. into the ground and an electric pump delivered water through it at the rate of about 500 gallons an hour. The pump had not been used for nearly a year. The catches were distributed over the two days.

(2) One of the two springs emerging in fields (owned by my colleague, Dr. J. A. C. Nicol) at Moortown, near Tavistock, Devon, on the slope of Dartmoor, produced a single adult *Bathynella*, when sampled on May 3. This site is at 560 ft. O.D., on metamorphosed slate, and about half a mile from the edge of the main granite outcrop. At the point of issue of the water a rough conduit had been made with flat stones, under which the water flowed over a small accumulation of stones and gravel some inches thick. Handfuls of the latter were swilled in a bucket and the water strained off.

(3) A pit was dug in a gravel spit alongside the River Plym, a little way above Cadover Bridge, in the granite area on the south-west side of Dartmoor, at 696 ft. O.D. Ground-water was reached just below 1 ft. and the gravel dug out for another foot. The gravel removed, consisting mainly of granite fragments with little detritus, was washed and the washings strained off, as well as much water that constantly seeped into the pit. The fauna collected included twelve specimens of *Bathynella*, mostly juvenile. A second pit, dug close at hand on May 27, in warmer weather, yielded two specimens at the lowest level dug. These observations are of interest in showing that *Bathynella* tolerates the acidic, peaty and very soft water for which Dartmoor is notorious.

On these findings, scanty as they are, it is difficult to escape the conclusion that *Bathynella natans* must be very widespread in water-holding strata through much of Britain. The gravels of the London basin may be expected to hold a large population. The zoological departments of some universities must be in a position to obtain a ready supply by simply boring into the gravels on which their buildings rest.

Niphargus glenniei Spooner. This small member of the amphipod genus of 'well-shrimps' was described from the limestone caves of Buckfastleigh and Ashburton (Devon)[6], which had remained the only known habitat. Several examples, however, have been obtained during this investigation. Two came from the Moortown spring that produced the *Bathynella*, and five from another spring at 700 ft. O.D. and nearer the granite. The second pit dug by the River Plym, in granite gravel, produced four specimens. Another example was got from a well on the edge of Dartmoor, near Wrangaton. Evidently, therefore, this species must be widely spread, in Devonshire, in interstitial ground-water, tolerating a wide range of *p*H. It seems to be the counterpart of *Niphargellus* spp. on the Continent.

Crangonyx subterraneus Bate. One of these amphipods was obtained from the water pumped at Whitchurch (as described above). Since first recognized by Spence Bate more than a hundred years ago, from a pump, there seem to be only two subsequent occurrences in Britain (one from a well, and one from a cave pool). The apparent scarcity would be explained if this species also proved to be primarily an inhabitant of interstitial water, and accidental elsewhere.

Granted that crustaceans of this sort, along with certain copepods, ostracods, mites, oligochaetes, nematodes, etc., regularly inhabit interstitial water, it may be asked how far do they range; or how deep into the geological strata do any of them penetrate.

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Soil *p*H and Mycorrhiza Development in *Pinus*

MYCORRHIZAS of *Pinus* will not develop profusely on trees growing in neutral or alkaline soils, and it has usually been assumed that this is so because the mycorrhizal fungi are acidophilic. During investigations of the fertility status of a nodular podzolic soil from the coastal lowlands of southern Queensland, I have found that formation of mycorrhiza in pine seedlings is not always inhibited by a rise in soil *p*H, and when it is, the effect can usually be explained in terms of host nutrition. The direct effect of alkalinity in restricting the growth of the fungus may be less important than was formerly thought. These results are derived from a series of pot experiments designed primarily to test for nutrient deficiencies, but also to study the effect of nutrient supply on the degree of mycorrhizal infection of seedlings. Comparison of relative mycorrhiza development was based on the number of mycorrhizas expressed as a percentage of the total number of short roots per seedling ('mycorrhiza per cent'). The criterion of infection used was the presence of dichotomous branching or forking of the short roots. Since dichotomy of short roots of *Pinus* is not always associated with mycorrhizal infection, some care is necessary in accepting forked short roots as mycorrhizas. Experience has shown that it is possible to recognize mycorrhizas of *Pinus* on the basis of their external morphology. In the work reported here, counting was carried out initially under a stereo-microscope (× 12·5), and after experience had been gained in this way a binocular loupé was used.

The forked mycorrhizas of the seedlings examined were predominantly of Melin's type *A* which, as described by Robertson[1], occur in acropetal succession on the parent long root. Type *B* mycorrhizas were far less common and these were counted along with the former type. Where any doubt existed, the short roots were regarded as uninfected. This also applied

Table 1. EFFECT OF AMMONIUM NITRATE AND CALCIUM CARBONATE ON SOIL NITRATE-LEVEL, SOIL pH, AND MYCORRHIZA DEVELOPMENT OF *P. caribaea* (EXPERIMENT SOWN, APRIL 30, 1957)

Treatment	Nitrate as p.p.m. nitrogen (June 10, 1957)	pH (Nov. 19, 1957)	'Mycorrhiza, per cent'* (Nov. 19, 1957)
Nil	12·7	6·3	19·5 (25·83)
Calcium carbonate, 16 cwt./acre	21·3	7·5	8·7 (16·86)
Ammonium nitrate, 3 cwt./acre	71·4	5·8	2·0 (7·21)
Calcium carbonate, 16 cwt./acre + ammonium nitrate, 3 cwt./acre	115·3	6·9	4·3 (8·19)
Significant difference			
($P < 0{\cdot}05$)	12·8	0·2	(5·26)
($P < 0{\cdot}01$)	17·6	0·3	(7·25)

* Mean arc sine transformations shown in brackets.

to unbranched mycorrhizas: while these could frequently be recognized under the stereo-microscope, their identification by means of the binocular loupé was by no means certain. Full details of this work will be published elsewhere, but the meantime the results can be summarized as follows: (1) Lime-induced chlorosis of *P. taeda* L. occurred at pH 7·5 and the seedlings had relatively few mycorrhizas. Normal mycorrhiza development was restored by adding the iron complex of ethylene diamine tetra-acetic acid to the soil without altering its pH; (2) both ammonium nitrate and calcium carbonate reduced mycorrhiza development in *P. caribaea* Mor. Calcium carbonate promoted nitrification, and as the nitrate-level in the soil increased, 'mycorrhiza per cent' declined, irrespective of whether soil pH increased or decreased (Table 1); (3) ammonium nitrate greatly reduced 'mycorrhiza per cent' of *P. taeda* growing at pH 5·5, but sodium carbonate did not affect mycorrhiza development even though it raised soil pH to 7·4; (4) sodium nitrate retarded mycorrhiza formation in *P. taeda*, and the greater the amount added, the longer the delay in mycorrhiza formation; (5) in an experiment designed to separate the effect of the sodium and nitrate ions, it was found that sodium ions did not affect mycorrhiza development in *P. taeda* even though they increased soil pH, whereas nitrate ions greatly reduced 'mycorrhiza per cent' (Table 2).

These experiments were all carried out using the closed pot system, that is, the pots were undrained. Because of this, no nitrate could be lost through leaching; nor was any evidence of denitrification encountered. It seems therefore that mycorrhiza formation in *P. taeda* and *P. caribaea* is not inhibited in neutral or slightly alkaline soils, provided the soil nitrate-level is low. That this has not been recognized previously may be due to the fact that mineralization of nitrogen proceeds rapidly in such soils.

Table 2. EFFECT OF SODIUM NITRATE AND SODIUM CARBONATE ON SOIL pH AND MYCORRHIZA DEVELOPMENT OF *P. taeda*

Treatment	pH	'Mycorrhiza, per cent'*
Nil	6·5	33·6 (35·28)
Sodium nitrate	7·0	9·2 (14·92)
Sodium carbonate	7·0	22·9 (29·29)
Sodium nitrate + sodium carbonate	7·3	11·3 (17·14)
Significant difference		
($P < 0{\cdot}05$)	0·1	(7·60)
($P < 0{\cdot}01$)	0·1	(10·19)

* Mean arc sine transformations shown in brackets. 10 ml. *N*/10 solution of each salt applied per pot.

The depressive effect of readily available nitrogen on mycorrhiza development may also explain why mycorrhizas are less abundant in mull soils than in mor. Although not necessarily more alkaline than mor soils, mulls nitrify well and have a higher content of ammonium and nitrate nitrogen in the natural state[2].

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ENTOMOLOGY

'Preferred Temperatures' of Land Arthropods

STUDIES on arthropod behaviour have often been concerned with the 'preferred temperatures' of the animals and attempts have been made to correlate results with the microclimatic changes in the animal's natural environment. It appears, however, that insufficient attention may have been paid to the other physical factors the variations of which cannot be easily eliminated from a temperature gradient. These factors have sometimes been assumed to be unimportant or have been disregarded altogether, and a preference shown by the animal for a particular zone within a temperature gradient apparatus has been attributed solely to temperature.

The main purpose of this communication is to point out that variables may exist in most of the usual types of closed temperature-gradient apparatus. For example, even in a gradient apparatus designed for observing small arthropods of 1·5 mm. × 1 mm. or less, the air temperature differs by more than 3 deg. C. from that of the floor. The air will be cooler than the floor at the 'warm half' and warmer than the floor in the 'cool half' of the temperature gradient. These differences will cause a 'thermal flow' from the warm end to the cool extreme with, at high humidities, the deposition of water droplets on the roof of the warm region and on the floor of the cool end.

It is often assumed that 'saturated air' may be easily produced and thus uniform humidity conditions obtained throughout a temperature gradient. However, if the air temperature is higher than that of the floor, the air in fact will not be completely saturated, although if the floor is warmer than the air the relative humidity will be 100 per cent. The rate of evaporation at any point in the apparatus can be determined by introducing there a modified Piche micro-evaporimeter; because the warm air flows into the cool region, evaporation may be much higher at the cool end than at the warm end.

Animals introduced into such an apparatus will consequently be acted on by (*a*) floor temperature, (*b*) air temperature, (*c*) thermal air currents, (*d*) differences in relative humidity and (*e*) evaporating gradients. All these factors, in addition to others depending on the physiological state of the organisms, must be considered in the interpretation of any results.

I have been concerned with aspects of the physiology and ecology of certain free-living mites (Oribatoidea, Acarina). Batches of fifty adult *Belba*

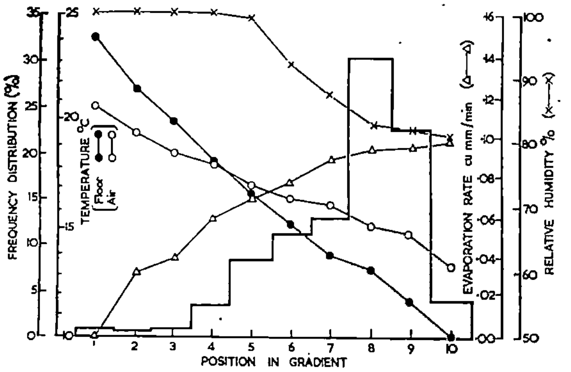

Fig. 1. Distribution of adult *Belba geniculosa* in a temperature gradient in 'saturated' air (mean of three experiments)

geniculosa Ouds., a common oribatid mite of oak-litter, were acclimatized to constant conditions and offered a linear temperature range under 'saturated' conditions. Their positions were recorded at fixed time-intervals to determine where they aggregated.

The apparatus consisted of a copper bar 22 cm. × 4 cm. × 0·2 cm. embedded at each end into insulated copper boxes, which contained heating and cooling agents. The upper side of the metal strip was surrounded by narrow glass sides (1 cm. deep) and covered with a glass plate marked at intervals to facilitate numerical records. The metal strip was covered with moist filter paper and floor and air temperatures within the apparatus were determined at definite intervals by thermistors; evaporation-rates 1 mm. above the floor were measured by a micro-evaporimeter. The relative humidity in the 'cool half' was calculated from aqueous vapour pressures derived from the floor and air temperatures; the atmosphere in the 'warm' half remained completely saturated. Except when recording, the apparatus was kept in darkness.

In Fig. 1 the frequency distribution of adult *B. geniculosa* in a 'saturated' temperature gradient after periods of 3 or 6 hr. is related to the temperature gradient and other physical variables.

By introducing desiccating and humidifying agents into the apparatus, the behaviour of the mites could be recorded under a range of conditions of temperature and humidity. Regardless of changes in relative humidity and evaporating gradients, *B. geniculosa* always aggregated within a floor temperature zone of about 12–14° C. (air temperature 14–16° C.). These results are somewhat surprising for the mites will quickly move towards the region of highest humidity offered to them in a linear relative humidity chamber at constant temperature. Thus temperature seems to have a greater effect than other physical factors and the mites seem to select a zone where death from desiccation will not long be delayed. However, other experiments indicate that desiccation is slow in humidities above 80 per cent, so the situation is more complicated than might be expected.

Temperature is not always the most important factor. Wellington[1,2], for example, found that larvæ of the spruce budworm, *Choristoneura fumiferana* (Clemens), aggregated within certain regions of evaporating gradients independent of temperature and these results were explained in terms of their micro-habitat[3].

Laboratory findings with *B. geniculosa* have been treated somewhat academically in this report and in further work it is hoped to correlate behaviour distributions under controlled conditions with diurnal changes and long-term micro-climatic phenomena in their natural environment.

It is suggested that in future more importance should be attached to all possible physical factors other than temperature in certain types of temperature gradient apparatus. In this way it may be possible to explain some of the existing inconsistencies between field and laboratory observations.

I gratefully acknowledge the receipt of a post-graduate Agricultural Research Council grant during the course of these investigations.

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Mite Orientation and Survival on Flies

PHORESY among arthropods has been widely noted and its importance in population dispersal and survival appreciated. Hypopi, the phoretic stage of mites, have been studied for the most part isolated from their hosts, and little is therefore known of their interaction. We have recently described some behavioural adaptations of the hypopus of *Myianoetus muscarum* (Anoetidae) to fly factors which favour the mite's dispersal[1]. Hypopi are attracted to a volatile substance produced by the pupæ of several fly species, including *Musca domestica, Muscina stabulans* and *Stomoxys calcitrans*, and cluster on the anterior end of the pupal case from which the fly is due to emerge. This communication describes the behaviour of the same mite on adult *M. stabulans*.

Hypopi can be found throughout the fly-breeding medium and may cluster on pupæ well down as well as on the surface. Their distribution, however, is mainly on the surface and in large infestations the surface is covered with cinnamon-coloured patches containing thousands of mites. A strip of paper towelling partially inserted into the medium will show a sharp boundary of mites just above the surface—presumably an indication of a preference for moisture. Aggregates of hypopi remain on the pupal case until eclosion, at which time they transfer to the emerging fly. After a brief excursion on the host, the mites occupy characteristic locations for attachment. One of the choice sites is under the dorsal conjunctival folds at the base of the abdomen; another is at the base of setæ. Horizontal rows of mites build outward and toward the rear. In moderate infestations, mites collect on areas bearing setæ and avoid smooth cuticle (Fig. 1). On all areas of the abdomen mites uniformly face toward the rear. This orientation is directly correlated with the pattern of setæ and conjunctival folds which are directed towards the posterior. I have observed hypopi back to the base of an overhanging seta or into a fold in the cuticle, presumably to obtain maximum contact with their anal suckers. This would serve to explain their aversion to smooth cuticle. Mite orientation on the thorax is obscured by its more complex chætotaxy.

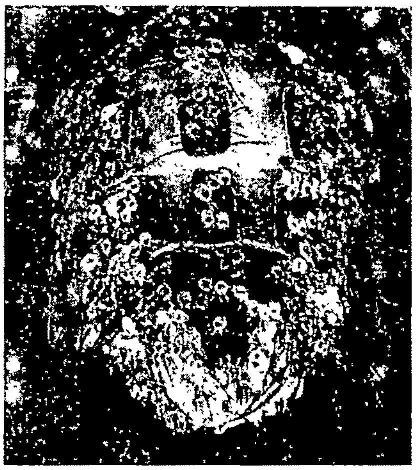

Fig. 1. Ventral abdominal aspect of fly showing preference of mites for areas bearing setæ and their orientation toward the rear

Once settled on the fly, hypopi remain sessile. There is a non-adaptive aspect of this behaviour which is strikingly demonstrated in two ways. The majority of mites will remain attached despite numerous visits of their hosts to a suitable breeding medium. Furthermore, the mites will remain attached to a dead host until they too succumb. Thus, for the majority, phoresy may be a one-way ride. Some of those mites which do transfer to a breeding area remain active for two weeks or longer without moulting. Others soon become quiescent and distended as a result of what appears to be a considerable intake of water. I measured the idiosomal heights of hypopi before and after contact with moisture and found them to be 67μ and 110μ, respectively. The mechanism of water absorption is not known, as the mouthparts are considered to be non-functional. A minimum of two days is required for the appearance of the tritonymph which may moult to the adult stage one day later (at 24° C.).

I have found dessication to be the most crucial factor in mite survival. The following results are based on three replications using about 200 mites in each test. The various stages of the mite were placed in a dry Petri dish at 24° C. and 50 per cent relative humidity. Under these conditions, the feeding stages suffered 100 per cent mortality within 2 hr., while the hypopi completely succumbed after 30 hr. The longevity of hypopi on caged flies kept under the same conditions was a minimum of 47 days. In my experiments the flies died before the mites. It is noteworthy that the microclimate of the fly is most favourable for the survival of hypopi. This is additional evidence of the adaptive value of the phoretic habit.

This work was supported by a National Science Foundation grant, *G*9934, and conducted, while a guest investigator (1960–61), in the University of Illinois College of Pharmacy. I am grateful to Prof. P. D. Carpenter, for advice and assistance during the course of this work, and to Dr. L. de la Torre of the College of Agriculture for the photograph.

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MICROBIOLOGY

Action of Mycophenolic Acid on Unstable Diploids of *Penicillium chrysogenum*

Diploids of *Penicillium chrysogenum* heterozygous for the genes y/Y (yellow versus green conidia) and w/W (white versus green conidia) have green conidia. On plating, these give rise to a majority of green colonies, together with a small percentage (less than 1 per cent) of yellow or white colonies, or green colonies with white and/or yellow sectors. The proportion of these 'segregant' colonies and sectors is strongly increased (by more than 20–30 per cent) if the conidia are treated with the nitrogen mustard, methyl-*bis*(β-chloroethyl)amine (HN–2) or with X-rays[1]. When certain of the colonies with sectors are sub-cultured by the plating of green conidia, they give rise to a population of colonies with sectors; these colonies maintain the unstable character indefinitely in successive transfers. A dozen unstable clones were isolated, each of which showed sectors with a single phenotype (rarely two) characterized by the presence of nutritional markers as well as by the colour of the conidia. Manganous chloride, added to the agar medium at 40 m*M* concentration, inhibited the production of sectors with nearly all the unstable clones; the inhibition, however, was not of a permanent nature, and disappeared when the clones were subcultured on medium free from manganous chloride[2].

During one experiment, a chance contaminant colony of *Penicillium* sp. was found to grow on a plate of unsupplemented nutrient agar on which colonies of an unstable strain (*D*) produced by treatment with HN–2 were giving rise to yellow sectors. Around the contaminant colony, colonies of strain *D* did not show sectors. The contaminant strain was isolated and cultivated in submerged culture in a corn-steep – dextrin medium, and the filtered broth was sterilized by autoclaving. 1 ml. of broth added to the agar on which strain *D* was plated completely inhibited sectoring of the colonies. As with manganous chloride, when conidia from such colonies were replated on unsupplemented medium, they gave rise again to sectoring colonies.

By extraction with *n*-butyl acetate at acid *p*H, a substance was isolated from the broth which could be easily crystallized from water or from carbon tetrachloride and which reproduced the effect observed with the culture filtrate of the *Penicillium* sp. The chemical and physical properties of this compound agreed closely with those of mycophenolic acid, a well-known metabolite of several *Penicillia*. The identity of the substance was confirmed by comparing its physical, chemical and biological properties with those of a sample of mycophenolic acid kindly supplied by Prof. J. H. Birkinshaw.

Mycophenolic acid inhibits sectoring of strain *D* and other unstable diploid strains of *Penicillium chrysogenum* at a concentration of 1 μgm./ml., that is, at a molar concentration about 10,000 times less than the minimum concentration of manganous chloride required to produce the same effect. The abolition of yellow sectors is not due to a selective effect; yellow colonies derived by plating conidia collected from sectors formed in the absence of mycophenolic acid grew and sporulated normally on agar containing mycophenolic acid. Nevertheless, the formation of yellow sectors from unstable strains is prevented by this substance.

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Mycophenolic acid does not show any of the other effects which manganous chloride produces on *Penicillium chrysogenum* treated with mutagens[2], namely, reactivation of viability after treatment with HN–2 and reduction of somatic segregation induced by HN–2 or by X-rays.

The mechanism of genetic instability in diploids of *Penicillium chrysogenum* is still unknown, and therefore the mode of action of mycophenolic acid in this process cannot yet be investigated. The inhibition of the formation of yellow diploid sectors has also been observed with an unstable diploid of *Aspergillus nidulans*; in this strain the yellow sectors were due to a high frequency of somatic crossing-over in chromosome I, probably connected with a breakage-fusion cycle set up in chromosome II (ref. 3).

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The Mycolytic Phenomenon and Biological Control of *Fusarium* in Soil

THERE have been many attempts to make use of biological control measures for the suppression of plant pathogens, borne by soil. Frequently, these attempts involve the inoculation into soil of organisms which antagonize the pathogens *in vitro*; such procedures try to disturb the natural microbiological equilibrium by the simple introduction into the environment of individual isolates. The frequent failure of the added micro-organisms to become established is not surprising because the biological associations and antagonisms within the ecosystem determine the composition of the microflora, the climax population being a reflexion of the physical and chemical characteristics of the habitat. By modifying the habitat, however, it is possible to alter the composition of the microflora, and such microbiological alterations may destroy plant pathogens borne by soil.

The possibility that mycolytic bacteria, that is, organisms which lyse fungi, are involved in the suppression of certain plant pathogens has been proposed frequently. For example, a lytic factor has been implicated in the control of *Fusarium* by strains of *Achromobacter* and *Pseudomonas*[1] and in the suppression of *Helminthosporium halodes* by *Bacillus subtilis*[2]. In an investigation of the disappearance of *Fusarium oxysporum* f. *cubense* from artificially infested soils, a number of bacteria capable of lysing the fungus were obtained. For the isolation of the mycolytic bacteria, a modification of the method of Mueller and Durrell[3] was used in which living *Fusarium* cells in water agar were included in the enriching medium. The active bacteria grew readily in an inorganic salts medium containing living or dead cells of *F. oxysporum* f. *cubense* as the sole carbon source, but fungus cell-walls isolated by treatment with potassium hydroxide could be substituted for the intact mycelium as the source of carbon. Three of the lytic micro-organisms were identified as strains of *Bacillus cereus*, two others as *Bacillus megaterium*, and a sixth isolate as a strain of *Pseudomonas*. When added to a growing culture of *F. oxysporum*, these bacteria caused a lysis of the fungus. Similar results have been obtained with fungi of several other genera. Furthermore, the bacteria-free culture filtrate of a lytic strain of *Bacillus cereus* grown in a medium containing *F. oxysporum* brought about the lysis of both the living and the dead fungus.

All the mycolytic bacteria exhibited chitinase activity and developed in media containing chitin as the only source of carbon, but culture filtrates prepared from media in which chitinoclastic micro-organisms were grown did not lyse *F. oxysporum*. The latter organisms were obtained from soil by use of chitin-containing enrichment solutions. Similarly, a strain of *Pseudomonas chitinovorans* possessing strong chitinase activity did not lyse the fungus. It would seem, therefore, that chitinase may be implicated in the mycolytic phenomenon, but lysis does not appear to result solely from the activity of this enzyme.

It is possible that mycolysis is a natural means for the biological control of susceptible *Fusaria*. If the active flora could be stimulated selectively in soil, the population of pathogenic fungi might decline. In preliminary work with sterile soil, it was noted that the mycelium of *F. oxysporum* was digested following the addition of a lytic *Bacillus* strain, but, as expected, the addition of mycolytic bacteria to normal soil failed to disturb the microbiological equilibrium to an extent sufficient to result in control of pathogenic *Fusaria*. On the other hand, the fact that all isolates had in common the capacity to utilize chitin suggested that the addition to soil of this polysaccharide might provide a selective means of stimulating the mycolytic population.

To test the possibility for control by such treatments, soil infested artificially with *Fusarium solani* f. *phaseoli* was planted to kidney beans immediately following amendments with chitin, cellulose or ammonium chloride. After six weeks, the severity of root-rotting was determined using a disease index: 0 for no disease and a value of 100 for severe rotting, the final disease index being the mean of all the values for each treatment. The results summarized in Table 1 demonstrate that chitin at a rate of 200 lb. per acre markedly reduced the severity of the root-rotting. Similar results have been obtained with the vascular wilt of radishes caused by *Fusarium oxysporum* f. *conglutinans*. In the radish wilt, chitin reduced the disease index from 47 for infested, untreated soil to a figure of 10 for infested soil treated with 200 lb. chitin. Neither cellulose, another polysaccharide slowly decomposed by the soil microflora, nor inorganic nitrogen produced similar beneficial effects.

Table 1. EFFECT OF CHITIN, CELLULOSE AND AMMONIUM CHLORIDE ON THE SEVERITY OF ROTTING OF THE BEAN-ROOT CAUSED BY *Fusarium solani* f. *phaseoli*

Treatment	Disease index (per cent)
None	39
Cellulose, 182 lb./acre	47
Ammonium chloride, 54 lb./acre	43
Cellulose + ammonium chloride	33
Chitin, 200 lb./acre	23
Uninoculated, unamended	0

The influence of chitin may result from an increase in the number of mycolytic micro-organisms. Chitin, however, is known to be one of the few substances which markedly increase the actinomycete population[4]. Because actinomycetes are particularly active in antibiotic production, in culture media at least, the content of antifungal substances in chitin-amended soil was determined by the method described previously[5]. The results obtained indicate that the toxicity of aqueous extracts of chitin-treated soils is far greater than that in unamended controls (K. C. Lu and J. E. Dawson, personal communication). Further work is in progress on the mechanism of the chitin-induced suppression.

This work was supported partly by a grant from the United Fruit Co., Boston, Massachusetts. The strain of *Pseudomonas chitinovorans* was provided by Dr. Hans Veldkamp, to whom we express our appreciation.

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Biological Characteristics of Colicine *X*

THE name colicine *X* was given[1] to a colicine produced by the strain of *Escherichia coli* used industrially by Hageda, AG., Berlin-Köln, for the preparation of the emulsion 'Mutaflor'. This colicine can be distinguished from other types of colicines by the following characteristics:

(1) *Specificity of mutants resistant to colicine X.* Specificity to colicines is determined by receptors, which are specific for each colicine[2]. The sensitive bacteria can lose the specific receptors one after another by mutation. By successive mutations a sensitive strain may become resistant towards many colicines. In a series of experiments I used two strains sensitive to colicines X, V, B, E, I, K, S_3+I, D, S_4, $J+I$, A and C, and by successive mutations I was able to isolate 200 mutants resistant to one or more colicines. Loss of receptor for colicine *X* was never accompanied by a loss of receptor for another type of colicine, and cross-resistance between colicine *X* and one of the other 12 colicines was not observed. The experiments prove that mutants resistant to colicine *X* are specific.

(2) *Spectrum of activity.* Colicine *X* is very active against some groups of Enterobacteriaceae; but like the other colicines does not inhibit growth of *Staphylococcus aureus*, *Bacterium anitratum* or *Pseudomonas* (Table 1). The *Escherichia coli*, *Citrobacter* and *Klebsiella-Cloaca* group are more frequently sensitive to colicine *X* than to other colicines, while *Salmonella* and *Proteus* are generally resistant. Strains of *Escherichieae* and *Klebsielleae* isolated from animal fæces are more sensitive to colicine *X* than strains isolated from human fæces; but it is still impossible to explain this ecological difference[3].

Table 1. ACTIVITY SPECTRUM OF COLICINE *X*

Organism	No. of strains examined	No. of strains sensitive	Percentage
Staphylococcus aureus	30	0	0
Bacterium anitratum	20	0	0
Pseudomonas	27	0	0
Enterobacteriaceae			
Salmonella	40	1	2·5
Proteus	20	0	0
Escherichia coli	130	100	76·9
Citrobacter	16	13	81·2
Klebsiella-Cloaca	40	27	67·5

The sensitivity of *E. coli* isolated from animal fæces towards 13 colicines is reported in Table 2. The results show clearly that colicine *X* is more active against *E. coli* than any other colicine used in this work.

Table 2. SENSITIVITY OF 80 STRAINS OF *Escherichia coli* TOWARDS COLICINE *X* AND 12 OTHER COLICINES

Type of colicine	No. of sensitive strains	Percentage
X	74	92·5
D	60	75
F	30	37·5
V	29	36·2
B	25	31·2
S_3+I	24	30
E	11	13·7
$J+I$	10	12·5
I	4	5
C	4	5
K	3	3·7
S_4	3	3·7
A	3	3·7

(3) *Other characteristics.* (*a*) The production of colicine *X* is not influenced by growth of the colicinogenic strain in synthetic medium[1].

(*b*) The diameters of the inhibition zones in nutrient agar are usually 20–26 mm. The morphology of the inhibition zone is not characteristic, but resembles that of colicines *D* and *K*.

(*c*) It has been difficult to study the heat resistance of colicine *X*, because suspensions of this colicine with a high titre cannot be prepared by the usual methods (see *d*). Undiluted colicinogenic cultures in broth were used for heat-resistance experiments, and it was found that colicine *X* is destroyed after boiling for 15 min.

(*d*) Colicine *X* is rapidly destroyed by the addition of 2–4 ml. chloroform to 100 ml. of bacterial culture in 'Oxoid' nutrient broth. The strain 'Mutaflor' is sensitive to streptomycin but the addition of streptomycin to the broth culture does not offer any advantages for the preparation of suspensions of colicine, as the concentration of colicine *X* in broth is low. It is possible that colicine *X* does not diffuse easily outside the bacterial cell.

(*e*) Colicine *X* is resistant to the proteolytic enzymes, which are produced by some strains of *Bacilli* or *Proteus*.

The foregoing characteristics permit the distinction of colicine *X* from the other 12 colicines produced by the type cultures of P. Fredericq.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Wednesday, April 5

Royal Meteorological Society (at 49 Cromwell Road, London S.W.7), at 5 p.m.—Prof. T. Bergeron (University of Uppsala): "The History of Meteorology" (Evening Discourse).

Royal Society of Medicine, History of Medicine Section (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Dr. K. D. Keele: "Three Early Masters of Experimental Medicine—Erasistratus, Galen, Leonardo di Vinci" (Presidential Address).

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Dr. W. Domzalski: "Airborne Techniques in Petroleum Information".

Wednesday, April 5—Friday, April 7

Institute of Physics and the Physical Society, in collaboration with the Institution of Electrical Engineers (at Brunel College of Technology, Acton, London, W.3)—Symposium on "Electrical Contacts".

Thursday, April 6

Institute of Refrigeration (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. J. A. Cox: "Results of Practical Experiments on Relative Humidity Control".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. W. Casson and Mr. H. J. Sheppard: "Technical and Economic Aspects of the Supply of Reactive Power in England and Wales".

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Transistorized Television Receivers".

Institute of Metals, London Local Section (at 17 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Mr. K. M. Bills: "Physical Methods of Analysis for Major Alloying Constituents".

Society for Analytical Chemistry, Biological Methods Group (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "Principles Involved in Collaborative Studies" opened by Mr. S. A. Price.

Friday, April 7

Institution of Electrical Engineers, Medical Electronics Discussion Group (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "The Conversion of Biological Data into Electrical Signals".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer in Mathematics—The Principal, Nottingham Training College, Clifton, Nottingham (April 10).

Demonstrator in Chemistry—The Registrar, University College of North Staffordshire, Keele, Staffs (April 14).

Assistant in the Department of Zoology—The Secretary, The University, Edinburgh (April 15).

Assistant Lecturer in Inorganic Chemistry—The Registrar, The University, Manchester 13 (April 15).

Lecturer (suitably qualified science graduate) in Bacteriological Biochemistry—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne (April 15).

Lecturer (with medical qualifications) in Anatomy—The Secretary, Medical College of St. Bartholomew's Hospital, West Smithfield London, E.C.1 (April 15).

University Demonstrator in Botany, with special reference to cytology and genetics—Dr. C. B. Goodhart, Museum of Zoology, The University, Downing Street, Cambridge (April 17).

Lecturer (suitably qualified graduate with experience or interest in the problems of agricultural research) in Statistics—The Secretary, Wye College (University of London), near Ashford, Kent (April 21).

Assistant Lecturer, Grade B (preferably with a university degree or equivalent qualifications) in the Department of Science and Metallurgy, to teach physics and mathematics mainly to Ordinary National Certificate classes—The Principal, North Lindsey Technical College, Kingsway, Scunthorpe, Lincs (April 22).

Senior Lecturer or Lecturer in Veterinary Anatomy; a Senior Lecturer or Lecturer in Veterinary Histology; a Senior Lecturer or Lecturer in Veterinary Medicine; and a Senior Lecturer or Lecturer in Veterinary Pharmacology at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 22).

Lecturer in Electrical Engineering; and a Lecturer in Mechanical Engineering—The Registrar, Trinity College, Dublin (April 24).

Lecturer or Assistant Lecturer in Philosophy—The Registrar, The University, Hull (April 24).

Reader or Lecturer in the Philosophy of Mathematics—The Secretary of Faculties, University Registry, Oxford (April 24).

Research Fellow (with a Ph.D. degree or equivalent experience) in the Chemistry Department, for fundamental work on compounds of potential use in the paint industry—The Professor of Chemistry, The University, Hull (April 24).

Assistant Lecturer (preferably with an interest in theoretical or experimental plasma physics) in Physics—The Secretary, The Queen's University, Belfast (April 29).

Lecturer in Applied Mathematics—The Principal, Jesus College, Oxford (May 1).

Lecturer in Histology and Embryology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 5).

Research Demonstrator (graduate in botany), to engage in research, preferably in plant physiology and biochemistry, plant pathology and ecology; and a Research Assistant (graduate in botany or graduating this year), to assist the Professor in the establishment and maintenance of a collection of plant organ and tissue cultures and in research involving these cultures—The Registrar, University College, Singleton Park, Swansea (May 13).

Chair of Mathematics (R.A.A.F. Academy), University of Melbourne—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 15).

Assistant Lecturers (with appropriate qualifications and teaching and research experience) in Applied or Pure Mathematics at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, May 19).

Lecturer (preferably with the ability to offer courses in methods of teaching chemistry and/or biology) in Education at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, May 19).

Lecturer (with an honours degree in zoology and preferably some experience of teaching and research) in Zoology at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, May 30).

Bacteriologist (graduate in science or veterinary science) at the Animal Health Trust Farm Livestock Research Centre, Stock, Essex, to assist in a programme of research into enteric diseases of young farm animals, with special reference to pigs—The Scientific Director, Animal Health Trust, 14 Ashley Place, London, S.W.1.

Glassblower—Dr. J. K. N. Jones, Chemistry Department, Queen's University, Kingston, Ontario, Canada.

Head of the Department of Science and Electrical Engineering—The Principal, College of Technology, Broadway, Letchworth, Herts.

Laboratory Technician (Grade III) in the Department of Physiological Chemistry, to assist with classes in practical physiology and some experimental work—The Assistant Bursar, The University, Reading.

Postdoctoral Fellow in the Department of Chemical Engineering, for research in flame spectroscopy or a related subject—Dr. A. G. Gaydon, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

Research Assistant (preferably with a good chemical background) in the Department of Chemical Engineering, for work on the fractionation and analysis of lipoprotein extracts—The Secretary, University College London, Gower Street, London, W.C.1.

Research Fellow in the Department of Electrical Engineering, for work on instrumentation and telemetering for physiological measurement on athletes—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 20/AF/2.

Research Fellows (2) (preferably with a Ph.D. degree in engineering or physics, or equivalent research experience) in the Department of Electrical Engineering under Prof. A. L. Cullen, for research in the field of millimetre wave techniques—The Registrar, The University, Sheffield 10.

Scientific Officer, Senior Scientific Officer or Principal Scientific Officer (preferably with experience in tissue culture techniques), for research work in virus diseases—The Secretary, Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh 9.

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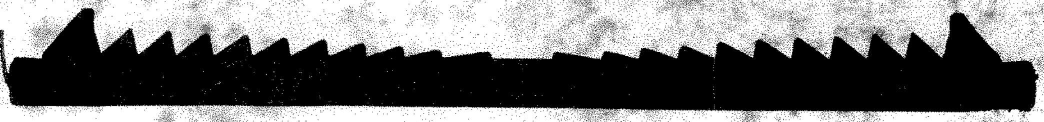

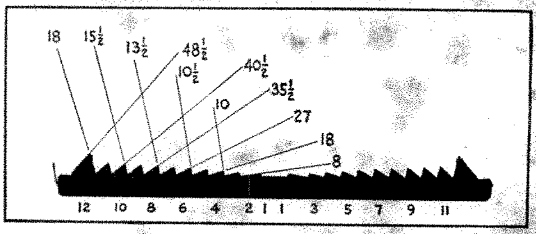

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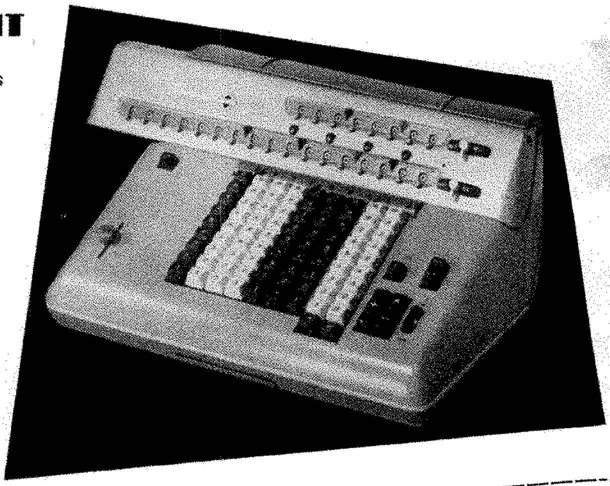

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A TIMELY REVOLUTION IN TECHNOLOGICAL EDUCATION

A SURVEY has been made to find out specifically what training a chemical engineer requires*. The results are interesting, since they provide a basis from which one may deduce a general principle regarding education of the technologist.

Chemical engineering is defined, in the *Encyclopædia Britannica*, as that branch of engineering which relates to the "design, construction, erection and operation of industrial plants in which matter undergoes a change of state or composition". The science, or craft, of the chemical engineer has been, and still is, the prime mover in developing the processes which serve to satisfy our technological needs. How best to fit the developing engineer for his task is, therefore, an important problem. The subjects which comprise his curriculum, as well as the time devoted to them, will determine, to a large extent, his ultimate effectiveness as a useful, well-integrated operator in industry.

With characteristic zeal for technological matters, the Americans, already in 1952, started to evaluate engineering training of all kinds†. An investigation was made by the American Society for Engineering Education. The method consisted in directing inquiries to a range of engineering colleges, industries and various professional organizations throughout the United States. The survey lasted three years; the replies were the considered opinions of authorities. Analysis revealed that, in a college engineering curriculum, 25 per cent of the time should be spent on mathematics and basic science, 50 per cent on engineering technology and the remainder on social studies, which include management techniques and applied economics. With its wide experience in industry and teaching, the Institution of Chemical Engineers in Britain recommends the following scheme: 45 per cent of the time to be spent on pure science and mathematics, 51 per cent on technological subjects and the remaining time on the social sciences, report writing and foreign languages.

No doubt these opinions are very useful, but they are nevertheless opinions. Realizing the need for factual information, Prof. R. E. Johnstone undertook an ingenious survey to find out in what way and to what extent the subjects taught at universities actually were being used by chemical engineers employed in British industry. With the aid of Mr. C. B. Lax, head of the Statistical Section at the Springfield Works of the United Kingdom Atomic Energy Authority, a questionnaire was so designed that replies would be "factual, quantitative, and amenable to statistical analysis". The questionnaires were sent to all corporate and graduate members of the Institution who were resident in the British Isles, except those engaged in full-time teaching. From a list of twenty-four sciences and techniques, engineers were requested to indicate which of these they had applied during the previous working week. To supplement information gained readily from almost two thousand engineers, Prof. Johnstone analysed his personal diary, a record of work done in industry over a period of twenty-eight years. This included most chemical engineering jobs in an eminently representative set of industries.

Results from these two complementary sources have been used, most elegantly, to design a suitable scheme of study for the engineer. Prof. Johnstone derived two limits within which the curriculum should lie. One limit, the "applied bias", represents a course of study in which the applied, technological type of subject predominates; the "pure bias", in contrast, stresses subjects for which there is not necessarily direct application. These limiting cases are summarized; so, too, is the scheme of the Institution as follows:

Subject	Per cent time of curriculum		
	Pure bias	Applied bias	Scheme of the Institute
Chemical engineering science	45	44	42
Pure science	22	14	34
Management and economics	6	19	2
Higher mathematics	13	9	11
Other engineering science	8	8	9
Report writing and foreign languages	6	6	2

Agreement with the scheme recommended by the Institution is remarkably close, except for pure science. Even if an engineering course with a leaning toward the 'non-applied' is required, the amount of time recommended for pure science by the Institution is rather excessive (34 per cent versus 22 per cent). At the other limit, namely, with an applied bias, more than twice as much time would be assigned to the study of pure science than is actually required by industry. There would be a consequent deficiency in time spent on training in management and economics.

Prof. Johnstone points out that much of the chemistry and physics used by practising chemical engineers is not above university entrance-level. Time allocated to such subjects probably could be spent far more effectively on topics of real engineering virtue. Moreover, as Prof. Johnstone states, "a scientist can practise his profession at a university, the engineer cannot". Engineering is a "large-scale activity which requires considerable resources in men, materials, accommodation and equipment before it can be carried on at all". Not only is a university engineering laboratory a very different place from an industrial factory, but also the work done is very different. Much of this information serves to confirm what experienced engineers have known for some time. Nevertheless, the advantages of such a survey are: "that it places the facts of the situation beyond reasonable argument" and "it provides quantitative data in place of judgments that have hitherto been largely qualitative", as Prof. Johnstone concludes. He and his colleague are indeed to be congratulated on their survey.

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* *A Survey of Chemical Engineering Education and Practice*, presented by Prof. R. Edgeworth Johnstone at a meeting of the Institution of Chemical Engineers, January 24, 1961.

† *Report on Evaluation of Engineering Education*, 1952–1955. American Society for Engineering Education, Illinois.

Now, the facts are plain. So, too, is the inescapable question: Would it not be of greater advantage to teach technology elsewhere rather than at a university?

There have been numerous complaints from industry that universities do not produce the kind of engineer required. From the academic side come weak rejoinders that industry does not know what is good for it. At last the solution is clear. Considering the demands of industries (and this survey has proved their demands most reasonable) the training of an engineer could probably be accomplished best by institutes of advanced technology, working in close liaison with industry. They would be similar in function to the practice-school of engineering at the Massachusetts Institute of Technology. Students there spend a large portion of their time in selected industries, but preserve close contact with the Institute.

The advantages are clear. Student engineers would have far more confidence when finally they are ready to begin their professional careers, due to their familiarity with actual processes of production. They will have learned, among a host of useful techniques to do working designs of fractionating towers with all the non-idealities involved, heat-balances on commercial furnaces, density measurements on fluid catalytic reactors and regenerators in operation, process 'trouble-shooting', and investigations on the idiosyncrasies of large-scale automation. They may have delved into marketing strategy, into the operation of consumer demand and/or company policy in determining the industrial processes to be used and into the methods of design for the best 'pay-out' on investment of capital. Moreover, they will gain an insight into the art of managing personnel and process operations. Along with this, they will develop an intuition for the enlightened guess-work which characterizes much of technology. Industry would welcome technologists who have been nurtured under its wing, so to speak.

Apart from the immeasurable financial advantage of having more efficient engineers in industry, the State would spend less in educating them. The unit cost of training a technologist is conceivably far less at a technical institute than at a university. Since industry will be supplying the *ne plus ultra* of engineering equipment, no longer will universities have to budget for expensive, though inadequate, substitutes. No longer will the academic staff suffer the malaise of not knowing whether to emphasize the 'applied' or the 'pure' aspects of learning. Happily, they will be free to encourage disinterested inquiry, the real function of a university.

If it seems better to teach technology in institutes separate from universities, then the question arises whether the engineering research which is carried out by universities should rather be done elsewhere. It is well to examine how the engineer aids technology in his research. In general, chemical engineering research appears as a stage after pure chemical research. When a new compound or a new way of synthesizing or converting known compounds has been discovered by a research chemist, and a market demand is established, the research engineer takes up the tale. He has to specify ultimately the type, size and operating conditions of equipment to yield the products required. Bench-scale experiments or pilot-plant work is used. Although pilot-plants are very expensive, it is often safer to base commercial designs on them, since scale-up from small experiments tends to be risky.

The theoretical problems involved are often quite abstruse. For example, the kinetics of the reacting system is usually a far cry from ordinary chemical kinetics, yielded by test-tube experiments. Complexities arising from heat, mass and momentum transfer must be taken into consideration. Engineering systems are non-ideal and hence not readily amenable to formulation. But here is where engineering research has displayed its particular genius in setting up mathematical models, often rough, but which serve, nevertheless, as reasonable approximations in describing physical systems. However, as the research engineer delves deeper into the theoretical aspects of these problems, he finds that he needs the tools of the applied mathematician and chemical physicist more and more. He needs to command a knowledge of mathematical probability theory, stochastic processes, variational principles, the absolute differential calculus, statistical mechanics and perhaps topology as well, to probe effectively into physical problems. Formal engineering education tends to fit him rather inadequately for this.

To do advanced experimental engineering research is a very costly business; consider items like shock-tubes, equipment for investigating turbulent shear flow, electronic computers, the apparatus for study of plasma-jets and so forth. Not only does industry have a personal commercial incentive to do such work, but also it commands the resources. It is unjust to say that industry is concerned entirely with empirical reseach. Consider, for example, from whence hails the revolutionary theory which explains the effect of interfacial turbulence on mass transfer. Large-scale industry is sophisticated enough to appreciate that to have research workers with advanced theoretical background in physics, chemistry and applied mathematics doing fundamental research can lead to unexpected results with vast technological implications.

Bearing all this in mind, it seems very obvious indeed that not only technology but also research in technology are out of place in the universities. Thus, the following system is recommended: technical institutes and industry will co-operate in producing efficient technologists; universities will play their part in educating first-rate applied mathematicians and chemical physicists to do research work in industry.

Sir John Cockcroft has stressed, on several occasions, the need for institutes of technology quite separate from universities proper. This timely transfer of technologies from the domain of the university is also contemplated by Dr. S. C. Curran in his article on p. 128 of this issue of *Nature*. Like winged *Drosophila*, they will take off to flourish in a new environment, leaving their erstwhile neighbours in the university to breathe more freely, too. The function of an institute of technology must not be confounded with that of a university. To preserve efficiency in this increasingly competitive world we must follow inevitably the economic dictum of 'division of endeavour'.

The ideal division thus seems to be with universities on one side. They will stress sound instruction and lecturing of the highest quality in the 'pure' fields. Research at the university should be of secondary importance and more of a fundamental, theoretical kind. The tenor of 'the academic market-place', it is hoped, will disappear. Academic staff will no longer be compelled to 'publish or perish'. On the other side, there will be the institutes of technology, well integrated with industry, which will thus exert a greater influence in determining the educational background of its technological force. Industry should not be denied this privilege. After all, it does supply the purpose for training technologists. The traditional, though lately diminishing, sovereignty of the universities should remain intact.

The net effect will be a stream-lining of physical science and a more potent research force. Technologists will be the fully fledged empiricists whom industry requires and they will know precisely what technological questions to ask. In the research departments of industry there will be a complementary force of well-equipped research workers. They will know where to seek the answers. Altogether, it seems reasonable that this would be a far more efficient, more manageable, neater and more powerful system than to have semi-empiricists of the twilight zone who neither know exactly what questions to ask nor how to set about answering them.

THE WORKS OF PAUL EHRENFEST

Collected Scientific Papers

By Paul Ehrenfest. Edited by Martin J. Klein. Pp. xii+632. (Amsterdam: North-Holland Publishing Company, 1959.) 90*s*.

PAUL EHRENFEST, the Dutch theoretical physicist, was born in 1880, took his doctor's degree under Boltzmann in 1904, and succeeded H. A. Lorentz in the chair of theoretical physics at Leyden in 1912; he remained there until his death in 1933. His active life therefore spanned most of the great period of transition from classical to modern theoretical physics, and his collected papers show the important part he had in helping on, interpreting, and clarifying this transition.

The earlier papers are mainly on classical statistical mechanics; in particular, Ehrenfest was much concerned with elucidating the basic ideas of Boltzmann's theory. To the same period belongs the great article on the fundamentals of statistical mechanics which was written in collaboration with his wife when he was in Leningrad, and was published in the *Encyclopädie der Mathematischen Wissenschaften* in 1912. In the later papers he dealt mainly with quantum statistics, methods of quantization and related topics. In particular, his famous paper on adiabatic invariants, which had so considerable an influence on the development of quantum theory, is also an outcome of his work on statistical mechanics.

His inaugural discourse at Leyden in 1913 is a good example of some characteristic features of his work, although it is only a semi-popular exposition. His subject was the downfall of the theories of the æther. He pictures an observer situated at the centre of a vast hollow reflecting sphere. The observer has by him a powerful lamp which he uses to send out a short flash of light uniformly in all directions. A little later the observer will see the whole inner surface of the sphere lit up simultaneously. Next, Ehrenfest asks us to think of the same vast sphere (and the observer) moving uniformly. If Newton were the observer, he would again expect to see the whole inner surface lit up simultaneously. Fresnel, however, would expect to see just a narrow band of illumination on an equator of the sphere. The illuminated band would then rapidly broaden until it reached the poles. Finally, Einstein, with the help of Lorentz, would expect to see the same as Newton. Here we have the elegant model used to illustrate a physical idea and to bring out the essential features of the problem. The discourse also provides an example of Ehrenfest's constant concern with the careful discussion of basic principles.

The short paper on the rotating dipole—written in collaboration with G. Breit in 1922—illustrates very well the difficulties of the period up to 1926 during which Ehrenfest was striving to clarify the old quantum theory of Bohr. It was a time when physicists stuck very closely, and very literally, to classical concepts. Ehrenfest and Breit of course had a model. It was an electric dipole which could rotate freely about an axis which was perpendicular to the dipole and passed through its mid-point. When the angle of rotation became $\pm f.2\pi$ where f is any large number (rational or irrational) the dipole is thrown back elastically. It is perhaps not surprising that Bohr's quantization rules could not cope with this system in a reasonable way. (It has a somewhat curious period and a quasi-period). However, Ehrenfest and Breit showed, in an elegant analysis, that the correspondence principle could be used to make the quantization rules yield the correct value for the angular momentum.

Ehrenfest admired H. A. Lorentz and held him in great respect; his obituary notice for Lorentz is included here. However, it is in an appreciation of Lorentz as a research physicist that we see his celebrated sense of fun for once managing to get into his writing. This appreciation was written on the occasion of Lorentz's seventieth birthday in 1923. The third paragraph is as follows:

"Perhaps too our ear suddenly recalls the variety of nuances of sound with which we have heard the name Lorentz pronounced by brilliant men of all nations. If an American pronounces it, or a

Frenchman or a Russian or a Scandinavian, or Planck, Boltzmann or Einstein, one can surely notice that each one means his own Lorentz. If a Netherlander has to mention the name Lorentz, he does it with pride, but softly, as if he fears that 'Perhaps Mr. Lorentz will be able to hear that he is being spoken about'." J. Hamilton

GENERAL PLANT CELL PHYSIOLOGY

Plant Physiology

A Treatise. Edited by F. C. Steward. Vol. 1*A*: Cellular Organization and Respiration. Pp. xxvii+331. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 13 dollars.

THIS is the first volume in order of numbering of the treatise on plant physiology in process of production under the general editorship of Prof. F. C. Steward, although it is actually the second to appear, the volume numbered 2 having already been noticed in these pages. Volume 1*A* contains three articles, the first on the plant cell and its inclusions, the others on some aspects of general plant cell physiology. It is desirable, indeed necessary, that a treatise on plant physiology should begin with an account of the cell, the system in which physiological actions take place, and R. Brown gives an account of this which is generally adequate and up to date. The conclusions drawn during recent years regarding cellular membranes, largely as a result of electron microscopy, are properly stressed, but as in all probability comparatively few readers will have had actual first-hand experience of the electron microscope, a brief description of how results are obtained by its use, and the reasons for the accepted interpretation of the structures so observed, might not have been out of place. These conclusions, it may be noted, in so far as they refer to the plasmatic membranes, fall well into line with those suggested by the conception of free space due to Briggs and Robertson.

The second article, by B. Vennesland, is entitled "Proteins, Enzymes, and the Mechanism of Enzyme Action". There is reason to believe that protoplasmic proteins, at least in many cells, are largely held in enzymes, and this account deals mainly with enzyme structure, which includes the structure of protein molecules, and the mechanism of enzyme actions. No classification of enzymes has ever received universal acceptance, and the author does not attempt a new one. However, in dealing with enzyme reaction mechanisms three different kinds of enzymes are considered separately. These are: (1) oxidation-reduction enzymes containing firmly bound prosthetic groups; (2) enzymes containing thiamine or pyridoxine derivatives; (3) pyridine nucleotide-linked dehydrogenases. Other aspects of enzyme reactions considered are their kinetics, inhibitors, group transfers and the problem of the active site. The article provides a good and succinct account of the present position of our knowledge of enzyme structure and action.

The final article deals mainly with what is generally called the mechanism of respiration and gives satisfactory accounts of the various ways by which it is now supposed the respiratory substrates are broken down, though perhaps more attention might have been given to the action of lactic dehydrogenase leading to the formation of lactic acid. I cannot agree with the statement that the production of lactic acid from pyruvic acid is "relatively rare in higher plants, though traces of lactic acid are found in potato fermentation . . . and in wheat and rice". Actually the production of lactic acid has been observed in other plants, while in potatoes Barker found that at the beginning of a period of anaerobiosis the production of alcohol and carbon dioxide might be negligible in comparison with the production of lactic acid. In tomato stems a production of lactic acid recorded as amounting to 13·7 per cent of the carbon dioxide produced can scarcely be described as a trace. Again, I do not think that the acceptance of the universality of the EMP pathway was as surprising and uncritical as the authors suggest. And, was such a universality as dogmatically asserted as they say? Surely it was no more than a tacit acceptance, a view which was readily modified when definite evidence of an alternative pathway was forthcoming.

The production of the volumes of this treatise is of the high quality we have come to associate with the publications of the Academic Press.

Walter Stiles

BONDS, BANDS AND SEMICONDUCTORS

Progress in Semiconductors

Vol. 5. Edited by Dr. Alan F. Gibson, Prof. R. E. Burgess and Dr. F. A. Kröger. Pp. vii+316. (London: Heywood and Co., Ltd., 1960.) 63*s*. net.

IS the band theory of semiconductors the modern phlogiston theory? Lavoisier's discovery of oxygen brought about the downfall of the latter; by some eyes the discovery of vitreous and liquid semiconductors is seen as the herald of the fall of band theory. Others, less extreme, see a better historical analogy to the current band-bond battle in the wave-particle war and consequently expect an amalgamation of the two theories. Band theory has reached a high degree of sophistication, as is evidenced by most articles in the latest addition to this invaluable series of progress reports. The chemical bond approach discussed by E. Mooser and W. B. Pearson is by comparison in its infancy. The authors discuss rules derived from it for predicting semiconductivity (their own rule appears in a modified form which allows for cation-cation bonds), and then attempt to classify semiconductors on the basis of their structures. In the concluding sections some intriguing empirical graphs relating semiconductor and structural parameters are reproduced. Some discussion of alloy semiconductors, especially as regards superlattice formation, might have been included.

Optical properties, as the editors admit, predominate in this issue. T. P. McLean reviews recent work on the absorption edge spectrum of semiconductors, in particular of germanium and silicon. B. Lax and S. Zwerdling have obviously striven to minimize the mathematics in their survey of magneto-optical phenomena. In the well-balanced meal they produce there is both meat for the specialist and gravy for the beginner. T. S. Moss, in a short article on indium antimonide, omits chemical and structural work in his haste to reach the optical properties where, of course, his interests lie. The sixty-nine references might well have been considerably increased. His final section on applications is of special interest.

The electrical properties of semiconductor surfaces, a very difficult subject, is ably reviewed by T. B. Watkins, with 26 figures in the text and 99 references. He concludes with some pertinent comments on the effects of surface conditions in semiconductor devices.

J. Appel's article on thermal conductivity of semiconductors is concerned almost exclusively with the theoretical aspects of the subject, which are developed from two angles: first, phenomenological, and secondly, quantum mechanical. The conclusion touches very briefly on the practical problem of thermoelectric cooling. The author is to be congratulated on the clarity of his exposition since English is not his native tongue.

R. R. Haering and S. Mrozowski give a masterly survey of the band structure and electronic properties of graphite crystals. The authors divide the properties they discuss into two groups: those which can be explained on a band model and those which cannot as yet. This article should inspire further work as it stresses unsolved problems.

A welcome addition at the end of the book is a table of contents of past and future volumes. Many interesting articles on the properties of semiconductors in general and the elements germanium, silicon, carbon, tellurium and bismuth in particular are in preparation. There are, however, several bad gaps in the programme. Compound semiconductors and their alloys seem ignored entirely. Interest in these stems especially from thermoelectric cooling and thermoelectric energy conversion, and so it is not surprising to find these topics absent too. No proposed article deals specifically with any topic in semiconductor chemistry or crystallography. This may be deliberate policy, but, if so, perhaps the editors should change the title of the series to *Progress in Semiconductor Physics*.

B. R. PAMPLIN

MICROSCOPIC STUDIES OF REFLECTING SURFACES

Surface Microtopography

By Prof. S. Tolansky. Pp. viii+296. (London: Longmans, Green and Co., Ltd., 1960.) 55s. net.

PROF. TOLANSKY describes here a series of investigations made with the collaboration of his research students over the past fifteen years or so. Most of the work has involved the use of a microscope, in some cases with phase-contrast techniques, but more often with the multiple-beam interference methods for which Prof. Tolansky is famous. The space devoted to each topic is necessarily short, but each is illustrated by many beautiful photographs printed on the glossy paper used throughout the book.

As the author remarks in the preface, the book is a kind of atlas, in which the 359 excellent pictures show 'maps' of various physical features of the structure of solid surfaces and their behaviour under the action of physical agencies. It is remarkable how wide a field of study is open to the application of multiple-beam interferometry in spite of the rather stringent limitations set by the need for the specimen to be able to withstand the cleaning and vacuum evaporation of silver without change. A large part of the book is concerned with crystals, particularly diamond, and the investigations cover aspects of their growth, cleavage, and etching, as well as studies of hardness and wear in relation to the crystalline structure. Other sections of the book include selected features of work on thin films, metal crystals and hardness testing, quartz crystals, silicon carbide, mica, and piezoelectric oscillators. The methods used are capable of extremely high sensitivity, and it is claimed that, in favourable cases, height differences of only a few angstroms can be estimated.

Something of value can be found here by those engaged in any of the branches of study included in the book, but in addition the book as a whole is valuable to students of optical techniques and to anyone who is likely to be inspired and stimulated by a fascinating account of highly informative experimental work carried out with very simple means. The text conveys the enthusiasm Prof. Tolansky so obviously has for this subject, and the way in which the story of each investigation is related holds the imagination and carries the reader along from the unravelling of one knot in the mystery to the next.

The first five chapters are concerned with the basic techniques of multiple-beam interferometry, and include better illustrations and diagrams of the equipment than have been published before. The diagrams, however, are still barely adequate to give more than just an idea of the arrangement of the apparatus required, and in some respects are actually misleading. One or two near-scale drawings of the apparatus would have been much more helpful to anyone wishing to try the technique, although this book is obviously not intended to be an instruction manual.

The remaining eighteen chapters are arranged in six groups, covering the topics already mentioned, and accompanied, where necessary, by further description of special techniques. The volume is concluded by two addenda introducing two more special interference systems, and two bibliographies confined to the publications of Prof. Tolansky and his students, and to the theses of his students. Other references are given in the text of the book as they occur. Some misprints and distortions of language seem to have escaped the proof-reading and editing, and this is annoying in a book of this price and quality; in a few cases the account of an experiment seems to have been shortened to an extent which makes the argument difficult to follow. A possible case of this occurs on p. 194, concerning the electro-deposition of layers, where the explanation seems to show an inconsistency with the illustrations. The point at issue here would best be resolved by line diagrams of the profiles of the edges of the layers, as in Plate 242. More profile diagrams of this kind would be helpful at other places too.

These minor defects do not detract much from the value of the book, if at all, as its chief function would seem to be as an introduction to the unique studies described more fully in the papers and theses on which it is based, and as a fascinating and stimulating account of a great deal of masterly experimental work.

J. W. GATES

Gasdynamic Discontinuities

By Wallace D. Hayes. (Princeton Aeronautical Paperbacks, No. 3.) Pp. v+68. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 1.45 dollars; 11*s.* 6*d.* net.

Small Perturbation Theory

By W. R. Sears. (Princeton Aeronautical Paperbacks, No. 4.) Pp. v+63. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 1.45 dollars; 11*s.* 6*d.* net.

Higher Approximations in Aerodynamic Theory

By M. J. Lighthill. (Princeton Aeronautical Paperbacks, No. 5.) Pp. v+147. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 1.95 dollars; 16*s.* net.

High Speed Wing Theory

By Robert T. Jones and Doris Cohen. (Princeton Aeronautical Paperbacks, No. 6.) Pp. v+243. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1960.) 2.95 dollars; 24*s.* net.

THAT there is a strong case for publishing 'The Sonnets' independently of the 'Complete Works' is scarcely to be disputed. There is at least as strong a case for separate publication of some, if not all, of the parts of the heterogeneous and overlapping collection of papers by dozens of authors which will eventually make up the twelve volumes of the Princeton Series on "High Speed Aerodynamics and Jet Propulsion". From the pleasantly rounded presentation of the individual contributions it appears that the ties of co-operative endeavour did not too severely constrict the individual authors. Consequently, the six paper-back extracts so far republished should meet a lively demand from those who have been attracted by the two-inch-thick volumes of the original series and from those who have been daunted by them; they will be warmly welcomed by students.

Numbers 1 and 2 in the paper-back series are concerned with rockets. Numbers 3, 4, 5 and 6 are respectively Vol. 3 Section *D*, Vol. 6 Section *C*, Vol. 6 Section *E* and Vol. 7 Section *A* of the Princeton Series, all widely applauded when they first appeared a few years ago. Number 6, by Mr. and Mrs. Jones, stands particularly well on its own as a really excellent text-book. In subsequent paper-backs in this series the publishers ought briefly to explain their system of cross-references.

Beiträge zür Physik und Chemie des 20 Jahrhunderts:

Lise Meitner, Otto Hahn, Max von Laue zum 80 Geburtstag. Herausgegeben von O. R. Frisch, F. A. Paneth, F. Laves und P. Rosbaud. Pp. xii+285+3 tafeln. (Braunschweig: Friedr. Vieweg und Sohn, 1959.) 29.50 D.M.

THIS is a collection of essays which was published to mark the eightieth birthday of three distinguished scientists, Lise Meitner, Otto Hahn and Max von Laue. About two-thirds of the book is in German and the remainder is in French and English. Some of the essays are historical and others contain accounts of very recent work; all are designed to demonstrate the importance of the early fundamental work of the three great pioneers. Many of the papers undoubtedly make extremely important contributions to knowledge; the present writer is much more concerned, however, with another aspect. Prof. Hooykaas of Amsterdam, speaking at the "Technology and Purpose" Conference at Oxford in 1959, deplored the impersonal nature of modern scientific writing. He pointed out as an example that in early chemical text-books the experimenter was portrayed in full academic robes whereas in modern texts the experimenter is eliminated; the test-tubes apparently support themselves.

The inestimable value of this collection of essays is that it begins to sketch in the character and personality not only of the scientists honoured but also of the many contributors. For example, we find Prof. Przibram recounting anecdotes about other learned professors, we find Dame Kathleen Lonsdale throwing stones into a pond and we find a paper by Prof. Rosenblum on "Spectres Magnetique des Particles Alpha" beginning "Je regarde mon pouce . . . ".

I can read German only with great difficulty and continual recourse to a dictionary; but in spite of this three vivid impressions have been obtained. The first is of the explosive growth of science in the fifty or so years covered; the second is of the immense fruitfulness of the work of the three great pioneers which stimulated this growth; the third is that perhaps the greatest tribute of all is simply that they should inspire the contributors to write in such warm human terms on subjects that would normally be regarded as completely objective and impersonal.

C. A. TAYLOR

Fonctions Sphériques de Legendre et Fonctions Sphéroidales

Tome 3. Par Dr. Louis Robin. (Collection Technique et Scientifique du Centre National d'Études des Télécommunications.) Pp. viii+289. (Paris: Gauthier-Villars, 1959.) n.p.

THIS is the concluding volume of Dr. Robin's encyclopædic treatise on Legendre functions and related topics; for the English reader, it supplements Hobson's 1931 treatise by its account of work done in recent years. The main sections deal with addition theorems, zeros, problems relating to a spheroid, torus or cone, and with functions allied to the Legendre functions, such as the Gegenbauer polynomials and functions. There is also a section on the spheroidal functions, mainly an epitome of the full account in Meixner and Schäfke, *Mathieusche Funktionen und Sphäroidfunktionen*, and a brief note on spherical Bessel functions. There are numerous bibliographical references, and useful lists of available numerical tables. The text, with its inevitable mass of complicated formulæ, is scarcely light reading, but the book forms a most valuable work of reference, well deserving a place in any mathematical library.

Biochemical Preparations

Vol. 7. Edited by Henry A. Lardy. Pp. ix+102. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1960.) 42*s.* net.

THE series of *Biochemical Preparations* needs no special commendation in these columns. It will be sufficient to say that Volume 7 extends and enhances the reputation of established usefulness and merit which the series has already gained, and that it betrays no sense of strain or dilettantism in the choice of matter forming the list of contents.

Of particular interest in the present volume are the methods for the preparation of N-acetylneuraminic acid from human milk; for preparing phosphorus-32-labelled adenosine di- and triphosphates by both chemical and enzymic means—the latter neatly adapted to give A-P-P*-P and A-P-P-P* as well as A-P-P*-P* ; and for a preparation of flavine adenine dinucleotide from the mycelium of *Eremothecium ashbyii* which gives better yields than the more usual preparations from animal tissues or yeast. The other methods included are for : β-amino*iso*butyric acid ; carbamyl phosphate ; (−)-carnitine chloride ; cerebrosides ; coproporphyrin III tetramethyl ester ; 6-deoxy-6-fluoro-D-glucose ; dihydroxyacetone phosphate ; D-fructose 1-phosphate ; 3-hydroxy-L-kynurenine ; hydroxypyruvic acid phosphate ; methylmalonic semialdehyde ; potassium dihydrogen $L_s(+)$-*iso*citrate ; S-palmityl coenzyme-*A* ; stearic acid, methyl stearate and homologous compounds ; stigmasterol ; and tetrahydrofolic acid.

This simple enumeration will be sufficient proof of the pertinence to the needs of the research worker which the volume maintains. Since a different editor-in-chief is appointed for each volume of *Biochemical Preparations* it is fitting to record here our thanks to Prof. Lardy, who has so successfully compiled and presented the volume under review.

F. H. Malpress

Hospital Infection

Causes and Prevention. By R. E. O. Williams, R. Blowers, L. P. Garrod and R. A. Shooter. Pp. x+307. (London: Lloyd-Luke (Medical Books), Ltd., 1960.) 35*s*. net.

A SYSTEMATIC approach to the causes and prevention of hospital infection is much to be welcomed. Accurate records are meagre and the problem is one which belongs to everybody and, consequently, to no one. This new book by four well-known specialists can fairly claim to provide a comprehensive review of how infections are spread, how they can be controlled and, more apposite, how they can be prevented.

Since streptococcal infections now cause no real difficulties—they still respond to penicillin and have acquired no resistance to the drug—the book is mainly concerned with the staphylococcal infections which, because of their resistant strains, are the main source of infection and worry in hospitals today. For a medical publication, the cautious and almost agnostic approach is refreshing—no views are put forward unless they can be substantiated with demonstrable facts. The text is clear and logically presented, and adds to the value of a book which should be useful not only to pathologists and bacteriologists but also to surgeons, pædiatricians, sister tutors, hospital administrators, and equally important, hospital architects.

Kathleen M. Hawkins

An Introduction to Statistical Thermodynamics

By Terrell L. Hill. (Addison-Wesley Series in Chemistry.) Pp. xiv+508. (Reading, Mass., and London: Addison-Wesley Publishing Company, 1960.) 74*s*.

THIS introductory text on equilibrium statistical thermodynamics is based on the elementary principles of quantum mechanics and covers a wide field of applications in both chemistry and physics. Classical statistics are not developed independently, but there is a short chapter on the transition from quantum statistics in which the treatment, however, is so curtailed as to be of doubtful value to the uninitiated. Indeed, because of the extensive coverage of modern developments, it has been necessary throughout the book to condense the mathematics in some measure, and the student who is specializing in either chemistry or physics might feel that the inclusion of much material, which inevitably must be outside the scope of his course, does not compensate for this lack of mathematical detail.

The book is divided into four parts, the first of which is devoted to a discussion of the various ensembles and to the statistical-mechanical treatment of the second and third laws of thermodynamics. The fundamentals having thus been established, their applications to systems of independent particles are next considered, and in the third part, which is concerned with interacting particles, there appears a comprehensive survey of the problems encountered in the study of liquids and solutions. Finally, distributions such as those of Fermi–Dirac and Bose–Einstein are derived.

Misleading statements noted include a reference to the temperature of a single molecule (p. 71) and an implication that the dimensions of both an area and a volume in classical phase-space are the same, namely those of Planck's constant (p. 113).

H. Steeple

British Scientific and Technical Books, 1953–57

Edited by L. J. Anthony. Pp. ix+251. (London: James Clarke and Co., Ltd., 1960. Published for Aslib.) 56*s*. net.

THIS volume is essentially a continuation of *British Scientific and Technical Books, 1935–1952*, following the pattern of that earlier bibliography and including entries for the more important and useful books on science and technology published in the British Commonwealth between January 1953 and December 1957. Entries are based on those in the *Aslib Book List* and relate primarily to books published through normal trade channels by commercial publishers. Government publications, monographs, and reports and papers issued by societies and institutions have been included only if they represent important contributions to the knowledge of the subject not otherwise obtainable. Entries for later editions of books noted in the earlier volume are included only if the new edition contains significant alterations or additions ; straightforward reprints are omitted. Besides including those books in such fields as documentation, libraries, psychology, planning and architecture which are of interest to technical readers, every effort has been made to include in the scientific and technical sections books which assist the understanding of the subject by non-technical readers.

The subject headings in the classified bibliography follow the order of the Universal Decimal Classification, but some modifications which have come into use since the issue of the latest Abridged English Edition have been used. Under each class heading entries are arranged alphabetically by author and the entries are annotated to indicate their scope and suitability for three different classes of readers or whether they are reference works such as dictionaries, encyclopædias, etc. There are an author index and a subject index. The volume is admirably produced, but its value would be appreciably enhanced if the time lag between the date of the latest entries and that of publication of the volume could be reduced ; on the present basis of compilation it does not seem too much to expect that this interval could be reduced by at least a year.

R. Brightman

PROPOSED MOLECULAR MODELS OF GRAMICIDIN S AND OTHER POLYPEPTIDES

By DR. DONALD T. WARNER
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Introduction

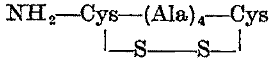
ONE of the most interesting problems in protein and polypeptide chemistry is the tertiary structure or conformation of the amino-acid chain and the relation of this structure to the biological activity of the compound. Some of the probable forces holding the polypeptide chain units in position have been studied extensively; but since the initial work of Pauling *et al.*[1] the hydrogen-bonding forces between —C=O and —NH groups which are believed to result in helical configurations have played a dominant and almost overwhelming part in nearly all the proposed tertiary structures of even the simplest polypeptides. Although the hydrogen bonds between NH— and —C=O groups are important forces, there is at least some reason to question their stability in the aqueous system where biological activity is nearly always encountered. Edsall and Wyman[2] suggest the possibility that "in aqueous solutions a helical polypeptide chain is rather likely to unfold into a more open and random type of coil". In a similar manner Schellman[3], from a study of the urea-water system, has concluded that hydrogen bonds, taken by themselves, give a marginal stability to ordered structures, which may be enhanced or disrupted by interactions of side-chains.

These questions about the relative stability of —NH——O=C— hydrogen bonds in the competitive aqueous system have opened the way for the serious consideration of other forces which may be operative under biological conditions to influence the tertiary structure of proteins. The interesting work by Kauzmann[4] concerning the probable effect of the hydrophobic attraction of the non-polar side-chains of peptides or proteins is an example of a new emphasis in the problem of tertiary configuration. His recent review[5] of the forces responsible for maintaining the native configurations of proteins includes a more complete exposition of the hydrophobic attractions as well as the dispersion forces (London forces) which are closely related to them. Equally provocative has been the extensive work with poly-L-proline[6a-g]. In this polymer all the usual considerations, such as peptide —NH——O=C— hydrogen bonds, disulphide linkages, and electrostatic attractions, are completely absent so far as can be seen, and yet the poly-L-proline exists in two definite stable configurations which are seemingly dependent for their stability upon the solvent systems employed. One stabilizing force which could apparently be present in this polyamino-acid in aqueous solution would be the hydrophobic attractions of the non-polar side-chains. Thus the nearly planar pyrrolidine rings of the L-proline units in a molecular model can be piled above each other like a series of staggered plates with a spiral of carbonyl oxygen atoms going up around the outside of the pile. (Cowan and McGavin[6a] actually indicate that in the pyrrolidine ring the γ-carbon atom is about 0·5 Å. out of the plane.) Since the distance between these carbonyl oxygens on the spiral is such that a water molecule fits between two adjacent oxygens almost exactly in the model, it is perhaps not out of order to consider that such a spiral could be stabilized by a combination of hydrophobic attractions of the rings and hydrogen bonding of water molecules between carbonyl oxygen atoms to yield the so-called *trans* form of poly-L-proline (poly-L-proline II)[6g]. I have prepared such a model, but prefer not to present it for discussion since with available construction kits it is impossible to represent the γ-carbon displacement (0·5 Å. out of the plane of the ring), an item of considerable importance in a structure containing only proline units. It is interesting that poly-L-proline II (stable in the aqueous system) can be converted back to poly-L-proline I by acetic acid–propanol[6d], a solvent system which might be expected to disrupt hydrophobic attractions.

In my work on molecular models I have been most concerned with some of the natural products of intermediate size and well-established structure containing relatively few amino-acids. These smaller polypeptides have frequently been characterized not only with regard to their amino-acid sequences but also in other fine points of structure such as possible ester linkages, ε-amino lysine linkages, and the presence of D-amino-acids. Many of the polypeptide antibiotics and hormones are in this category. Since most of these molecules have interesting biological activity, and lend themselves fairly conveniently to the construction of molecular models, it was hoped that such a study would give some clue to structural similarities other than amino-acid sequence which might be the basis for a common biologically active unit.

Theoretical Considerations

In attempting to define a common structural feature in these highly reactive polypeptides, I first of all considered the possible conformations of the cyclic ring which is present, for example, in a molecule such as oxytocin. This disulphide stabilized structure has 22 atoms in the ring, and the intact cycle is essential for maximum activity in molecules which possess this system. However, in my opinion, one of the interesting structural features in this cycle is the presence of six amide linkages, and the fact that these groupings can be arranged in a strainless hexagonal configuration with the carbonyl oxygen atoms at the corners of the hexagon. For the purpose of illustrating this concept, a molecular model of a relatively simple (and hypothetical) cystine-stabilized structure,



$$\underbrace{NH_2\text{—Cys—(Ala)}_4\text{—Cys}}_{\text{S—S}}$$

is pictured in Fig. 1*A*.

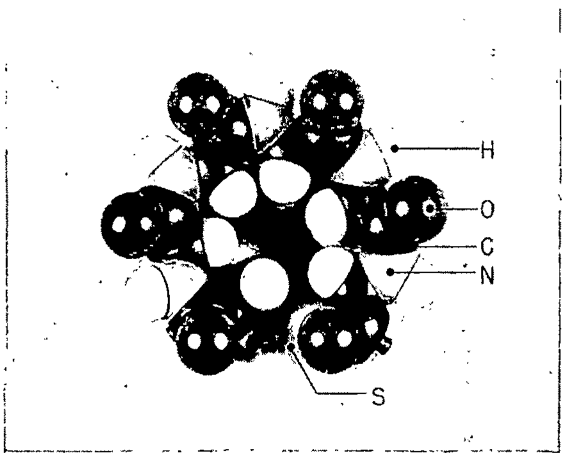


Fig. 1*A*

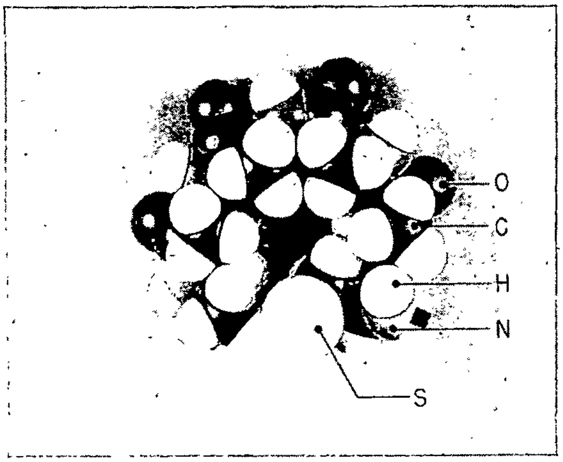


Fig. 1*B*

This amide side of the hexagon, which I shall refer to as the 'front' or hydrophilic side, shows a very compact arrangement of the α-carbon atoms as a central ring within the hexagon and the α-hydrogen atoms of the L configuration pointing inward to form a nearly circular pattern around an open central area. (In all these models, the absolute configuration for the L-amino-acids as determined by J. Trommel and J. M. Bijvoet[7], was used. All the models illustrated here were made with the Catalin space-filling molecular models (Catalin, Ltd., Waltham Abbey, Essex, England).) The other side of this hexagonal model, which I shall refer to in this and subsequent discussion of other models as the 'back' side or hydrophobic side, is shown in Fig. 1*B*. It will readily be seen that the arrangement of amide-groups suggested for the 'front side' has the consequence of bringing all the non-polar methyl groups on the 'back side' into very close proximity. Thus the possibilities for maximum hydrophobic attractions which are suggested as desirable for an aqueous environment by Kauzmann[4,5] are perhaps more rigidly satisfied by this hexagonal structure than by other possible conformations. In addition to these favourable non-polar interactions on the 'back side' of the model, the carbonyl oxygen atoms on the 'front side' of the plane are almost exactly 4·8 Å. from each other[8], which would be the proper distance to hydrogen bond one water molecule based on an oxygen–oxygen distance of about 2·90 Å. in liquid water (at 1·5° C.) and an H—O—H angle of 109°. Thus the proposed hexagonal configuration of Figs. 1*A* and *B* could be the favoured form in an aqueous environment by virtue of the co-operative combination of (*a*) 'back side' hydrophobic attractions and (*b*) 'front side' hydrogen bonding of water by adjacent carbonyl oxygen atoms.

In joining the disulphide linkage of the cystine unit, we have taken into consideration the most stable conformation of the —CH_2S—SCH_2-linkage with a dihedral angle of 90° between the methylene groups as discussed by Calvin[9]. This dihedral angle imposes certain restrictions on the approach distance of the two carbonyl oxygens of the cystine unit and may be a probable factor in the uniform spacing of these two atoms. Other implications of the configuration of the disulphide linkage on chain structure are discussed by Haurowitz[10]. Although Figs. 1*A* and *B* picture a hypothetical polypeptide containing the simplest non-polar side-chain of alanine with a cystine unit in order to present the general principles of our proposal, the models could represent a possible general conformation of the oxytocin ring.

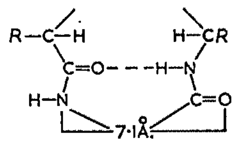
A second consideration in applying this concept of hexagonal structures as probable reactive units can be observed with the cyclic decapeptides such as gramicidin *S* (ref. 11), circulin[12], and tyrocidin *A* (ref. 13) or *B* (ref. 14). In one of the possible conformations, these molecules can be shown to be capable of existing as two fused hexagons somewhat analogous to the six-member and ten-member benzene–naphthalene situation. This approach to the structure of the cyclic decapeptides also offers some interesting clues to the structural necessity of certain D-amino acids, which I hope to point out as the discussion progresses.

A third consideration in this concept of hexagonal structures as reactive units concerns the open central position in the hexagon, which is readily apparent in Figs. 1*A* and *B*. Since this open central position is in a definite fixed position in the hexagon, it could serve as an opening through which a reactive side-chain group (for example, δ-NH_2 group of ornithine, γ-CO_2H of glutamic acid) could enter the 'front' side of the hexagon at a rigidly determined position. This possibility too will be discussed in greater detail for specific compounds.

Keeping in mind then the three concepts of (*a*) a hexagonal configuration with an amide or hydrophilic 'front' side and a side-chain or hydrophobic 'back' side, (*b*) the possibility that two or more hexagons may appear as fused units and (*c*) a fixed open centre in the hexagon for side-chain reactive groups, I propose to examine the complete models of some of the simpler naturally occurring polypeptides. In particular we are interested in gramicidin *S*, since a proposed conformation for this antibiotic has recently been published[15,16]. I would also like to extend this discussion to the antibiotics etamycin and actinomycin *D* (C_1) for reasons to be presented later.

Gramicidin S

The primary structure of gramicidin *S* is probably one of the best-established sequences of the polypeptide antibiotics[17] and in addition some of the X-ray dimensions and the overall shape of the molecule have been determined[15]. The proposed conformation of Hodgkin *et al.*[15] as adopted by Schwyzer[16] is based entirely on internal hydrogen bonding to hold the structure together. A model of their conformation is illustrated in Figs. 2*A* and *B*. This suggested structure places the component amino-acids essentially in two parallel chains joined by proline units at the ends, presumably because proline has been frequently associated with directional changes in the polypeptide chain. Only four of the eight possible —NH groups are actually engaged in intramolecular hydrogen bonding. The two proline residues, of course, have no —NH groups, and the other four —NH groups are presumed to be hydrogen bonded intermolecularly. On the 'front side' of this model (Fig. 2*A*) the two side-chains of ornithine are located above the plane of the amide linkages far from any association with other side-chains. Since these two side-chains are not apparently restricted to any position, the reactive amino-groups will not have a definite fixed place in this conformation. When the model is examined on the basis of Kauzmann's[4,5] discussion of hydrophobic attractions and dispersion forces, the Hodgkin–Schwyzer conformation[15,16] seems to offer little opportunity for maximum hydrophobic interaction of the alkyl side-chains. Not only is there considerable open space between non-polar groups on the same side of the principal plane of the molecule, but also some side-chains are actually on opposite sides of the plane. In fact, it would appear from the models that, in general, the *trans* configuration of the amide-group is less favourable for maximum hydrophobic attraction of side-chains on adjacent amino-acid units since the α-carbon atoms carrying the side-chains are always farther apart when the amide linkage is *trans*. In a similar manner, although Kauzmann indicates that aromatic rings will be in the most favoured position when they are close together, their configuration[15,16] finds the two benzene rings of the respective D-phenylalanine units as far apart as they could possibly be, at the opposite ends of the parallel chains. The structure indicates no particular geometrical advantage in having the D-form of phenylalanine instead of the L-isomer at that position. The overall dimensions of the model do not seem to be particularly close to the rectangular dimensions of 11 Å. × 19 Å. suggested by Hodgkin's X-ray data[15]. Indeed, if one lays out diagrammatically the distances involved between the two parallel chains (taking a liberal value of 3 Å. for the —O——H—N hydrogen bond):



then the average width of the proposed rectangle is about 7 Å. rather than the 11 Å. value determined by the X-ray method. One or two of the unbonded carbonyl oxygens may be about 10 Å. apart, but that would represent the maximum width at one or two places only. The overall length of the model as shown would correspond to a value of about 24 Å., although this could be varied somewhat by pointing the benzene rings downward in an isolated position.

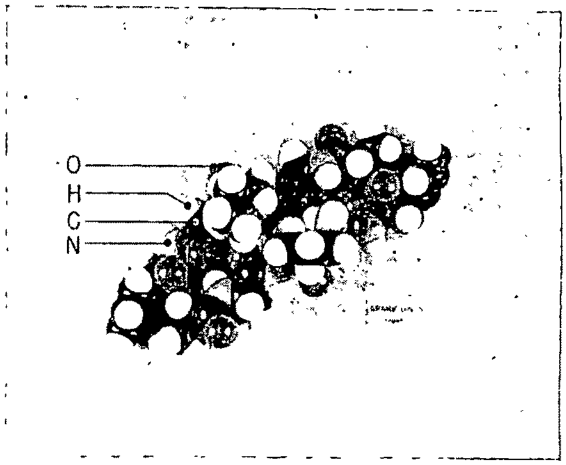


Fig. 2*A*

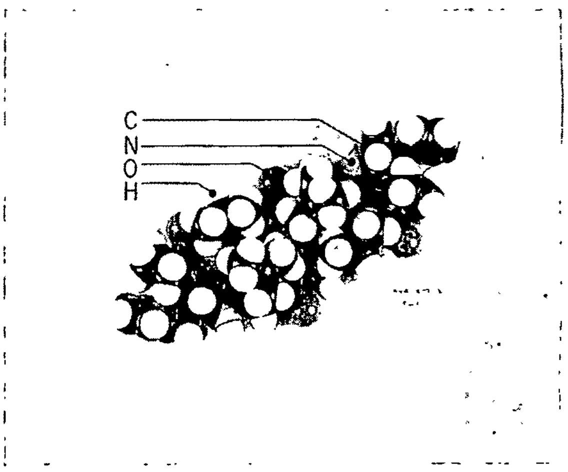


Fig. 2*B*

As an alternative suggestion for the conformation of gramicidin *S*, I would like to discuss the structure which is indicated in Figs. 3*A* and *B*. The formulation of this model has been based on my previous observation that the cyclic decapeptide ring can be readily arranged in the shape of two fused hexagons, a virtually strainless structure employing the usual bond-angles. In this fused system, the carbonyl oxygen atoms again form the corners of the hexagons, and the α-carbon atoms form two closely knit rings within the individual units. Now in a model of such a cyclic decapeptide, if all the amino-acids were L-amino-acids, the α-carbon atoms would project only a ring of α-hydrogen atoms toward the centre of the molecule, and at the fusion point of the two hexagons there would be a considerable open space. However, in the case of the gramicidin *S* decapeptide there are two D-phenylalanine molecules in the cycle just five units apart. If these two components are placed at the juncture position of the two rings, the opposite D-configuration at that position projects its non-polar side-chain (instead of the single α-hydrogen atom) into the central open area and the open space is neatly filled. Indeed, the —CH_2— groups of the two D-phenylalanine side-chains in this suggested conformation so direct their respective benzene rings into the central space that the two rings are exactly side by side. Not only does this projection of the side-chains completely fill the open fusion junction but it also fulfils elegantly Kauzmann's suggestion[5] that "the aromatic rings of phenylalanine will prefer to be close to one another" in the most desirable hydrophobic arrangement. The smaller central open area in the individual hexagonal units is still available for the entrance of the δ-amino ornithine groups from the 'back side' of the structure. It will be noted that, if one prefers to presume that proline units are associated with a change in the direction of the polypeptide chain (a point which may still be open to question), then the proline units adjacent to the D-phenylalanine units are also located at the proper positions. In this conformation a look at the 'back side' (Fig. 3*B*) of gramicidin *S* is even more revealing, since it clearly shows the attractive possibilities for maximum hydrophobic bonding. With the two benzene rings fitted nicely side by side, these aromatic substituents are also flanked by the nearly planar rings of the two proline units to form four compact layers. On either side of this central compact layer we have the non-polar groups of valine, ornithine, and leucine fitted closely together with maximum hydrophobic attraction. The δ-amino groups of the two ornithine side-chains are thus brought firmly into position in the two small open centres of the individual hexagon units, and appear in a fixed position in the 'front side' of the structure (Fig. 3*A*). The general shape of the 'back side' of gramicidin *S* in our proposed model is roughly rectangular. Because of the layered arrangement of the pro-phe-phe-pro side-chains it is possible to calculate the width of this rectangle from the standard approach distances of aromatic nuclei and the van der Waals's radii of methylene groups[18]. This calculated value is 10·8 Å., very close to the observed value of 11 Å. The length estimated from the Catalin model is about 16–17 Å., which is not in complete agreement with the observed value of 19 Å. However, these dimensions do not take into account the possible contribution to the overall size by three moles of water and two moles of ethanol known to be present in at least one of the crystals examined[15]. I would like to emphasize again that I am proposing this conformation as the one which might be stabilized by forces operating in an aqueous system, which may or may not be different in their magnitude or direction from forces operating in the crystal state or in a non-aqueous environment.

The points which seem to favour our conformation of gramicidin *S* (Figs. 3*A* and *B*) may be summarized as follows:

(*a*) The overall size and shape of this conformation as indicated from the model and actual contact distances seem to fit the X-ray data perhaps somewhat more satisfactorily than the previously proposed conformation of Hodgkin and Schwyzer[15,16].

(*b*) The structure gives maximum aggregation of hydrophobic groups, together with a most favourable placing of the aromatic rings of the D-phenylalanine units.

(*c*) The fused hexagon arrangement indicates a probable specific structural advantage or requirement for the presence of the two D-phenylalanine units in the molecule, a point that is not so apparent in the alternate arrangements thus far proposed.

(*d*) The smaller open central areas in the two hexagonal units provide spaces for the rigid arrangement of the two δ-amino-ornithine groups at fixed distances from each other on the 'front side' of the molecule. This 'front side' may be regarded as the hydrophilic side of the structure, and may be the reactive surface of the molecule.

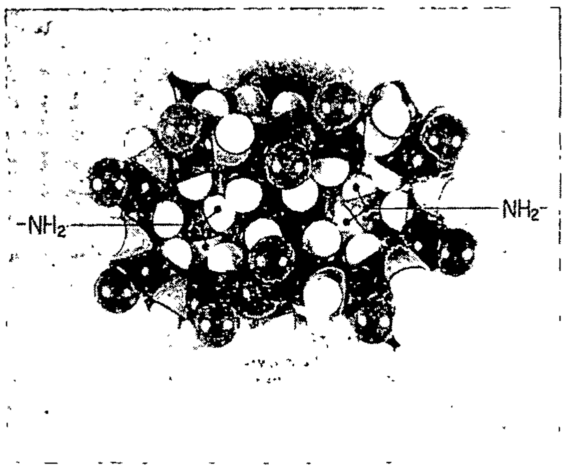


Fig. 3*A*

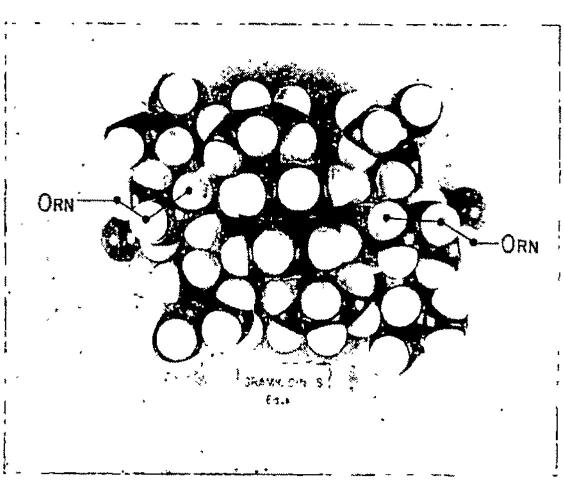


Fig. 3*B*

One other feature of the 'front side' (Fig. 3*A*) of gramicidin *S* may be worth mentioning. Schmidt[15] emphasizes in a discussion of the X-ray data that "there appear to be many groups about 4·8 Å. apart". Similar reflexions of approximately 5 Å. were observed by Cowan and Hodgkin[19] in their work with insulin and gramicidin *B*. It may be suggested that eight of the ten oxygen atoms at adjacent corners of the fused hexagons are approximately 4·8 Å. apart as estimated from the models and from diagrams based on measured angles and interatomic distances. The two centrally located amino groups are also at a similar distance from the carbonyl oxygen groups.

Etamycin and Other Polypeptides

The complete lack of —NH groups already noted for poly-L-proline is a feature which is shared to a somewhat lesser extent by at least two classes of naturally occurring polypeptide antibiotics. Thus, for example, in etamycin[20] and the whole spectrum of actinomycin antibiotics[21a–d] a majority of the amino-acids in the molecule have secondary amino groups (such as sarcosine, N-methylvaline and others) which prevent these units from participating in —NH bonding with other carbonyl groups. Therefore the tertiary structure of these compounds is more probably determined by other forces such as hydrophobic bonding, at least when compared with compounds having a full quota of —NH groupings. Indeed, as if to augment the low number of —NH bonds, the percentage of amino-acids with non-polar (that is, hydrophobic) side-chains is also very high in etamycin as well as the actinomycins, so that both effects would operate in an aqueous system toward configurations with a high degree of possible hydrophobic bonding. In these two types of structures, salt linkages and disulphide bonds are also absent. Both compounds also contain D-amino-acids which I think may be structurally expedient to promote closer hydrophobic fitting as in the case of gramicidin *S*.

Using these concepts of maximum hydrophobic bonding in aqueous systems, I have prepared a model of etamycin and photographed it from the 'front' (amide side) in Fig. 4*A* and the 'back' (hydrophobic side) in Fig. 4*B*. Fig. 4*A* shows the overall compact configuration which this proposed arrangement permits. Into the centre of the hexagonal unit it brings the phenolic hydroxyl group of the 3-hydroxypicolinic acid, held in that position by the benzene ring of phenylsarcosine. It will be noted that, with the exception of the ester-linked L-threonine, each of the other L-amino-acids of etamycin contributes its single α-hydrogen atom to the central ring surrounding the hydroxyl group of the hydroxypicolinic acid. However, the D-allohydroxyproline, having the opposite configuration, points its β-methylene group into the central area, which also brings the γ-carbon containing the hydroxyl group alongside the pyridine ring of the picolinic acid. This hydroxyl group of the D-allohydroxyproline assumes a very interesting and significant position in the proposed conformation. First of all, it lies immediately adjacent to the nitrogen atom of the pyridine ring; and consequently the —N——H—O— bonding observed by Pressman[22] for water molecules with the ring nitrogen of *iso*nicotinate, nicotinate, and picolinate, will be a highly probable attraction. When the hydrogen atom of this hydroxyl group is pointed toward the ring nitrogen, the oxygen atom is turned in the proper direction so that it can hydrogen-bond very neatly on its 'back side' with the —NH group of the picolinate–threonine amide bond as well as the —NH group of the threonine -D-leucine bond. Some of these possible interactions are seen more clearly on the other side of the model (Fig. 4*B*). The —NH grouping of the D-leucine amide link to threonine is best accommodated in the ring structure in the *trans* configuration. With two D-amino-acids adjacent to each other in the etamycin chain sequence, steric interference permits only one of the side-chains to be arranged in the central area of the hexagon. The three probable interactions which were mentioned for the hydroxyl group of D-allohydroxyproline strongly favour its inclusion in the central area instead of the D-leucine. As a consequence the rather bulky non-polar side-chain of D-leucine is pushed to the outside, a situation which is best accomplished by the indicated *trans* amide linkage at the threonine-D-leucine junction, and then simultaneously favoured by the resulting hydrogen bond with the hydroxyl group of its D-allohydroxyproline neighbour. So far as the 'front side' of the conformation (Fig. 4*A*) is concerned, the presence of two adjacent D-amino-acid residues produces a 'change of direction' in the amino-acid chain so that the carbonyl oxygen of D-leucine is crowded outside the hexagon formed by the other six carbonyl oxygen atoms.

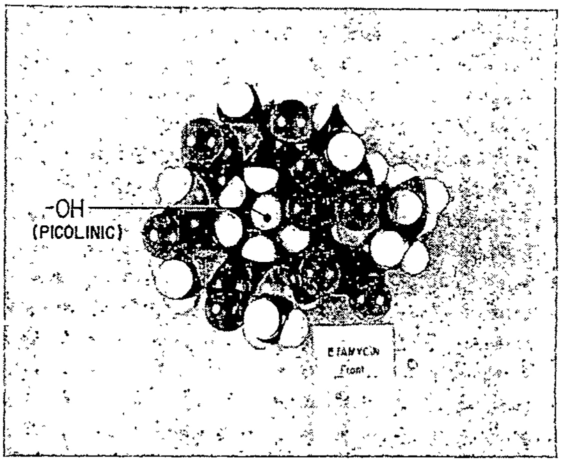


Fig. 4*A*

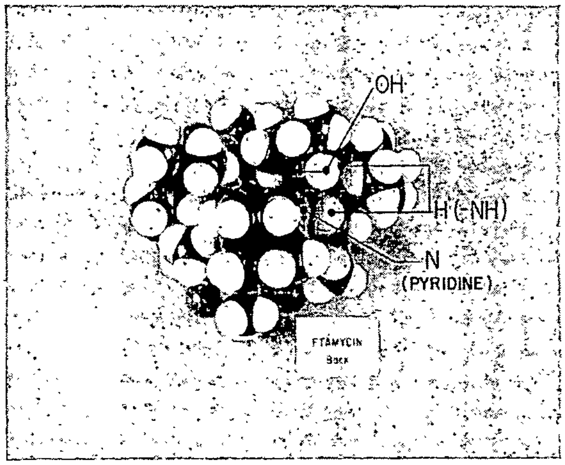


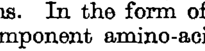
Fig. 4*B*

Other features of the hydrophobic 'back side' of etamycin (Fig. 4*B*) are the very close fitting of the aromatic rings of benzene (phenylsarcosine) and pyridine (picolinic acid) placed side by side, and the other non-polar groups compactly arranged around the two rings. The D-allohydroxyproline ring is particularly well fitted against the pyridine ring, and the entire conformation agrees well with Kauzmann's suggestions[5] about hydrophobic bonding and dispersion forces. In this proposed conformation of etamycin, hydrophobic attractions and hydrogen bonding possibilities can both be present to a maximum extent, although the suggested bonding would not involve a carbonyl oxygen, but the hydroxyl group of D-allohydroxyproline. This model of etamycin also shows quite nicely that the hydroxyl group in the D-allo isomer is in a better position for hydrogen bonding than the hydroxyl group of the more common hydroxyproline.

The third molecular model I shall discuss in detail is actinomycin *D* (C_1). From the work of Brockman *et al.*[21a–c] and Bullock and Johnson[21d], the actinomycins have been shown to contain a substituted phenoxazine chromophore attached to two individual cyclic pentapeptide lactone rings through the amino-group of threonine, while the hydroxy group of threonine forms the lactone juncture. Models of the structure of actinomycin *D* as it appears in the literature can be made; but the two pentapeptide rings are quite strained, and there is, of course, a certain amount of 'free rotation' about the amide bond of the threonine–chromophore junctions. Consequently the entire conformation has little structural rigidity and a low degree of hydrophobic interaction, especially between the chromophore and the non-polar side-chains. However, if one examines the chemical evidence for the two individual lactone rings, the methanolic sodium hydroxide hydrolysis[23], consuming two equivalents of alkali, is equally consistent, in my opinion, with a decapeptide dilactone ring in a continuous cycle and attached at two points to the chromophore. Similarly, the alkaline peroxide degradation of the chromophore[21d] to yield two pentapeptide fragments would proceed equally well from a decapeptide dilactone. Even the recent synthesis of actinomycin C_3 by Brockman and Lackner[24] is not unequivocal proof for the two individual pentapeptide lactone rings. Schwyzer and Sieber[25] have demonstrated previously that the pentapeptide, val-orn-leu-phe-pro (L,L,L,D,L), preferentially forms the cyclic decapeptide, gramicidin *S*, instead of the expected cyclic pentapeptide. Consequently the formation of the decapeptide dilactone should be considered in the actinomycin C_3 synthesis. It is interesting that Brockman[26] also considered the possibility of the decapeptide dilactone in his earlier work, but discarded it later because of his views that the chromophore is formed biologically from the oxidative fusion of the pentapeptide lactone of 3-oxo-4-methyl-anthranilic acid[21c].

Because of the distinct possibility that the actinomycin molecule may be a decapeptide dilactone, I would like to present a model of this alternate structure for consideration as a very likely primary structure and conformation of actinomycin *D* (C_1). The suggested amino-acid sequence of the decapeptide dilactone is given in Fig. 5.

The positions of the abbreviated amino-acid symbols correspond approximately to the carbonyl oxygen positions of the amino-acids in the molecular model (Fig. 6*A*), with the



$$\underset{\displaystyle O}{\overset{\|}{—C}}—\underset{\displaystyle R}{\overset{|}{CH}}—NH—$$

direction counter-clockwise. In the case of the threonine, the —NH points toward (and above) the chromophore while the —OH forms the lactone with N-methylvaline.

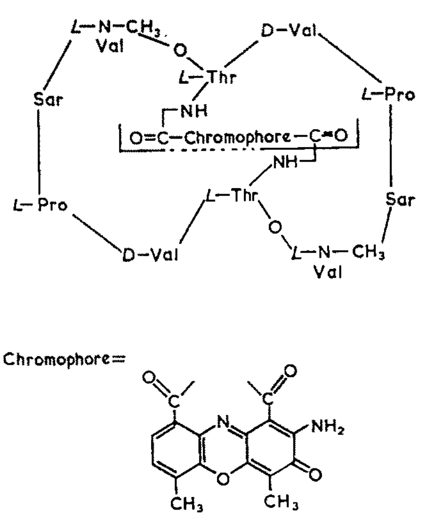


Fig. 5. Probable amino-acid sequence of actinomycin *D* (C_1)

The molecular models of actinomycin *D* (C_1) are illustrated in Fig. 6*A* ('front side') and 6*B* ('back side'). This proposed structure for actinomycin places it in the same class of compounds as gramicidin *S*, circulin and the tyrocidins. In the form of the decapeptide dilactone, the component amino-acids surround and support the chromophore whereas in the alternate case of two pentapeptide lactones these rings circled above the chromophore. The decapeptide dilactone therefore gives maximum hydrophobic interaction. This is apparent also from the 'front side' (Fig. 6*A*). The two molecules of D-valine bring the —CH and one CH_3 group of the side-chain into the central open space next to the chromophore, thus effectively shielding this resonating system as well as supporting it in the central portion of the molecule. (Substitution of L-valine in the model gives a much less satisfactory fit.) Thus, our proposed configuration of the molecule indicates again a definite, structural, space-filling preference for the D-amino-acid. This utility was also suggested for the D-phenylalanine units in gramicidin *S* and for the D-allohydroxyproline and D-leucine in the case of etamycin. It will be noted that here, too, as in the etamycin and gramicidin *S* models, the D-amino-acid is associated with a change in direction of the amino-acid chain; for in order to accommodate the bulky D-valine side-chain in the centre of the structure, the threonine carbonyl group points toward the centre and consequently projects its side-chain outward toward the ester linkage with N-methyl-L-valine. Although actinomycin *D* has only four possible —NH groups for hydrogen bonding, it will be noted that two of these —NH——O=C— hydrogen bonds are nicely formed between the —NH group of one threonine unit and the carbonyl oxygen of the other threonine unit at the junction of the two hexagons.

With the rather rigid chromophore grouping in actinomycin *D*, it should be possible to measure quite accurately the distance between the carbonyl oxygen atoms at the centres of the decapeptide dilactone. Although interatomic distances for phenoxazine are not recorded, a fair estimate may perhaps be obtained from the values for phenazine and benzoic acid[27]. The value so obtained would be about 7·2–7·4 Å., depending on the angles selected between the carbonyl group and the aromatic ring. The value estimated from the Catalin models is about 7·3 Å. (measured tangentially to the two carbonyl oxygen atoms).

The hydrophobic 'back side' of the actinomycin *D* (C_1) (Fig. 6*B*) shows a central location of the chromophore. The symmetry of the picture is quite apparent, being roughly rectangular or elliptical in shape with matching corners and sides. Here, too, the resonating chromophore system is seen to be well protected by non-polar, hydrophobic groups, almost like an electrical insulating layer. I should perhaps emphasize again that this structure would, in our opinion, be a probably preferred configuration for the aqueous (or biological) system, and might not be present with solvents which would rupture hydrophobic bonds. This 'back side' of actinomycin *D*

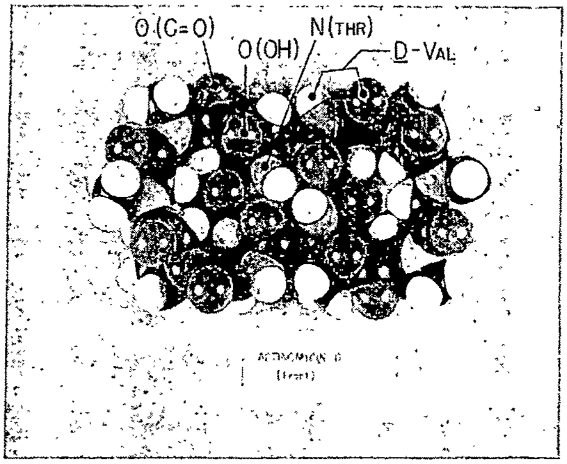


Fig. 6A

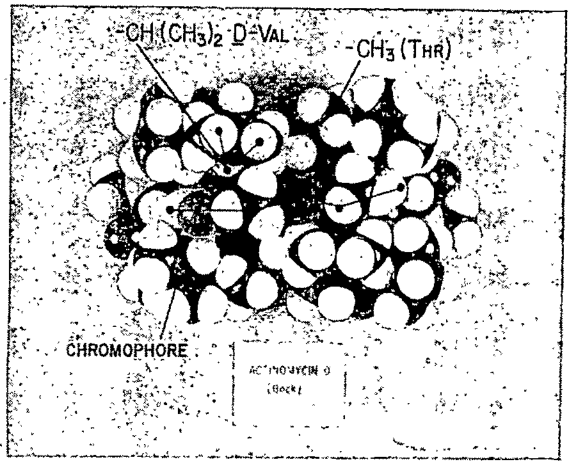


Fig. 6B

shows a slight open space between the threonine methyl group (which was purposely moved slightly away from the chromophore to pinpoint its position) and the adjacent methyl group of D-valine. When this particular valine methyl group is replaced by an ethyl group, the space along the sides of the chromophore is even more uniformly filled, and the resulting amino-acid turns out to have the structure of D-allo-*iso*leucine[28]. It was of interest to us that the configuration of D-allo*iso*leucine was predicted from consideration of the open space in the model before we had checked the actual assignment of the structure, but this result could, of course, have been fortuitous. The model containing two moles of D-allo*iso*leucine would be actinomycin C_3.

One other feature of the 'back side' of actinomycin *D* (Fig. 6*B*) is perhaps worth indicating in this conformation. The long, slender shape of the chromophore group requires as much space as possible at the ends to accommodate the benzene rings and especially the amino substituent of the phenoxazine nucleus. This space is conveniently provided in this particular conformation by having sarcosine units with their minimum-sized α-CH_2 groups exactly in line with the ends of the chromophore at that point. The rigid arrangement of the ends of the chromophore relative to the sarcosine units may also be an important point in the recent acid degradation studies with actinomycin C_3. In these experiments[29a] concentrated hydrochloric acid at 37° selectively splits the molecule in only one place at a sarcosine–methyl valine linkage. From Fig. 6*B* it is apparent that the sarcosine–methyl valine juncture at the left end of the chromophore is near the chromophore amino group, but the similar amide bond at the right end of the chromophore does not have a similar neighbouring group. Such adjacent amino-groups are known to contribute an increased acid stability to the glycosidic linkage in neobiosamines[30] and we have observed a similar stabilizing effect in the very slow acid degradation of the glycosamine polymer, chitosan[31]. Consequently this particular decapeptide dilactone conformation also offers a plausible explanation for the selective acid hydrolysis of one of the sarcosine–methyl valine bonds and the relative stability of the other.

In the course of our work with these molecular models we have prepared similar proposed conformations of other structures such as the complete oxytocin molecule and the cyclic decapeptide, circulin. The circulin model may be especially interesting because it shows how a single D-amino-acid (D-leucine) can effectively fill the open centre of the two fused hexagons which may be the preferred conformation for the cyclic decapeptide antibiotics. The recent disclosure of the formulæ of circulin *A* and *B* by Koffler and his associates[32a,b] has prompted me to include a single model (Fig. 7) showing mainly the decapeptide ring, with only the α-hydrogen atoms (but not the side-chains) of the nine L-amino-acids, and the $—CH_2CH(CH_3)_2$ side-chain of the D-leucine. The D-isomer side-chain may also function as a structural spacing unit to separate the two central carbonyl oxygen atoms. This may also be true for the two D-phenylalanine side-chains in gramicidin *S*.

Concluding Remarks

It will, of course, be appreciated that the proposed 'fused hexagon' conformation which has been discussed for cyclic decapeptides of the antibiotic type may also be capable of extension to the active centre of an enzyme, such as trypsin, where a disulphide-stabilized decapeptide is presumably part of the reactive unit[33]. If the two reactive centres in such a trypsin cyclic decapeptide are at approximately the same distance as the two centres of the actinomycin chromophore (about 7·3 Å.), then it could perhaps be significant that the distance between

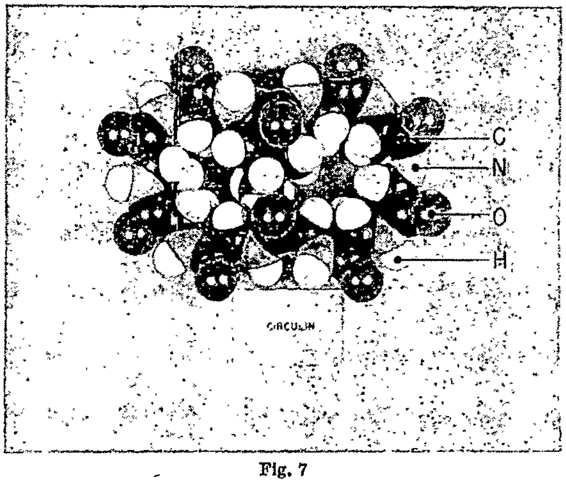


Fig. 7

the ε-amino group of lysine and the carbon atom of its carbonyl amide group (where anion attack in hydrolysis might be expected) is definitely approximately 7·2–7·5 Å. as judged by the Catalin model of the extended chain. This dimension was also estimated at 7·4–7·5 Å. diagrammatically from the interatomic distances and angles given for DL-α-norleucine (ref. 27, *M* 207), arbitrarily using the α-NH_2 angle with the α-C atom as a rough approximation for the angle of the ε-amino group with the ε-carbon atom in the extended chain configuration. Such a space separation of the reactive groups would allow attachment of the ε-amino group of lysine at one point and hydrolysis of the amide linkage at the other point. With the incomplete information thus far available concerning the active centre of trypsin this is admittedly a very speculative consideration, but the indicated dimensions are at least consistent.

Our considerable experience with these models in the construction of decapeptides and other slightly smaller polypeptides substantiates clearly the points raised by Platt[34] in his discussion of the need for better models. We noted difficulties especially in the gramicidin *S* model, where the bulky groups had to be held in place by small bolts and rubber bands to simulate hydrophobic attraction. As a result small spaces sometimes appear between the joined atoms in the photographs which are the result of mechanical weakness of the model bonds and slight differences in bond-angles rather than steric hindrance in the actual structure. No doubt these difficulties can be surmounted, and the problem is strictly an economic problem so far as the model suppliers are concerned. In spite of these difficulties, the models have been a great aid in visualizing the conformations which I have presented for gramicidin *S*, etamycin and actinomycin *D*. I think that the conformations show quite clearly the compact hydrophobic attractions which can be present in molecules with fairly complex side-chains, and therefore suggest that these conformations may probably be favoured by the aqueous, biological systems. Whether *cis* amide bonds (allowing the closest hydrophobic attraction of non-polar groups) or *trans* amide bonds (which seem to be at present favoured but offer less compact hydrophobic attractions) are actually present in the aqueous system is probably a major consideration. The comments by Pauling[35] concerning the probable instability of the *cis* amide linkage which is associated with the presumed orbital overlap of α-hydrogen atoms were based on a van der Waals's radius for hydrogen of 1·2–1·4 Å. This argument should perhaps be re-examined on the basis of the somewhat smaller 'interference radius' of hydrogen which some workers regard as a more accurate measure of possible steric hindrance[36a–d]. The models also clearly show that, in a cyclic hexapeptide with *cis* amide linkages, the carbon–hydrogen bond at the tetrahedral α-carbon atom projects upward at about a 20° angle with respect to the main plane of the hexapeptide ring. Consequently a C—H distance of 1·07 Å. is equivalent to an 'in-plane' projection of about 1·00 Å., so that any crowding of neighbouring α-hydrogen atoms is further relieved. In the light of these considerations the *cis* configuration of the amide bond may be consistent with a desirable close-fitting structure instead of a sterically unstable conformation. Its presence is certainly consistent with maximum hydrophobic interactions which may be highly effective in the aqueous system.

I wish to thank many of my colleagues at the Upjohn Co. for their helpfulness in the preparation of this manuscript and their encouragement of the work. I am particularly grateful to Prof. C. H. Li for his suggestions and interest. I want to thank Mr. Eugene Beals of our photography group for his patient help in preparing the pictures of the molecular models. The co-operation of Mr. J. E. S. Whitney of Catalin, Ltd., in preparing the special model atoms of peptide nitrogen for use in constructing these conformations is appreciated.

Note added in proof. Since this manuscript was submitted for publication, papers, by Klotz and Franzen[37] on the stability of interpeptide hydrogen bonds in aqueous systems, and by Tanford *et al.*[38] on hydrophobic bonds and the structural stability of native proteins, have appeared which are pertinent to the validity of the conformations proposed.

With regard to Figs. 3*A* and 3*B*, the possibility that the L-leu-D-phe amide junction may involve a *trans* amide bond with the —NH— pointing toward the hexagonal centre is suggested. Similar considerations could apply to Fig. 7 and minor changes in the arrangement of the D-leu unit in Figs. 4*A* and 4*B* might improve the hydrophobic picture.

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THE TECHNOLOGICAL UNIVERSITY

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School Education

OUR schools, particularly the Scottish schools, are in a critically dangerous state so far as the flow of properly trained students to the universities is concerned. This is affecting the number of entrants to science and engineering, which are the principal fields of study in a university biased towards technology. Early action of a radical kind is required if pupils in our senior secondary schools are to be given a fair opportunity to enter the most expanding part of the national education scene. Most schools seem to be desperately short of staff adequately trained in mathematics and physics, and to a lesser extent chemistry. The two fundamental sciences of physics and chemistry must be given the status of full subjects and staffed accordingly as otherwise physicists are penalized.

The length of training, four years in an honours course, followed by a year of study at a training college, is much less attractive than the shorter passage to teaching in England. The deadlock must be broken, as otherwise Scottish pupils will suffer severely by missing the opportunities available to them in science and technology. Salary differentials of an attractive nature might be one answer, and this might be effected by the special staffing of advanced-level schools set up at chosen centres. These schools could take pupils from approximately the Ordinary Level to the Advanced Level and the Scottish and English patterns thus brought nearer to equivalence in spite of the special difficulties encountered in Scotland. These stem to a large extent from the fact that the population is more widely dispersed so that the sixth form is a sham in most instances. It is impracticable and very expensive to set up good sixth forms in the majority of Scottish schools.

The higher-level education in technology depends on school performance in mathematics, physics and chemistry, and the drive towards specialization in these subjects in the English schools has been examined recently by scientists who advised some reduction in extreme specialization in all fields. In Scotland there is no specialization in this sense, and whereas the advice offered to English schools was to cut the study in science or classics or any other single specialized field to half the total, the Scottish schools simply do not, indeed can not, offer that much. If they did the staffing position would become still more desperate.

Some outlet for the natural appetite of pupils of 16–18 years of age must be offered. They are then at one of the peaks of their mental activity, they are intensely interested in fashioning their future career and the lack of adequate facilities for the young people in the upper reaches of our schools is bound to depress and thwart them. Many are being lost and more will be lost unless we find a plan and are prepared to carry it out immediately. There is no reason to decry the wish of the young people to choose their subjects with a vocation in mind. Interest is the sure key to learning and in turn to understanding. If the right subjects are taught well it is all to the good if the vocational aspect is obviously attractive. This is one of the great points of strength of mathematics and science. They are necessary for entry to so many fields in which trained men and women are required.

A Modern University

There seems to be an obvious need for a form of university that is not, so far, part in its own right of our British university system. This might be termed the technological university though this is something of a misnomer. If one takes a thoroughly acceptable form of definition of a university training it is clear that an education in science and engineering of the right kind satisfies the conditions extremely well. A proper understanding of these subjects is both cultural and vocational at one and the same time. The technologist requires too a 'rounding-off' of his education and this can be very appropriately provided by study of the social sciences or humane studies in which a keen interest is for him a natural extension of his more professional studies. This is recognized in universities of this type, for example, the Massachusetts Institute of Technology, where those responsible for the courses in the social studies are of the highest calibre. In the hands of such real experts in their fields the courses are of great value. In such a university, too, the reverse is readily possible although not so frequently practised. A study of the social sciences with a good grounding in science or engineering offers an excellent kind of degree course.

Such an institution has great strength in its freedom from some of the difficulties facing the really comprehensive multi-faculty universities. The administration of those is a task of great difficulty, but there could be some uniformity of purpose in the more modern form of university. This could enable it to organize more easily inter-departmental programmes at both undergraduate and postgraduate level.

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* Substance of a lecture delivered before the Royal Society of Edinburgh on March 6.

Research

At present there is great danger that universities may lose their traditional role of leadership in fundamental research and this might well be taken over by large research groups under governmental control. A university operating in science, engineering and the social sciences has a form lending itself to the proper handling of real research programmes, and so research leadership of a more valuable type could be retained.

Such a university gives its graduates a real appreciation of the wholeness of our Western civilization. The departments would have a real sense of their common aim. The cultural target would be clearly established. The subjects would not be treated as segments of our intellectual heritage operating in comparative isolation, a situation not uncommon in our universities.

The position in Britain is not at all satisfactory. In the United States and on the Continent of Europe the situation has been faced squarely during the past century. Large institutions devoting themselves to science and technology and associated fields such as the humane sciences have long granted their own degrees and stood alongside other types of university. Thus we have, for example, the Massachusetts Institute of Technology, and institutes of comparable standing at Zurich and Aachen. The industrial strength of the nations depends more and more on such institutions and they are an absolutely vital part of the higher educational system. We ignored the vital role of science and scientific research so that France and later Germany far excelled us. About a century ago we finally decided to turn professional and leave amateurism in pure science, and our universities admitted the sciences. But applied science and engineering have never been adequately recognized and we must act now, see to the full recognition and acceptable status. This will mean that the training of scientists and technologists can be organized on an adequate scale to satisfy the vital needs of Britain for graduates and for thoroughly good research.

The number of scientists and engineers required is such that the balance of the orthodox form of university structure would be upset. Some five or so applied scientists are required for each pure scientist; but the faculty of science itself is usually large as faculties stand. Clearly it would be wise to recognize that the real demand cannot be met by existing universities, and that in meeting it well it is wise to set up universities of a pattern suitable to meet the need in the most appropriate way. The new institution will more readily provide the men with the type of education that is truly of the best university type even if the institution is not of the orthodox pattern of universities with comprehensive subject coverage. It has as its primary objective the provision of a true university education in a limited number of fields without the weaknesses inherent in one and the same place attempting to provide it in all fields. Conditions are such that to try to do so in all fields, especially by including technology as one of the many, is to court disaster.

We live in a technological age, and the number of technologists required should only be limited in the future by the number of those with the adequate capacity for the subjects and the taste for them. Given an education of an integrated type the men graduating from such a university will scarcely be aware of the so-called division between the two cultures. They will have learned to absorb both and should continue to do so throughout their lives. These are the views which hold sway at the Royal College of Science and Technology, Glasgow, and we have applied them already in our courses and we will apply them increasingly in the future.

SIGNIFICANCE OF CARBONACEOUS METEORITES IN THEORIES ON THE ORIGIN OF LIFE

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At the meeting of the New York Academy of Sciences on March 15, 1961, B. Nagy, W. G. Meinschein and D. J. Hennessy reported their findings of mass spectrographic analysis of carbonaceous material from the Orgeuil meteorite of 1864 (*Nature*, 189, 967; 1961). They concluded from this that the distribution of hydrocarbons in the high-temperature distillate was so similar to that found for animal products such as butter or organic materials in sediments as to justify the conclusion, admittedly preliminary, "that biogenic processes occur and that living forms exist in regions of the universe beyond the earth".

It was this conclusion that, quite naturally, gave rise to much publicity. There is a danger that this will, in serious scientific circles, cause the observations of the three authors to be overlooked, which would be a great mistake, for whatever the interpretation put on them they are of cardinal importance to science.

It would be premature to discuss here the detail of these observations in advance of the publication of the full report; but enough has appeared to understand their general tenor, and it is important that they should be widely known, for a reason which will be discussed more fully later, otherwise careless study of other meteoritic material may well destroy irreplaceable evidence.

The study of carbonaceous material in meteorites is not new. The first detailed analysis to be published was that of G. Mueller in 1952 on the Cold Bokkevelt meteorite, which seems similar to that in the Orgeuil meteorite. Using conventional methods of analysis, he showed that a large part of the black material was soluble in organic solvents and the recovered material showed a somewhat unusual composition: hydrogen, 8 per cent; carbon, 24 per cent; nitrogen, 4 per cent; oxygen, 49 per cent; sulphur, > 9 per cent; chlorine, 6 per cent. The high oxygen content suggests a complex organic acid. If it was to be compared with any material produced by living systems, it would be with the humic acid of peat. Mueller drew no cosmic conclusions from his work, and it attracted little attention at the time.

The recent work is consistent with this picture but completes it in a significant way. Using mass spectrographic analysis of a distillate of the meteorite, they found the largest portion of the 250°–400° C. fraction to consist of paraffinoid hydrocarbons of carbon numbers ranging from 15 to 24 with peaks at 18 and 23 carbon atoms, as well as some cycloparaffins. This distribution is similar but by no means identical with that found by the authors for butter and recent sediments using the same apparatus.

There can be no serious doubt that the Orgeuil material is from a genuine meteorite, and that it has been examined in an uncontaminated state. The mass spectroscopic evidence should also be accepted in view of the experience of Dr. Meinschein in precisely this type of work in the petroleum field.

The major question that arises, therefore, is as to the interpretation of the results. Though the mass spectroscopic evidence indicates a composition of meteoritic hydrocarbons within the range of those given by material of organic origin on the Earth, from this it only follows that the meteorite material *may* be of organismal origin and not that it *must* be so. The actual hydrocarbon fragments are admittedly artefacts produced from some more complex source by the rather drastic distillation and electron bombardment processes. However, as there is little possibility that they are synthesized by these processes, the original material must contain paraffinoid elements similar to those produced in living matter.

Here the onus of proof is on synthetic chemists who must show that these have been, or could be, produced by purely inorganic condensations from smaller carbon-containing molecules. A possible mechanism for this is suggested below but requires experimental test.

The vital question could be settled once and for all if specific molecules such as porphyrins or nucleotides were found. A counter indication at the moment is the extremely low phosphorus content of the meteorite.

Pending a settlement of this question by analytical or synthetic methods, it may be worth while, in order to direct further experimentation, to speculate on alternate hypotheses for the formation of carbonaceous material in meteorites. These alternatives are either that they are produced by some form of life or that they are produced by wholly inorganic processes.

If the material is derived from life, this must have occurred on the planetary parent body from which by general consensus we believe meteorites to have been derived by fragmentation.

The time of flight of the Orgeuil stone has not, so far as I know, yet been measured; but it is unlikely to be more than 200 million years on analogy with other meteorites. This would mean that in recorded geological time there must have existed a planet on which life processes had evolved sufficiently to produce long-chain hydrocarbons.

Meteoritic parent bodies, however, as most recently demonstrated by E. Anders[1], appear to be located in the asteroid belt between Mars and Jupiter, a region where mean surface temperatures are approximately – 50° C. This would not appear to permit liquid water and hence any chemical evolution. Further, even if hot enough they would not be large enough to hold water vapour gravitationally and, therefore, would be dry bodies like the Moon.

The only likely place for the genesis of such hydrocarbons would be a planet the size of the Earth at about the same distance from the Sun. Why not the Earth itself, if we are not allowed any longer to postulate Pythagoras' anti-Earth? The Orgeuil meteorite would then be a piece of primitive Earth shot off some hundreds of millions of years ago and again united to its parent body. Even more fanciful would be the idea that the stone derives from an earth-like planet of another solar system.

If the first alternative appears difficult to accept, the second only avoids some of these difficulties to encounter others. In this hypothesis, following Mason[2], the nuclei of condensation would be the little platelets of hydrous iron magnesium silicate formed directly from the solar gases. Originally, in the cool and opaque dust cloud surrounding the Sun, these would accumulate ice, methane, ammonia and hydrogen sulphide in solid form from the more volatile solar gas components. As the cloud cleared by particle agglomeration, these would evaporate leaving, however, a thin skin of polymerized carbonaceous material produced by the action of cosmic ray and solar particles. In favour of this we have the evidence from Duchesne[3] that carbonaceous meteorites contain free radicals. There is also the observation of G. Mueller[4] that the very similar Cold Bokkevelt meteorite resisted boiling hydrofluoric acid for 140 hr., indicating the complete carbonaceous covering of each small silicate particle.

The crucial question is now whether it is possible to imitate the carbonaceous material of the meteorites by the action of radiation-work here for atomic piles and high-energy machines. If this question can be answered in the affirmative, the carbonaceous meteorites become the *primary accumulation* of elements and free energy for the first synthesis of life *on this Earth*[5] rather than the end products of chemical evolution on some other celestial body, which in any event would simply remove the problem of the origin of life by another great move in time and space.

We can speculate about these problems now, but what is clearly needed is more careful experiment and critical evaluation. Thanks to the pioneer work of Nagy, Meinschein and Hennessy, there is every reason for believing that this will take place in many laboratories throughout the world, but if this happens without planning and co-operation the results may be scientifically disastrous.

There are probably not more than 50 kgm. of carbonaceous meteorite material in the world to-day; it could easily be used up in a year if each investigator looking for one thing at a time—be it helium-3, porphyrin or radicals—uses up a piece of meteorite in a way to make it unavailable to other workers. What is needed is a carefully worked out flow-sheet needing the minimum of material, so arranged that only the best equipped and experienced workers do the operation of analysis in which they are expert. This would require an overall organization that fortunately is to hand in the Commission on Space Research and in its constituent organizations in other countries.

The study of meteorites is one involving nearly all the disciplines in science and, like the International Geophysical Year, could be another excellent trial ground for co-operation in science.

[1] Anders, E., *Astrophys. J.*, 132, 243 (1960); *Geochim. Cosmochim. Acta*, 19, 177 (1960).

[2] Mason, B., *J. Geophys. Res.*, 65, No. 9 (September 1960).

[3] Duchesne, J., and Depireux, J., *Nature*, 182, 931 (1958).

[4] Mueller, G., *Cosmochim. Geochim. Acta*, 4, 1 (1953).

[5] *Nature*, 186, 693 (1960).

NEWS and VIEWS

Bacteriology at St. Bartholomew's :

Prof. L. P. Garrod

For more than thirty-five years bacteriology at St. Bartholomew's Hospital has been associated with the name of L. P. Garrod, who is due to retire in this academic year. During all these years, Prof. Garrod has contributed much in the field of medical bacteriology. Anyone who knows the cramped conditions in which he has worked will be astonished at what he has achieved. Prof. Garrod belongs to a generation in which it was still possible to be a first-class pathologist and bacteriologist, as well as a first-class clinician, and any who remember his lectures on 'worms' will agree that he is also no mean helminthologist. He has contributed many important articles or chapters in almost every branch of medical bacteriology—for example, actinomycosis, staphylococcal infections and endocarditis, to mention only a few. But Garrod has also kept abreast or even ahead of new developments. In recent years he has often led the way in the critical appraisal of new antibiotics and sometimes in exposing exaggerated claims that have been made. He has been especially interested in the validity of laboratory tests for the sensitivity of bacteria to antibiotics, and he has always tried to show how these tests should be employed so as to give information which may be of real clinical value. He has also been interested in the mode of action of antibiotics, in the differences in bacteriostatic and bactericidal activity, and in the development of antibiotic-resistant strains. He has exerted a profound influence on the development of bacteriology during what may prove to be the first phase of the era of chemotherapy, which is now showing signs of passing from a medical to a more biochemical and biological phase. It is to be hoped that Prof. Garrod will continue after his retirement to work in the field in which he has contributed so much.

Dr. R. A. Shooter

Dr. R. A. Shooter, whose appointment to succeed Prof. L. P. Garrod as professor of bacteriology starts in October 1961, has already made his mark in the field of hospital bacteriology. In much of his work he has been closely associated with Prof. Garrod, but in recent years he has developed a special interest in problems of cross-infection and hospital hygiene and epidemiology. This work has been especially important at a time when surgical wards and theatres and medical wards, too, have been invaded by antibiotic-resistant staphylococci and other organisms, and when the first flush of success in the antibiotic era has given place to a more sober realization of what antibiotics can and cannot be expected to do. Dr. Shooter has paid great attention to problems of staphylococci, of masks, of air and dust and of ventilation in the spread of staphylococcal infection. In the past few months Prof. Garrod and Dr. Shooter have combined with Dr. Blowers and Prof. Williams to produce an excellent book on hospital infection. This has met a very real need and is a fitting conclusion to years of fruitful co-operation in this special branch of bacteriology.

Technical Operations at the International Atomic Energy Agency :

Dr. P. L. Balligand

Dr. Pierre Louis Balligand has been appointed to succeed Dr. Hubert de Laboulaye as deputy director general in charge of the Department of Technical Operations of the International Atomic Energy Agency. Dr. Balligand, who is a graduate of the Ecole Polytechnique, is at present head of the Division of Large Research Reactors of the French Commissariat à l'Energie Atomique, which was established to run the operation of the test reactor *EL* 2 and the start-up and development of the high-flux reactor *EL* 3. The service will also include the operation of the test reactor *Pegase* at the new French reactor centre at Cadarache. Dr. Balligand was concerned with the organization of a test reactor research group, the function of which was normalizing in-pile experiments and arranging dosimetry and flux measurements studies. This group has collaborated closely with similar efforts in other European countries ; an experimental programme is now being organized in Belgium at Mol, and others are being developed in Italy, Switzerland and Yugoslavia. Reactor safety regulations have been a main concern of Dr. Balligand and the development and application of the first provisional safety measures for experimental reactors at Saclay were his responsibility. However, these regulations are now the responsibility of a Reactor Safety Committee, of which Dr. Balligand was a member. Dr. Balligand will commence his new duties towards the end of March, when Dr. de Laboulaye returns to a senior position with the French Commissariat à l'Energie Atomique.

Industrial Engineering at Toronto :

Prof. Arthur Porter

Dr. Arthur Porter, dean of the College of Engineering in the University of Saskatchewan, has been appointed the first professor of industrial engineering in the University of Toronto. Prof. Porter is an authority on the electrical control systems which, among other things, make possible large-scale automation of industry. He worked in Toronto during 1949–55 as head of the research division of Ferranti Electrical, Ltd., the company which installed the University of Toronto's first large electronic computer in 1952. Before becoming dean at Saskatchewan in 1958, he was professor of light electrical engineering at the Imperial College of Science and Technology, London (see *Nature*, 181, 1178; 1958). He is the first member of the University of Toronto staff to be associated solely with the new course in industrial engineering, which superseded the engineering and business course two years ago. Until now, the industrial engineering course has been administered and staffed entirely by professors attached to the Mechanical Engineering, Mathematics and other Departments. The new course covers areas of fundamental engineering and science, but is heavily weighted toward mathematics. Its undergraduates study operations research, electronic data processing, control theory, numerical analysis and probability and statistics. They also are introduced to modern work in organizational structure, financial control and industrial psychology.

Agricultural Economics at Nottingham :

D. K. Britton

Mr. D. K. Britton, at present general manager, Market and Economic Research Department, Massey-Ferguson (U.K.), Ltd., has been elected to the new chair of agricultural economics at the University of Nottingham as from June 1. Mr. Britton will be head of the Department of Agricultural Economics and a provincial agricultural economist. Mr. Britton graduated at the London School of Economics and shortly afterwards was appointed statistician in the Ministry of Agriculture and Fisheries. From 1947 until 1952 he was a member of the research staff of the Agricultural Economics Research Institute, University of Oxford, after which he accepted an appointment with the United Nations Food and Agriculture Organization in Geneva. In this work he travelled widely in Eastern and Western Europe. In 1959 he returned to the United Kingdom to take up his present position.

Security Measures and Freedom of the Individual

An immediate repercussion of the recent spy trial in London was the announcement in the House of Commons on March 23 that the First Lord of the Admiralty would set up a committee of inquiry to ascertain what security weaknesses existed at the Admiralty Underwater Weapons Establishment and to determine where responsibility lies for such breaches of security as have occurred. The Prime Minister, in replying, showed himself fully aware of the difficulty of holding the balance between national security and the freedom of the individual from perpetual surveillance in a free society. He quoted the Lord Chief Justice's words that ultimate security must depend always on the honesty of those who are put in a position of trust; and if a person suddenly and for gain becomes dishonest, no security measures are adequate. Existing security procedures which apply to the Government Services were reviewed comprehensively by the Committee of Privy Councillors in 1956, and Parliament accepted these procedures as appropriate and adequate for their purposes. They are kept continually under review by the appropriate authorities, and to adopt a different procedure would involve a much greater range of inquiry about the activities of individuals. Nevertheless, Mr. Macmillan undertook to look at the matter again, and to consider whether the procedures laid down in 1956 should be tightened in any way.

The International Union of Geological Sciences

At the twenty-first International Geological Congress meeting held in Copenhagen on August 23, 1960, it was decided to form an international union of geology. The International Union of Geological Sciences was founded by thirty countries at a Constitutive Assembly held at Unesco House, Paris, on March 9–10, 1961. The statutes prepared by the Organizing Committee in Stockholm in December 1960 were ratified, and the following Executive Committee was elected: *president*, J. M. Harrison (Canada); *vice-presidents*, I. I. Gorski (U.S.S.R.), L. Hawkes (United Kingdom), A. R. Lamego (Brazil), J. Lombard (France), B. C. Roy (India) ; *secretary*, Th. Sorgenfrei (Denmark); *treasurer*, J. A. Dons (Norway). The object of the Union is to promote international co-operation in geology, and of geology with other sciences. Application has been made for admittance to the International Council of Scientific Unions at its meeting in London in September 1961.

Export Problems and the Technical Chemist

A booklet issued by the Association of British Chemical Manufacturers (86 Strand, London, W.C.2), entitled *European Free Trade Association: Origin Rules, Certification and Segregation of Stocks*, comprises a report of meetings in London and Manchester in June 1960. It includes addresses by W. A. M. Edwards on recent developments concerning the European Free Trade Association and the Common Market, by W. G. Harrold on the need for origin rules and by H. W. Vallender on the operation of the value rule and the associated basic materials ; and also N. E. Wallace's paper on the operation of the process rules for organic chemicals, and for other sectors of the chemical industry, and those of L. J. White on the certification and control of the origin of goods and on the segregation of stocks of materials by accounting methods. There is also a note on the provision of information on origin of chemicals purchased for use in the manufacture of products for export to the European Free Trade Area. Intended primarily to assist those concerned in these export problems, these papers, and particularly that of N. E. Wallace, illustrate the technical difficulties with which the chemical manufacturer has now to contend. There is also a short bibliography of official publications, including the key document "Classification of Chemicals in the Brussels Nomenclature 1958".

Funds for Research and Development in Industry in the United States

A survey, *Funds for Research and Development in Industry, 1957 : Performance and Financing*, made for the National Science Foundation by the Department of Commerce, Bureau of the Census, is the first in a series of annual surveys to be conducted in the United States by that Bureau for the Foundation (Surveys of Science Resources Series. Pp. xiv+119. Washington, D.C.: Government Printing Office, 1960. 65 cents). More than half the total 7,200 million dollars was for research and development in the aircraft and electrical equipment industries, and 52 per cent of such work was financed by the Federal Government ; 241 million dollars was for basic research ; 1,514 million dollars for applied research ; and 5,401 million dollars for development. Of the total, 6,060 million dollars was expended by companies employing 5,000 or more persons. Chemical and allied products ; stone, clay and glass products; petroleum refining and extraction; and primary metals financed 90 per cent or more of their research and development. The average cost per scientist and engineer in research and development was 34,300 dollars, varying from 20,200 dollars in food and kindred products to 42,300 dollars in aircraft, and from 20,400 dollars in companies with fewer than 1,000 employees to 37,400 dollars in companies with 5,000 or more. Wages and salaries averaged 51 per cent of research and development costs in 1957, ranging from 46 per cent in the electrical equipment industry to 67 per cent in the paper and allied products. Company-financed research and development (excluding that federally financed) averaged 1·6 per cent of net sales, and varied widely from less than 1 per cent in food and kindred products, paper and allied products, petroleum refining and extraction, and primary metals, to 6 per cent in scientific and mechanical measuring instruments; but including federally financed research and development the average was 3·3 per cent of net sales.

Employment of Librarians in the United Kingdom

The recent annual report of the Manchester Public Libraries Committee referred to difficulties which were being experienced through the rapid turnover of staff and the progressive weakening of professional experience. The Library Association has been increasingly disturbed in recent years by the extent to which it has been necessary for public libraries to fill professional posts with partly qualified staff; whereas in 1957 only 16 per cent of administrative, professional and technical posts were occupied by unqualified staff, in 1960 it had risen to 27 per cent, and at the end of 1960, out of a total of 2,738 posts, 277 were vacant and 998 occupied by unqualified staff. This situation led the Library Association to seek information from 484 public libraries regarding their turnover of staff since January 1959, and replies received from 352 showed that 47 per cent of qualified staff leaving were lost to the service, only 56 per cent of the vacated posts were filled by qualified librarians and 10 per cent remained unfilled. There was a loss of 299 qualified librarians to other types of library and public employment.

These and other figures were quoted by Mr. A. Fitch, who raised the subject in an adjournment debate in the House of Commons on March 1 and referred to the estimate in the Roberts report that an increase of more than 2,000 qualified librarians in the public library service was required for an efficient service. He suggested that the shortage was to be attributed to the unsatisfactory salary scales, and that the recommendations of the Roberts Committee were being ignored by the library authorities. In replying on the debate, the Parliamentary Secretary to the Ministry of Education, Mr. K. Thompson, referred to the Minister's announcement on December 15 that it was the Government's intention that the Minister of Education should have general responsibility for the supervision of the public library service, and that after certain preliminary work had been completed legislation would be laid before the House. Mr. Thompson said that this preparatory work was actively proceeding and broad measures of agreement already existed on many of the problems. Whatever new duties might be laid on the Minister of Education, the library service would continue to be an essentially local authority service, with Ministerial intervention limited to the minimum consistent with ensuring the maintenance of uniformly high standards. The Government accepted the view of the Roberts Committee that standards of staffing should be high both in numbers and quality, but he insisted that salary scales and conditions of service must be negotiated between librarians and the authorities employing them and that the Minister had neither the power nor the duty to intervene. As regards wastage of staff, Mr. Thompson thought that in so far as this applies to staffing of libraries elsewhere it might be to the advantage of the library system as a whole and encourage inter-library co-operation.

Abstracts of Human Developmental Biology

Excerpta Medica has issued the first of a new monthly journal called *Abstracts of Human Developmental Biology* (1, No. 1; January 1961. Pp. 52. Abstracts No. 1–164. Published monthly. Amsterdam and London: Excerpta Medica Foundation, 1961. Subscription rate: £6 18*s*. per annum). The new journal covers the experimental and clinical aspects of normal and pathological human development, including fundamental aspects of general morphogenesis, of reproduction, of transplantation in post-natal life, and of pathological growth, as compared with normal development, differentiation and growth. The new journal, developed and published with the aid of a grant from the United States Public Health Service, will contain approximately 2,000 abstracts a year, and will include classified subject and author indexes. It will thereby contribute to collaboration between specialists in the field of obstetrics, surgery, pædiatrics, social medicine, embryology, experimental teratology, genetics and immunology, by integrating information in all these disciplines so far as they concern the problems of normal and abnormal development, reproduction and transplantation.

Florida State Museum, University of Florida

The Director's report for 1959–60 of the Florida State Museum, University of Florida, has recently become available (Pp. 19+2 plates. Gainesville, Fla.: University of Florida, Florida State Museum, 1960). This Museum, which serves the people of the State and the University community in particular, is much hampered by lack of space and also of staff. The Department of Exhibits operates in three distinct areas of service. New exhibits are produced and loan exhibits are scheduled for the halls in the headquarters museum, a major reconditioning operation to modernize the archaic exhibits in the Seagle Building is in progress, and its influence also works in the aptly named 'out-reach exhibits'. Among the special exhibits are "You and the Atom", which illustrates the basic principles of nuclear energy and peace-time applications in medicine, agriculture and industry.

Control of Red Scale in Citrus Fruit

Red scale is the most important insect pest of citrus fruit in Australia. Standard control methods involving oil sprays or fumigation with hydrocyanic acid are by no means satisfactory. Mr. B. H. Martin and Dr. R. F. Black, of the Griffith Irrigation Research Station, have studied the possibilities of heat treatment as a control measure. An essential feature of any such treatment is that it will kill the scale without damaging the tree or the fruit. The experiments have been described in a recent issue of *Rural Research* (23, Sept. 1960). The technique used in the experiments consisted of heating scale-infested fruit in ovens at different temperatures and for different periods of time. The effect of heat on fruit was therefore examined but not the possible damage to trees. It was found that all the scales could be killed by a temperature of 70° C. maintained for 1 hr. This treatment, however, resulted in severe damage to the fruit. Equally effective 'kills' were obtained at a temperature of 50° C. kept up for 2½ hr. During this treatment the fruit was not damaged. Increasing the humidity during the 50° C. treatment did not alter the time necessary to kill the scale, and it is apparent that high temperature rather than dehydration was the direct cause of death. Further experiments have involved treating the trees themselves. The technique used was to enclose the trees in plastic sheets and utilize solar energy as the heat supply. Some difficulty has been experienced in maintaining temperatures at a safe level.

Congelifraction of Clays

THE clay fraction of soils is customarily regarded as a product chiefly of chemical rather than mechanical processes. While chemical alteration is undoubtedly necessary to produce clay minerals in the first place from the components of crystalline rocks, it now seems that at least one form of mechanical weathering, namely, congelifraction, can be effective in reducing particle size well down into the clay grade in unconsolidated sediments. I. C. McDowall (*N.Z. J. Geol. and Geophys.*, **3**, 337 ; 1960) has investigated the effects of alternate freezing and thawing on water-saturated samples of kaolinite, vermiculite, halloysite, and weathered clays containing illite and halloysite. The particle size distributions were determined at intervals by the Andreason pipette method, and it was found that appreciable reduction of particle size occurred during 800 cycles. The process appears to operate on particles at least as small as 1μ, and is most rapid in those minerals where the bonding between the basal planes of component sheets of the clay mineral is weakest. In cold climates the process might therefore be an important one in the formation of plastic clays, perhaps particularly of fireclays.

Navigation down the Centuries

A SHORT review of navigational methods from the earliest times to the present, with special emphasis on the newest techniques with Earth satellites, is made in a 24-page publication of the Coast and Geodetic Survey of the United States Department of Commerce, entitled *Use of Artificial Satellites for Navigation and Oceanographic Surveys*, by Paul D. Thomas (Technical Bulletin No. 12. Pp. i+24. Washington, D.C.: Government Printing Office, 1960. 25 cents). The first half of the publication is a historical review, beginning with Hipparchus and Ptolemy, touching on Galileo, the French expeditions of the seventeenth and eighteenth centuries, and the development of the marine chronometer, before coming to radar systems, *Loran* and inertia navigation. The second half of the paper is devoted to Earth satellites. Here the main interest is in the transit system of satellites, already in action on an experimental basis, from which position can be obtained by interpretation of the Doppler shifts of the radio-waves received from the satellites.

Use of Superconductors for producing Strong Magnetic Fields

THE production of strong magnetic fields by the use of superconductivity phenomena would appear to be considerably advanced by the development of a new superconducting compound of niobium and tin, Nb_3Sn, by the Bell Telephone Laboratories. The distinctive property of this material is its ability to retain superconducting properties even when exposed to fields of about 90,000 oersteds, and possibly even higher, while carrying currents of approximately 100,000 amp. $cm.^{-2}$ (see Kunzler *et al.*, *Phys. Rev. Letters* for February 1, 1961). The new material is very brittle, but by packing a narrow niobium tube with appropriate quantities of powdered niobium and tin, sealing the ends of the tube with niobium plugs, reducing the tube by swaging and drawing it, a wire of 0·015 in. diameter which can be wound into a solenoid coil is formed. On heating the wire to about 1,000° C., the inner materials react to form Nb_3Sn, and the necessary superconducting properties are developed. Owing to mechanical difficulties associated with the substance at high fields, a large solenoid of the material has not yet been wound, but it is anticipated that these difficulties will be overcome within the next twelve months.

The Iron and Steel Institute, the Institute of Metals and the Institution of Metallurgists

THE Councils of the Iron and Steel Institute, the Institute of Metals and the Institution of Metallurgists have recently examined the problems of closer co-operation between these three societies. After careful consideration they have concluded that amalgamation of the three societies would not be practicable within the foreseeable future. The three Councils, therefore, decided to set up a permanent Joint Consultative Committee of the three societies, which will meet regularly and advise their Councils on all activities and questions of common interest.

Lederle Medical Student Research Fellowships

THE Lederle Laboratories Division of the American Cyanamid Co. announces that it is making available fellowships to medical schools throughout the United States and Canada for the year 1961. These fellowships, in amounts not exceeding 600 dollars a year for any one individual, are intended to relieve in part the financial burden of students who desire to devote their summer vacations to research in the preclinical departments. Students who apply for Lederle Medical Student Research Fellowships must have the consent of the faculty member under whose supervision their research is to be conducted. Further information can be obtained from the Lederle Laboratories, American Cyanamid Co., Pearl River, New York.

Carlsberg–Wellcome Travelling Research Fellowships, 1961–62

APPLICATIONS are invited for the Carlsberg–Wellcome travelling research fellowships, 1961–62, the object of which is to encourage co-operation, on an exchange basis, between Danish and British research workers in any branch of the natural sciences which has a bearing on human and animal medicine. One fellowship annually is awarded to a candidate from the United Kingdom for a year's work in Denmark and one annually to a Danish candidate for a year's work in the United Kingdom. The stipend may range from £900 to £1,300 per annum (or the equivalent sums in Danish kroner), travelling and some incidental expenses being provided in addition. The tenure of the awards will start in September 1961. Further information can be obtained from the Assistant Scientific Secretary of the Wellcome Trust, 52 Queen Anne Street, London, W.1. Applications must be submitted before April 30.

Medical Research Fund for Australia and New Zealand

A FUND has been established by the Life Offices' Association for Australasia to promote and assist scientific and medical research. For the present it is intended to use the resources of the Fund to stimulate and support research in the field of cardiovascular function and disease. Grants from the Fund will be made as follows: *grants in aid*, which will be given to non-profit institutions with basic facilities and qualified personnel for medical research, and will range from £1,000 to £3,000; *research fellowships*, which will be open to medical graduates or those with equivalent scientific training, and will fall into two categories: (*a*) fellowships taken up in Australia and New Zealand for a period of one year (£2,200–£2,500 p.a.); (*b*) travelling fellowships, for a period of two years abroad, followed by a third year in an approved institution in Australia or New Zealand (£2,500–£3,000 p.a.). Further information and application forms can be obtained from Dr. H. M. Whyte, Life Insurance Medical Research Fund of Australia and New Zealand, 87 Pitt Street, Sydney. Applications for all awards must be received by July 1.

André Mayer Food and Agriculture Fellowships

THE Food and Agriculture Organization of the United Nations has announced that it will offer ten or twelve André Mayer fellowships in 1961. These awards will be made under the following two groups: *research*, for unusually promising persons with research experience behind them who would offer a guarantee for the carrying out of independent research work; *research training*, for younger and promising individuals who have demonstrated an inclination towards, and an aptitude for, research work. Due consideration will be given to geographical distribution, to ensure that a proportion of the awards is given to candidates from countries where research facilities are not highly developed and the need for trained research workers is great. The subjects presented must be in relation to the Organization's activities, which comprise: land and water development, plant production and protection, animal production and health, rural institutions and services, fisheries, forestry and forest products, nutrition (non-medical), atomic energy in food and agriculture, agricultural economics (commodities, statistics, economic analysis). Further information can be obtained from the National Committee for the United Kingdom of the Food and Agriculture Organization, Ministry of Agriculture, Fisheries and Food, Whitehall Place (East Block), London, S.W.1. The closing date for application is April 30.

University News: Birmingham

THE following appointments have been announced: Dr. M. Z. Atassi, to be Research Fellow in the Department of Chemistry; Dr. B. Cockayne, at present Research Fellow in the Department of Physical Metallurgy, to be lecturer in the Department of Physical Metallurgy; M. J. Hamlin, to be lecturer in the Department of Civil Engineering; Dr. D. A. Smith, to be lecturer in the Department of Microbiology. The title of reader in neuropharmacology has been conferred on Dr. P. B. Bradley, senior lecturer in electrophysiology in the Department of Experimental Psychiatry.

Edinburgh

DR. DUGALD GARDNER has been appointed as senior lecturer in pathology. Dr. R. C. Dorf has been appointed as a lecturer in electrical engineering, and T. Astarabadi as a lecturer in physiology.

Announcements

DR. C. J. JACKSON has been appointed chairman of the Water Pollution Research Board for the period April 1, 1961–March 31, 1966, in succession to Dr. F. H. Garner, who completed his term of office on March 31. Dr. Jackson is in charge of all water pollution problems for the Distillers Co., Ltd., both on the potable and industrial sides.

PROF. J. A. GOEDKOOP has been appointed managing director for research at the Reactor Centrum Nederland, where he was previously in charge of the Physics Department. Prof. Goedkoop has been at the Centrum since its foundation in 1955. For some years he directed the physics research at the Norwegian–Netherlands Joint Establishment for Nuclear Energy Research (JENER) at Kjeller, Norway. He is also a part-time professor at the Leyden State University.

A JOINT meeting of the Challenger Society and representatives from the Marine Laboratories (Development Commissioners' Scheme) will be held at the Royal Dublin Society, Ballsbridge, Dublin, during May 24–25. Further information can be obtained from the Challenger Society, c/o Dr. A. E. J. Went, Fisheries Division, Department of Lands, 3 Cathal Brugha Street, Dublin.

THE Nutrition Society (Scottish Group) is holding a symposium on "The Relative Values of Animal and Vegetable Fats in Nutrition" at the Physiology Lecture Theatre, Queen's College, Dundee, on April 22. Further information can be obtained from the honorary secretary of the Scottish Group of the Nutrition Society, Dr. J. Davidson, Rowett Research Institute, Bucksburn, Aberdeen.

THE annual general meeting of the Oil and Fats Group of the Society of Chemical Industry is to be held at Liverpool on April 25. It will include an address by Dr. A. T. James, of the National Institute for Medical Research, on "Applications of Gas Chromatography to Some Medical Problems". Further information can be obtained from Dr. H. Jasperson, c/o J. Bibby and Sons, Ltd., Research Department, King Edward Street, Liverpool 3.

ERRATA. Two mistakes in the list of new Fell
of the Royal Society of Edinburgh (*Nature*, Marc
p. 876) have been pointed out: Dr. K. B. Fr
senior lecturer in bacteriology (not botany)
University of Aberdeen; and the name of D
Gimingham, senior lecturer in botany in th
sity of Aberdeen, was omitted.

AFFORESTATION OF UPLAND HEATHS

FORESTRY Commission *Bulletin* No. 32 purports to deal with what are termed 'upland heaths', which are said to occur along the eastern side of Scotland and northern England, and to be distinguishable from lowland heaths formed farther south*. They are distinct from the peat-covered lands in the western parts of the country, which were the subject of an earlier *Bulletin*—No. 22—by the same author, entitled: *Experiments in Tree Planting on Peat* (H.M. Stationery Office, 1954). It reviews experimental work carried out over a period of thirty-six years during 1921–57, and records all that was attempted by the Forestry Commission, "with an assessment of the results so far".

The last phrase gives the clue to one of the major obstacles which research workers encounter when they undertake experiments on a field scale with the view of improving sylvicultural and management techniques. It is not possible to bring such experiments to a conclusion within less than a century, especially when they are conducted on such infertile sites as the degraded forest soils of early centuries, now covered with ericaceous vegetation, which go by the name of heaths. These soils, it is now generally accepted, have arisen as a result of forest destruction in the past, and of repeated burnings and heavy grazing which have killed off the woody vegetation. Whatever the original composition and structure of the former forest soils may have been, these have been lost, and the problem of the restoration of the soils to their original relatively fertile state is immense.

Since these heath sites are almost entirely unproductive from an agricultural point of view and should never have been deforested, it is only right and proper that they should, if possible, be reforested; and it is not to be wondered at that the Forestry Commission has acquired, and continues to acquire, extensive areas of such 'marginal' land, and rightly so.

The length of time which this kind of study requires has another important effect, namely, that no one individual can commence the work and bring it to completion in his life-time. If he could, it is more than likely that by the time he reached the end of his span he would have forgotten what the conditions were when he began his experiments. In these experiments no fewer than eight senior officers and three junior officers are credited with having played an important part, not counting the author himself. The difficulty of seeing the work as a whole and in its proper perspective is increased by so many changes over such a relatively short time. Another embarrassment to the forest research worker is that, no sooner do his experiments begin to indicate apparent success, than the inexperienced general practitioner draws his own conclusions and immediately applies a new method before it has been tested thoroughly. The older forester is more wary.

This particular experimental project was begun in 1921, and the major experimental areas were at first concentrated in six forests, four in Scotland, and two in the north of England. Later on experimental work was extended on a less-intensive scale to other forests. The kinds of experiment attempted were many, too many to be handled adequately, but the principal projects dealt with were: methods of ground preparation; various kinds of ploughing; trials of species; use of manures at time of planting; and later, use of nurse trees and mixtures of species, besides a variety of miscellaneous procedures. The result of all this was an accumulation of a vast amount of mensurational and other data. The earlier experiments were somewhat crudely set out, but later widespread use was made of approved methods of layout and of statistical methods of handling the results. Mr. Zehetmayr is to be congratulated on having presented his information in a very readable form and on treating the problem with admirable judgment. The *Bulletin* contains a mass of information on method and species. The conclusions are presented in an excellent summary, and it must be said that they do not differ essentially from those reached by tree-planters in Britain long before the Forestry Commission was born, except in respect of the newer species. The early work of the Commission was bedevilled by an idea that spruces, because they are highly productive on suitable sites, should be grown everywhere, even on upland heaths. Another herring drawn across the trail was the idea, imposed from above, that the deeper one ploughed the better. The unjustifiable opinion was held, and often still is, that the trees could be compelled to extend their roots down into the execrable material on which the heath grows. Fortunately, the experiments indicate that it is more effective to prepare the surface-layer than to bring up a mass of inert mineral matter, containing a small proportion of soluble minerals, which in time return to the depths whence they came. On sites with richer sub-soils nearer the surface, deep ploughing seems to be more useful—at least for a time. There is an undoubted stimulus to tree growth on ploughed land due to improved mineralization of the organic matter, but how long it continues remains to be seen. It is claimed that ploughing has extended the area on which planting is economically possible and has improved growth on moderately fertile areas. The possibility of future troubles is not overlooked.

It is interesting to find that the author believes, and rightly, that for these upland heaths, Scots pine will remain the most important tree. Corsican pine and Japanese larch—more exacting trees—do not seem to be suitable substitutes, but the shore or lodgepole pine from North America is a promising introduction.

This publication, like certain others produced by the Forestry Commission, can be criticized on a number of points. In the first place, some attempt should have been made to use the classification of heath-types dealt with in Chapter 1, and to treat them by classes. For example, upland heaths on the Red Sandstone areas of Scotland are radically different from those on the Jurassic of the Cleveland

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* Forestry Commission. *Bulletin* No. 32: *Afforestation of Upland Heaths*. By J. W. L. Zehetmayr. Pp. v+145+34 plates. (London, H.M. Stationery Office, 1960.) 17*s*. 6*d*. net.

Hills of Yorkshire. The so-called 'pans' differ markedly, depending on the looseness of texture of the underlying glacial deposits. The essential difference between the upland heaths of Britain and the continental heaths of Denmark, Germany and the Netherlands is clearly explained in Chapter 2. While such differences may justify the adoption of a completely independent attitude in Britain they do not excuse the neglect of the important work done in France by Duchaufour on heath soils or by Scandinavian, other than Danish, workers. Nor is the ignoring of the heath afforestation carried out in Great Britain long before the Forestry Commission was constituted excusable. Whether Continental heaths have "a longer history of systematic afforestation than our own" depends on what is meant by 'systematic'. The skilled tree-planters of Britain, in the eighteenth and nineteenth centuries, with a life-time of experience behind them, came to very definite conclusions about suitability of species, the value of ploughing and other techniques, and in forestry a life-time of experience in one locality may compensate for lack of intensive experiment at heavy cost, and the use of statistical methods to support the very obvious results which the intelligent observer sees and records in his memory, if not in his note-book. This is possibly rank heresy, but when one is confronted with the meagre conclusions from thirty-six years of intensive experiments, involving a multitude of measurements and calculations, one may be permitted to wonder if the capacity of the human brain is not nowadays underestimated.

Mr. Zehetmayr deserves great credit for the immense pains which he has taken to range over space, at least, and make himself conversant with heath-land planting. He has dealt with the enormous accumulation of experimental data in a masterly manner and makes good use of figures and illustrations. As a result of his breadth of vision, his conclusions are sound and are devoid of the exaggeration and puerile propaganda which sometimes detract from the value of forestry technical publications. He also places the important work so far achieved in its correct perspective historically, although perhaps his glance has been directed too far ahead and not enough backwards. Much more intensive soil research, as opposed to more-or-less empirical experimentation, is now required if further real progress is to be made. Meanwhile, adopting the same cautious attitude as the author, foresters and tree-planters would do well to be conservative, rather than rash, in their attitude, and to eschew the high-living tree species in favour of the abstemious. They should read and apply the lessons so far learned with discrimination, paying special heed to variation in locality conditions. In particular, deep ploughing on a large scale should not be used indiscriminately; it is often not only unnecessary but also harmful, especially in its effect on the drainage of catchment areas. The dweller in the lowlands is often unaware of what is happening in the uplands in these days. The excellence of Mr. Zehetmayr's interpretation will give great satisfaction to all who have participated in this project.

M. L. ANDERSON

REORGANIZATION OF BRITISH NATIONALIZED TRANSPORT UNDERTAKINGS

NEITHER in the White Paper on the *Reorganization of the Nationalized Transport Undertakings** nor in the debate on January 30, in which the Minister of Transport, Mr. E. Marples, asked the House of Commons to take note of the proposals for reorganization, was any reference made to the importance of research or of a sufficiency of scientific and technical man-power in Britain. Furthermore, the organization chart appended to the White Paper gives no indication as to how the organization for research is affected. The White Paper puts as the Government's aim undertakings soundly based on organization and finance, providing efficient services and giving a good livelihood and worth-while jobs to those who work in them. It directs attention to the fact that the railways are a vital basic industry, employing 500,000 people and representing an investment of nearly £1,600 million, still growing by more than £100 million each year. A railway system of the right size is regarded as an essential element in the British transport network, and will remain so for as long as can be foreseen, but the White Paper does not discuss the vital question: What is the right size? The related question of congestion on the roads is ignored, and while the idea of the railways as a social service is rejected, the question of uneconomic services such as branch lines is reserved for further consideration; it is merely stated that for the time being railway losses on any such services will be covered by the contributions proposed from public funds.

This point was stressed during the debate by Sir Toby Low, chairman of the Select Committee on Nationalized Industries, who, while agreeing that, in the main, the Government's proposals were in line with the recommendations of the Select Committee, was doubtful whether full control over the finances of the railway system could be achieved until the problem of dealing with uneconomical services had been decided. Sir Toby insisted that we could find out why losses are incurred, and he urged in conclusion that the real problem was not money but morale. Success can only be achieved by the vigorous use of every instrument of management and of science. Apart, however, from the Minister's announcement that the Government had decided that the main-line electrification scheme from Euston to Crewe, Manchester and Liverpool should go ahead as quickly as was consistent with the best use of the available resources, nothing in the debate clarified the uncertainty about the progress of modernization or the future of the uneconomical services. The Joint Parliamentary Secretary to the Ministry of Transport, Mr. John Hay, did not accept Sir Toby Low's challenge, and only stated that in 1960 the Treasury provided £160 million and in 1961 £140 million for investment in the railway industry, as a result of which more than seventy major projects had already been approved. Earlier, Mr. Marples had said that he would have to keep the electrification scheme (which is to cost £175 million) under close review to see how the estimates of cost and the rate of working compared, but that this did not necessarily mean that no other major main-line electrification scheme would be approved.

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* Ministry of Transport. *Reorganization of the Nationalized Transport Undertakings.* Pp. 14. (Cmnd. 1248.) (London: H.M. Stationery Office, 1960.) 1*s.* net.

The White Paper states that the main conclusions reached by the Government are that the activities of the British Transport Commission, as at present constituted, are so large and diverse that it is virtually impossible to run them effectively as a single undertaking. There has been confusion in judging between what is economically right and what is socially desirable, and the commercial capability of the railways is circumscribed by outmoded statutory obligations and restrictions on their trading operations. Mounting deficits (now totalling £500 million), the size of capital debt in relation to the earning capacity of the assets and the increasing burden of interest (already some £75 million alone) as modernization proceeds, present a situation detrimental to the morale of management and workers, to financial control and to hope of recovery. Accordingly, the Government has decided to replace the British Transport Commission and the existing organization by a new structure designed to overcome the main defects and disadvantages of the present organization, to reconstruct the finances of the Commission, and of the railways in particular, and to give the various undertakings the maximum practicable freedom of operation in their commercial affairs.

In the new structure, each of the main activities of the present Transport Commission—railways, London Transport, docks, inland waterways—will be managed by a separate Board holding its own assets and responsible for its own capital debt. The British Railway Board will be responsible for running the railways as an effective national system, but will perform only those central functions which are essential to the running of the railways as a single entity, including policies for safety, training and research, and the determination of the future size and shape of the railway system ; all other functions will be the responsibility of the Regional Railway Boards. Co-ordination of policy between the new Boards for railways, London Transport, docks and inland waterways will be the responsibility of the Minister of Transport, assisted by a new Nationalized Transport Advisory Council. His special duties in respect of safety, training and research will continue. As regards finance, the Government proposes to write off £400 million of the £2,000 million capital liabilities of the British Transport Commission, and when £400 million of the remainder has been transferred to the appropriate new Boards, to place in suspense £800 million of the £1,200 million still remaining against the railways so that it carries neither fixed interest nor fixed repayment obligations. The amount and treatment of this suspense account will be subject to review from time to time in the light of developments. It is expected that the railways will eliminate their present operating loss of £60 million a year. The importance of efficient operation and the most economic use of man-power is also emphasized, and the railways are to be freed from statutory control over their charges except for fares in the London Passenger Transport Area.

THE AGADIR EARTHQUAKE

FOLLOWING a detailed local study of the Agadir earthquake of February 29, 1960, P. Erimesco has published the conclusions in the *Bulletin de L'Institut des Pêches Maritimes du Maroc* (No. 5 ; September 1960). The author states that earth tremors in Morocco are not infrequent, although they very seldom attain an intensity of more than 5 on the Mercalli scale. In the list given in the text for the period from May 31, 1934, to October 28, 1951, eighteen earthquakes have intensity 4 or greater. Two of these were of intensity 7, and four had maximum intensity 6. The town of Agadir is situated at the intersection of two lines of marked seismicity. One line follows the region between the High Atlas Mountains and the Anti Atlas. The other crosses the High Atlas Range and continues to the coast of the Mediterranean.

During the night of February 29, 1960, after two slight tremors, an earthquake of unusually high intensity for the region (IX–X) devastated the town of Agadir (initial time 23h. 40m. 12s. G.M.T.). According to the map produced by the author, showing isoseismal lines for the shock, the small region of greatest intensity inside isoseismal X lies just on shore. The local estimate of depth of focus lies between 3 and 7 km., and of the magnitude of the shock 5·75 to 6. According to Vít Kárník, of Czechoslovakia, the average formula connecting magnitude (M), intensity (I_0) at epicentre, and depth of focus (h) for European shocks is :

$$M = 0{\cdot}67\, I_0 + 1{\cdot}7 \log h - 1{\cdot}4$$

For $I_0 = 10$ and $h = 5$ km. this gives $M = 6{\cdot}48$, which is somewhat higher than the local estimate.

Whole areas of the town, situated near the port and along the shore, suffered very heavy damage, and in certain places total destruction. A squadron of the French Navy, rushing help to the stricken town, registered on the way two hitherto unknown depths of 15 m. and 10 m. where charts indicated 900 m. and 90 m., respectively. To check these soundings taken visually, two vessels of the Navy made, the next day, a systematic search. At the places in question, no echo at all could be obtained.

At the moment of the earthquake, the trawler *Rolando*, heading south-west, passed approximately the place where later the 10-m. depth was observed. The crew reported that they felt three shocks, as if their boat had hit a solid object. The sea became very choppy for several minutes and a glow appeared in the sea, shifting rapidly towards the town of Agadir. Shortly afterwards they saw the lights in the town going out.

In spite of near-by badly damaged buildings, the quay walls were found practically without serious deformation, with the exception of two short oil wharfs. The slip for the fishing boats had not suffered at all. The tide gauge at the time of the earthquake registered the choppy sea in the harbour usual during that time of the year on account of wind from the south-west, but no exceptional waves. To investigate changes on the sea-floor consequent on the earthquake, P. Erimesco with his assistants examined the effects in the harbour of Agadir and along the shore. In the Bay of Agadir, systematic soundings were made and samples were taken from the bottom at the place where the 10-m. depth was observed. The soundings could not detect any variation from the depths marked on the charts; and the bottom samples at the place where volcanic activity had been suspected contained living annelids in perfect condition.

Thus, contrary to local reports at the time of the earthquake, we have evidence of a most unusual occurrence, namely, an area of intense earthquake destruction practically on the shore line, and virtually no effect at sea—neither the sea bottom nor the sea waves—except perhaps the rising of a shoal of zooplankton and a cloud of mud in the sea.

P. Erimesco seeks to explain this by reference to the local geology. He states that from the geological structure of the region it can be inferred that the almost vertical Cretaceous and Tertiary strata near the coast are dipping steeply underneath the sea bed. The covering Quaternary and Recent layers are sub-horizontal or horizontal and, in their upper part, not yet compacted, and are saturated with water. Earthquake waves in the Cretaceous, or in the underlying folded Palæozoic strata, are thus propagated mainly in the solid rock, and, by total reflexion, literally channelled towards the outcrop of these strata on the coast. In the unconsolidated and water-saturated material at the bottom of the sea, the energy of the shock waves is largely dissipated in the material, and only locally can a fraction of the energy released by the earthquake reach the surface of the sea. The trawler *Rolando* might have been at just such a place.

E. TILLOTSON

SEDIMENTATION IN OROGENIC BELTS

A LITTLE more than twelve years ago, Kuenen and Migliorini set out the evidence which had led them independently to the fruitful idea that certain marine sediments may be re-deposited in deeper water, possibly many hundreds of kilometres distant from their temporary resting places, by the action of turbidity currents. The speakers at the symposium "Some Aspects of Sedimentation in Orogenic Belts", held by the Geological Society at the Royal Institution on February 8, had a wide field over which to range, but it was remarkable how much of the work described stemmed directly or indirectly from this concept.

In opening the symposium the president, Prof. S. E. Hollingworth, reminded the audience how present-day views on re-sedimentation could be traced back to the work of Bailey and O. T. Jones in the 'thirties. The principal speakers in the first session dealt with work in the Alps (Prof. R. Trümpy), the Polish Carpathians (Prof. M. Ksiazkiewicz) and the Himalayas (Prof. W. D. Gill). In the second session Prof. A. Wood spoke on the Lower Palæozoic of Wales, Prof. Scott Simpson on the Upper Palæozoic of south-west England, Prof. R. M. Shackleton on the Dalradians and Prof. Gill on a Carboniferous basin in Ireland. Discussion followed in each session. A summary account will appear in the *Proceedings of the Geological Society*, and rather than duplicate this summary I propose to consider in a more general way some of the topics which came up during the meeting.

Prof. Trümpy, who has recently provided a welcome account of alpine sedimentation[2], opened with a warning note on the use of the term 'flysch'[1,2], which, he said, describes a facies group and not a rock. Flysch, he continued, is marine, thick (> several 100 m.), and essentially composed of sandstones and shales in proportions varying from about 5 : 1 to 1 : 10. Individual beds can be followed for long distances, and flysch is in general monotonous. The shales are silty and the sandstones are often felspathic and generally micaceous. True greywackes are rare. Breccias and conglomerates, though quite subordinate, are typical of many flysch deposits. Prof. Trümpy pointed out that graded calcarenites are not called flysch in the Alps.

The structural setting in which flysch appears was discussed by several speakers. Prof. M. Ksiazkiewicz[3-9] showed how flysch was deposited in various parts of the Carpathian basin at different stages of its evolution. Thus in the outer zone folded in the Lower Miocene the flysch formed from the end of the Jurassic without interruption until the Oligocene. In the middle zone flysch did not appear until the Lower Senonian shortly before the pre-Maestrichtian orogenic stage, while in the inner zone flysch rests on rocks folded at the end of the Cretaceous. In the Alps, Prof. Trümpy suggested, flysch marked a stage when tension gave way to compression. Prof. Scott Simpson made the point that flysch is not confined to fold belts, while Prof. Gill gave a striking account of a comparatively small and shallow epicontinental basin in the Visean of County Dublin, in which slides and turbidity currents had developed in a non-orogenic setting.

The difficult question of the depth of water overlying flysch deposits was approached in several ways. Prof. Trümpy placed the fossils of the Alpine flysch in three ecological groups; an authochthonous fauna of fossils such as fucoids and helminthoids derived from soft-bodied animals indicating deep water beyond the penetration of light; a transported benthonic fauna including the larger foraminifera, calcareous algae and bryozoa, preserved in graded sandstone beds; and thirdly, pelagic microfossils such as globigerinids and radiolaria. Dr. J. F. M. de Raaf reported that foraminifera similar to or identical with recent deeper water species had been found in pelitic rocks above individual turbidites from flysch deposits in Switzerland, France, Spain, Italy and Greece. Profs. Ksiazkiewicz, Scott Simpson and Trümpy gave evidence which indicated that deep-water conditions were established before deposition of flysch began. Where shallow-water sediments were followed by flysch an intervening group of deep-water sediments was often present. In this connexion an interesting parallel emerged between a succession from south-west England in which, during the Variscan orogeny, Upper Devonian shallow water sediments were followed first by deep-water shales, cherts and limestones and then by Namurian flysch, and certain Alpine successions in which shallow-water platform deposits were covered first by shales and fine-grained limestones with a purely pelagic microfauna and then by flysch. In both regions the arrival of flysch was heralded by a phase when sedimentation did not keep pace with downwarping. In contrast, Gill attributed the lack of flysch over the greater part of the Himalayas to the building up of land masses in Middle and Upper Eocene time.

Prof. A. Wood[10–12] described some of the variations in lithology shown by the Aberystwyth grits which he employed to demonstrate the changes which could occur in turbidites as they are followed down-current. He discussed the behaviour of the associated mudstone and suggested that under certain conditions muds were stripped off and added to the turbid suspension passing overhead. Detailed work of this nature and of the kind carried out by Radomski in the Alps is beginning to establish the relationship between changes in facies and the several environments which might be expected in a single basin and seems likely to improve our understanding of the mechanism of turbidity currents.

Later in the discussion Mr. W. A. Cummins suggested that the finer-grained chloritic fraction of some greywackes might in part be a secondary feature. The apparent lack of contemporary greywackes might be explained in this way. Dr. J. G. Ramsay and Dr. J. L. Knill mentioned the difficulties which may arise in establishing the original attitudes of deformed sedimentary structures. Although, as Prof. Wood pointed out, account is usually taken of the effects of plunging structures, so far as I am aware the effects of shear folding in which the angle between a sedimentary structure and the fold axis alters during folding have not been considered. The results presented by Ramsay[16] on this question should allow observations to be made in rocks deformed in this way.

Prof. Shackleton summarized present knowledge of Dalradian current directions[13–15], and commented on the distribution of the various Dalradian sediments, some of which remain remarkably consistent from western Ireland to north-east Scotland. Dr. B. Sturt described variations within the Dalradian of Perthshire and Prof. Shackleton mentioned the recent recognition of turbidites near the base of the Torridonian succession in Colonsay and Islay.

One of the most arresting features of the symposium was the sequence of maps presented by Prof. M. Ksiazkiewicz[3–9], which illustrated the results of measurements of current directions and facies analyses in the Polish part of the Carpathian flysch zone. This work, carried out by Prof. Ksiazkiewicz and S. Dzulynski, A. Slaczka, K. Zytko, W. Sikora, L. Koszarski and A. Radomski, has resulted in an elegant demonstration of the forms of the troughs and of the whereabouts and nature of the source areas. The varying directions taken by sediments were shown, and it was demonstrated, for example, that for a considerable time the axes of two neighbouring troughs sloped in opposite directions so that a northern trough was fed from the west and a southern from the east. These important results provide one of the most detailed accounts yet established of the movement of sediment within an evolving orogenic belt. I suggested, when summing up the day's discussion at the close of the meeting, that a synoptic account of comparable observations made in the British Palæozoic might be useful in bringing out what is known on this topic and what we need to find out in the future.

J. Sutton

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ROCK ENGRAVINGS IN NORTHERN ITALY

SOME years before the First World War, l'Institut de Paléontologie Humaine was founded by Albert I, Prince of Monaco. Its various publications, not least the series of *Archives*, are of importance to every prehistorian. *Memoire* 31, entitled *Le Grande Roche de Naquane*, has maintained the standard. Rock engravings in the Val Camonica have long been known, especially as the result of the publications of G. Marro and R. Battaglia, but the time was ripe for this detailed study by M. Anati to appear. The carvings are found on rocks, many hundreds of them, up the valley for about 30 miles from near Boario to Edolo. A line drawn on a map from Bergamo to Bolzano will pass not far from the art complex. The author has attempted to separate these stylized drawings into three series: the first dating may be as old as the late Bronze Age, a second belonging to the Iron Age, and a third, which was still being made when Rome conquered northern Italy. A glance at the illustrations—there are no less than 52 full pages—reveals that there are depicted in a pocking technique animals, mainly domestic but including stags, human beings and various weapons and signs. The author decided not to select his illustrations from various sites in the complex, but to concentrate on a single rock surface. There is thus given a complete account of at least one locality from among the 600 or so which have been identified.

The Val Camonica is a long valley running up into the Alps, and it is probable that it has always been a highway for people passing north and south. But why for 30 miles or so folk should have taken the trouble to cover rocks with stylized figures of animals, men and signs remains a mystery. It is easy to talk about ritual, but that does not answer the question. Why the ritual, and what was it ? Similar rock art complexes are known, the most famous being those surrounding Monte Bego in the Ligurian Alps. While there is a certain similarity with those of the Val Camonica, there are also notable differences (the characteristic halberds of Monte Bego seem to be replaced by spears at Val Camonica and I do not remember any stags at Monte Bego), and in any event their connexion would rather be with the earlier series at the Val Camonica than with the later. At Monte Bego there is a thunderstorm almost daily at 3 p.m., due to the fact that it is the first high mountain inland from the hot Riviera coast. Local peasants, when asked what the weather will be, still say, "Let me see what the devil at Monte Bego is doing". One can imagine a reason for a ritual to take place from time to time at that mountain, but at Val Camonica such a reason would seem to be absent.

M. Anati is to be congratulated on producing a very useful and much-wanted work. Two criticisms must be added. No general index is supplied, and while there are references to a few works in the text, there is no bibliography. This is a real nuisance. French publications seldom carry any index : a policy to be questioned. In the present case, its absence is a great inconvenience. But these two defects must not be allowed to detract from the real value of M. Anati's excellent work. MILES BURKITT

HYPOTHERMIA AND THE EFFECTS OF COLD

THE modern investigation of hypothermia in man can be said to date from the work of Temple Fay and G. W. Smith, carried out during 1938–39. They cooled patients suffering from inoperable carcinoma, in the hope that the lowered temperature would affect the new growth more than the rest of the body. The clinical work was complemented by physiological work by D. B. Dill and W. H. Forbes on hypothermic patients.

During the Second World War hypothermia became important as a factor in survival at sea. Shipwreck survivors rescued after prolonged immersion frequently died because of lowered body temperature. Much work on the effects of body-cooling was thus stimulated, with emphasis on the treatment of hypothermia rather than on its clinical use. After the War, A. H. Hegnauer and his associates at Boston started a systematic investigation of the physiology of hypothermia in the dog, and about the same time W. G. Bigelow began his work at Toronto with the possibility of using hypothermia as a surgical tool.

Since 1950, the subject has advanced very rapidly, particularly as the surgical advantages of hypothermia have become apparent. Smith and Fay reduced body-temperature to between 30° C. and 32° C., and this was a hazardous procedure. For a long time it was found to be dangerous to lower the temperature to below 27° C. Now C. E. Drew and his colleagues cool their patients to below 15° C.

A recent issue of the *British Medical Bulletin* contains a fascinating collection of articles describing present investigations and how the techniques of hypothermia are being used in medicine and surgery (17, No. 1 ; January 1961 : Hypothermia and the Effects of Cold. Pp. 1–78+xvii. London : British Council, 1961. 20*s*.). The articles include one by R. W. Salt on the resistance of poikilothermic animals to cold ; hibernation in mammals by Dr. L. Harrison Matthews ; local cooling in man by Dr. R. H. Fox ; physiological effects of continued cold on animals and man by J. S. Hart ; biochemical changes in exposure and acclimatization to cold environments by Dr. F. Depocas; the technique of induced hypothermia by Drs. I. K. R. McMillan and E. S. Machell; profound hypothermia in cardiac surgery by Dr. C. E. Drew. Other articles include accounts of experimental deep hypothermia and the circulation in hypothermia; metabolism in hypothermia; hormones in hypothermia; the central nervous system in hypothermia and the effect of temperature on the response to drugs; and sensitivity of hypothermic mammals to X-irradiation. Prof. A. S. Parkes has acted as co-ordinator of the whole series.

OPEN FORESTS IN TROPICAL AFRICA

PUBLICATION No. 52, issued by the Scientific Council for Africa South of the Sahara, contains selected papers presented at a conference of British, French, Belgian and Portuguese specialists held at Ndola, Northern Rhodesia, during November 17–23, 1959, and also the recommendations made and the conclusions reached*.

The conference considered the forestry problem of what is generally referred to as miombo woodland which, from Katanga (Congo) to Southern Rhodesia, covers some 670,000 square miles. The term miombo embraces several forest types ranging from dry high forest to savannah-woodland, but *Brachystegia* species are fairly typical of the tree canopy.

The sylviculture and management of the miombo woodland are made difficult by the large areas, the damage caused by fires, the low proportion of valuable species, the slow rate of growth, the long haulage distances, the limited uses of timbers which are not easy to work, slow to season, not durable and sometimes not possible to impregnate. *Pterocarpus angolensis* and the so-called Rhodesian teak, *Baikiaea plurijuga*, provide timber for constructional work and for joinery, but most of the other timbers are used for fuel-wood, mine-wood and smelter poles in the Copper-belt.

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* Scientific Council for Africa South of the Sahara. CSA/CCTA Publication No. 52. Pp. 126. (London: Bureau of Publications, CCTA/CSA Secretariat, Watergate House, York Buildings, W.C.2, 1960.)

As a result of burning experiments, covering a period of 26 years in Northern Rhodesia, there is ample evidence to confirm that late burning is most damaging to the vegetation and soil, complete protection is an unattainable ideal and that early burning is practical and beneficial by preventing damaging late fires, allowing the stocking of the trees to increase and not causing erosion.

Attempts are being made to resolve some of the problems of miombo woodland. There is a need for coniferous timber and it is being imported from Canada at great expense into the Copper-belt. In tropical and sub-tropical Africa mountain grassland is used largely for the planting of exotic conifers, but Northern Rhodesia has none. However, as a result of a well-planned, cautious programme, instituted some 20 years ago, tropical pines are being planted in suitable soils in miombo woodland.

A similar programme has been instituted in Katanga, but with eucalypts, and it is estimated that about one-hundredth of the area is required to yield the same amount of produce for the mines as can be obtained from miombo woodland.

It is good to note that not only were the problems affecting such large areas expressed at the Ndola Conference but also that steps have been put in train to exchange information between member Governments and to collect further information required for a fuller understanding of miombo woodland so that it may be put to better use.

AN A.C. MODULATED FLAME IONIZATION TRIODE

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A CONVENTIONAL flame ionization detector operates as a diode, the ionization current of which is measured with a d.c. amplifier. The effects of drift and fluctuations in gain associated with such d.c. systems may be avoided by the use of an a.c. system. This may take several forms: the diode may be supplied with an a.c. voltage[1]; the d.c. current out of the diode may be converted to a.c.; the flow of gas to the jet may be modulated with an oscillating diaphragm; or the diode may be converted to a triode and used as an a.c. amplifier.

In a recent account[2] of the discussion at the third International Symposium on Gas Chromatography, L. Ongkiehong is reported to have suggested the insertion of a third electrode into the flame ionization detector. We have been investigating the properties of such a triode system for some time, and preliminary results are presented here. The triode detector has a large output and needs no further amplification of the signal, allowing a considerable reduction in associated equipment. By a suitable choice of operating conditions, the detector can have a linear or approximately logarithmic response.

The system used consists of a hypodermic needle (0·4 mm. int. diam.) as a jet and a 1-sq. cm. 48-mesh platinum gauze anode situated about 9 mm. above it. The third electrode, termed by us the grid, is a cylinder 4 mm. int. diam., 2·5 mm. long, wound from 0·5-mm. platinum wire, placed coaxially and approximately level with the jet.

Such a system may be treated in the same way as a triode valve. Fig. 1 shows typical characteristic curves taken at a constant flow of 10^{-8} gm./sec. of ethylene. Only characteristics involving positive anode potentials and currents are given, superimposed on which are two load lines *BC* and *DE*, each of slope 2×10^9 ohms.

The way in which the system is used is as follows:

(1) Suitable values are chosen for the d.c. anode supply voltage and load resistor.

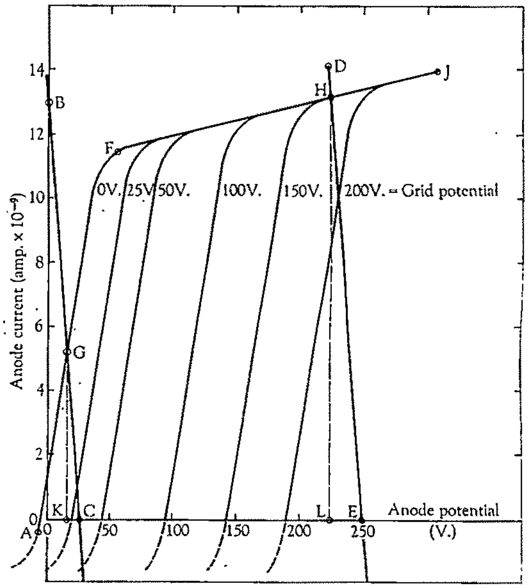

Fig. 1. Characteristic curves of flame ionization triode. Nitrogen flow, 50 ml./min.; hydrogen flow, 50 ml./min.; air flow, 800 ml./min.; ethylene flow, 10^{-8} gm./sec.; anode height, 9 mm.; grid height, 0 mm.

(2) A sinoidal voltage having a peak-to-peak value equal to the anode supply voltage is applied to the grid.

(3) A d.c. voltage equal to half the anode supply voltage is also applied to the grid, so that the voltage on the grid will vary sinoidally between zero and the anode supply voltage.

(4) The peak-to-peak a.c. voltage developed at the anode is transferred by a cathode follower to a phase-sensitive detector, the d.c. output voltage of which is related to the ionization current in the flame.

In Fig. 1, if C volts d.c. is supplied to the anode through a resistor of 2 × 10^9 ohms (corresponding to load-line BC), the peak-to-peak a.c. voltage developed at the anode is KC; similarly, if anode supply voltage E and load-line DE are chosen, the output voltage is LE.

If the ethylene flow-rate is doubled, the other flow-rates remaining unchanged, the height of the portion FJ of the curve doubles and the slope of AG doubles; consequently voltage LE is doubled but voltage KC only increases slightly. If anode supply voltage C and load-line BC are chosen, the detector will be approximately logarithmic for ethylene flow-rates greater than about 5 × 10^{-9} gm./sec. If anode supply voltage E and load-line DE are chosen, the detector will be linear up to, say, 10^{-7} gm./sec., and logarithmic for greater flow-rates. Thus, by suitable selection of anode supply voltage and load resistor the detector may be made approximately: (*a*) linear, (*b*) logarithmic, or (*c*) linear up to any desired flow-rate and logarithmic thereafter.

The flame ionization triode, like its vacuum counterpart, has both grid-anode capacitance and transit time effects. Because of the latter, considerable phase-shift occurs at frequencies above 20 c./s.; consequently it is preferable to apply sine waves rather than square waves to the grid. With the particular configuration used, the optimum frequency is 13 c./s. The grid-anode capacitance is of the order of 0·05 *p*F. which, at this frequency, gives a signal of about 1 volt peak-to-peak a.c. with no flame. This constant signal is removed by introducing an equal signal of opposite phase into the anode voltage supply. Constant background signals, caused by the hydrogen flame, are removed in the same way. This capacitance could be reduced by introducing further electrodes to form a tetrode or pentode; alternatively the grid may be earthed and the a.c. voltage applied to the jet.

One particular advantage of this type of detector is its high output. Applying 100 V. peak-to-peak to the grid and using a cathode follower with a measured input impedance of 2 × 10^9 ohms, followed by a phase-sensitive detector but without any intermediate amplifier, some 20 volts d.c. is available at the output of the phase-sensitive detector for an ethylene flow-rate of 10^{-8} gm./sec. The noise-level of the system in these circumstances is about 100 μV., which suggests an attainable detection limit of the order of 10^{-13} gm./sec.

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PHOTOLYSIS OF NITROMETHANE

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IT is reasonably certain[1] that the main primary step in the pyrolysis of nitromethane (at pressures below 200 mm.) is a breaking of the C—N bond and that a fraction of the methyl radicals produced form methane by hydrogen abstraction. Gray, Yoffe, and Roselaar[1] suggested that the mechanism of the photolysis is essentially similar, namely, a primary split:

$$CH_3NO_2 + h\nu \rightarrow CH_3\cdot + NO_2. \qquad (1)$$

On the other hand, Brown and Pimentel[2] found that when nitromethane in solid argon at 20° K. was illuminated it isomerized to methyl nitrite, and considered that all the products resulted from the secondary photolysis of the nitrite. They stated "there seems to be no evidence preventing the assumption that the primary act of photolysis of gaseous nitromethane is the same as in the (argon) matrix", and as they found no evidence for the products nitrogen peroxide, methane or ethane they considered that (1) did not occur. The authors of both these papers claim that their primary step explains the photolysis products found for gaseous nitromethane by Hirschlaff and Norrish[3]. It is not obvious how either postulate explains the appearance of nitrogen as a major product. It seemed useful, therefore, to re-examine the gas phase photolysis and, in particular, to analyse the products for methane.

22 mm. of degassed nitromethane were photolysed at 27° C. in a quartz cell. (I am indebted to Mr. I. Brown of these Laboratories for a sample of pure nitromethane. Its mass spectrum was essentially identical with that quoted in "Mass Spectral Data of the American Petroleum Institute Research Project 44", Carnegie Institute of Technology, Pittsburgh, Pa.) The light source was a 250-W. medium pressure mercury arc used both directly and through a Jena *BG*3 filter which cut out all light below 2500 Å. The products uncondensed by liquid air were expanded into 1-l. flask and analysed with a mass spectrometer. The results were recorded in Table 1 in units of 10^{12} molecules/ml. of illuminated volume/sec.

Table 1

Product Light	CO	N_2	NO	H_2	CH_4	C_2H_6
None		0·9	1·7			
,,		0·1	0·5			
Full arc	159	23	76	45	15·0	0·6
,, ,,	144	18	85	46	14·5	1·1
,, ,,	161	21	79	45	14·2	0·5
Filter	32	4·6	14	9·3	0·81	0·04
,,	13	4·2	20	5·3	0·86	0·07
,,	23	3·3	19	6·8	0·90	0·09

Although the amount of methane was small the analysis was quite unequivocal since it depended on peaks at masses 15 and 16 to which there was practically no contribution from other gases present. The analysis for nitrogen was less accurate since it depended on the residual at mass 14 after subtraction of the contribution from nitric oxide and methane. Nevertheless its identification was quite certain. Small peaks, about twice the height of the mass spectrometer background, at masses 26 and 27 were in the right ratio to be produced by ethane and were tentatively so assigned.

A second fraction, of products uncondensed at −79° C., was examined, but the mixture was too complex for quantitative analysis. Nitrogen peroxide, carbon dioxide, nitric oxide and methyl alcohol were identified positively. A peak at mass 29 together with other residuals seemed fairly certainly due to formaldehyde. After subtraction of the contribution from carbon dioxide, residuals at masses 44 and 45 were in the ratio of 25 : 1 which agreed exactly with the ratio of these peaks in a sample of formaldoxime. It appeared unlikely that any of the mass 44 peak was due to nitrous oxide.

A solid white polymer was also formed during the photolysis ; wildly erratic results for the carbon monoxide and hydrogen analyses were obtained until the polymer was removed after each run. The product obtained by heating this polymer gave major peaks at masses 45, 30, 29 and 15 consistent with the view that it was a mixture of formaldehyde and nitrosomethane.

Except for the nitrogen, all the products found are those to be expected from the primary split (1). Methane is formed by:

$$CH_3\cdot + CH_3NO_2 \rightarrow CH_4 + \cdot CH_2NO_2 \qquad (2)$$

and the $\cdot CH_2NO_2$ radical probably splits to give formaldehyde and nitric oxide:

$$\cdot CH_2NO_2\ (? + M) \rightarrow CH_2O + NO. \qquad (3)$$

Oxygen is present from the secondary photolysis of nitrogen peroxide, and it is well known[4] that oxidation of methyl radicals leads to carbon monoxide, carbon dioxide, methyl alcohol and formaldehyde. The reactions:

$$CH_3\cdot + NO_2 \rightarrow CH_3O\cdot + NO \qquad (4)$$

$$CH_3\cdot + CH_3NO_2 \rightarrow CH_3O\cdot + CH_3NO \qquad (5)$$

probably occur also. The hydrogen is produced by the secondary photolysis of formaldehyde. Finally, Coe and Doumani[5] have shown that the reaction of methyl radicals with nitric oxide leads to nitrosomethane, formaldoxime and their solid polymers. Thus the evidence obtained from these analyses gives convincing support to the view that (1) is the major primary step in the gas-phase photolysis. This postulate is consistent with the results of Brown and Pimentel[2] if it is assumed that in the gas phase methyl radicals and nitrogen peroxide disproportionate by (4) but that in the 'cage' of the solid matrix at 20° K. they combine to form methyl nitrite rather than the original nitromethane.

This work gives no support to the conclusion of Hirschlaff and Norrish that nitrogen is the major product of the photolysis. It is not clear whether their 'nitrogen' was analysed for methane and hydrogen. Nitrogen is, however, a minor product, as it is also in the photolysis of tetranitromethane[6]. Except that energy considerations would suggest a heterogeneous reaction, the mechanism for nitrogen production in these photolyses is obscure.

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BIVALENT METAL IONS AS THE COUPLING FACTOR BETWEEN CELL METABOLISM AND THE RATE OF CELL MUTATION

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ONE of the most important interactions which take place in the cell is transphosphorylation. The way phosphates enter into the energy cycles and their presence in nucleic acids has been investigated by a great number of workers. What has become clearer recently is the importance of the divalent metal ions in this process.

Thus, Ashwell and Hickman[1] showed that calcium accelerates the breakdown of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) in spleen homogenates. X-rays were found to have a similar effect. As the radiations are known to deactivate enzymes, their action was ascribed to the destruction of cellular material allowing greater contact between the enzymes and ATP. More recently, Lowenstein[2,3], has demonstrated that enzymes are not necessary for the hydrolysis of ATP to ADP. Divalent ions, for example, Ca^{2+} and Mg^{2+}, alone were sufficient to bring about the transformation :

$$\text{ATP} + \text{orthophosphate (P–32)} \xrightarrow{Ca^{2+}\text{ and }Mg^{2+}} \text{ADP} + \text{pyrophosphate}$$

where: orthophosphate $= 5 \times 10^{-3}\ M$; ATP $= 1 \times 10^{-2}\ M$; $Mg^{2+} = 1 \times 10^{-2}\ M$.

The formation of pyrophosphate at these concentrations in aqueous solution by means of a non-enzymic reaction is quite remarkable. Lowenstein explained the catalytic action of the bivalent metal ions by the complex they can form with ATP ; although he is not sure whether Ca-ATP, Ca-ATP-Ca or Ca-ATP-Mg is the important entity leading to ADP formation.

The hydrolysis of condensed phosphates such as pyro- and tri-polyphosphate has been investigated in some detail[4]. It is markedly affected by changes in *p*H and the presence of metal ions (particularly calcium). Van Wazer[4], describing work by Griffith at Monsanto, has explained this on the basis of an 'activated complex' which takes the form of the metal chelate. On that basis, Ca-ATP ought to be the complex which leads to ADP.

The behaviour of the condensed phosphates and their organic derivatives (for example ATP, ADP) with respect to calcium ions in aqueous solution appears to be similar[5]. This is not surprising because the dominant ionic feature is the polyphosphate chain. To this must be added what is now known of the interactions between condensed phosphates, 'hardness' in water and metals on surfaces[6]. Fig. 1 shows the effect of mixing solutions of condensed phosphates and calcium. It is possible to get an insoluble salt $Ca_2P_2O_7$, or a soluble complex $CaP_2O_7^{2-}$.

$$(1)\ \xrightarrow[\text{in phase diagram}]{P_2O_7\text{ into calcium}}\ (a)\ P_2O_7{}^{4-} + 2\,Ca^{2+} \longrightarrow \underset{\text{insoluble salt}}{Ca_2P_2O_7}$$

$$(b)\ P_2O_7{}^{4-} + Ca_2P_2O_7 \longrightarrow \underset{\text{soluble complex}}{2CaP_2O_7{}^{2-}} \xrightarrow{\text{hydrolysis}}$$

$$(2)\ \uparrow\ \begin{matrix}\text{Calcium into }P_2O_7\\ \text{in phase diagram}\end{matrix}\ (a)\ Ca^{2+} + P_2O_7{}^{4-} \longrightarrow \underset{\text{soluble complex}}{CaP_2O_7{}^{2-}} \xrightarrow{\text{hydrolysis}}$$

$$(b)\ Ca^{2+} + CaP_2O_7{}^{2-} \longrightarrow \underset{\text{insoluble salt}}{Ca_2P_2O_7}$$

Tripolyphosphate behaves in a similar manner, but does not show as great an insoluble phase. Whether the complex is hydrolysed or not will depend on the *p*H of the solution. Once the insoluble salt has been formed, it is difficult to bring it back into solution again. In fact, it has been found that five times the stoichiometric quantity of polyphosphate is needed[7]. It follows that, whenever the calcium phosphate system passes through an insoluble phase, a time-lag must occur before the calcium can be brought back into solution and catalytic service. Also, once the insoluble calcium salt has formed, we are really working with an inhomogeneous system, as observed in detergency[6]. If calcium were to be precipitated in the cell as another insoluble salt (for example, calcium fatty acid) the same problems of solubilization would arise. The exact position taken up by the phosphates, ATP and ADP, during transphosphorylation and the build-up of nucleic acids must therefore be determined by the positions of the divalent metal ions and local conditions of *p*H.

These results, then, explain why small changes in calcium and ADP concentration have such a marked effect on the control of respiration in the cell[8]. Furthermore, the control points in phosphorylation would in fact be positions where divalent metals are to be found[9]. It can be seen from this that the part which calcium and magnesium ions play in cell metabolism is of the utmost importance.

In addition to the above it has been found that in the nucleus calcium and magnesium ion hold together sections of chromosomes[10]. That is, changes in the concentration of the ions will determine the degree of chromosomal aberration. Ethylenediamine tetraacetic acid (EDTA) was found to produce breaks in the chromosomes of *Vicia faba*[11]. *Tradescantia* grown in calcium-deficient diets showed similar results[12]. If the effect of calcium and magnesium ion concentration on chromosomal aberration were to be added to that of its action on the phosphorylating metabolism the pattern depicted in Table 1 would emerge.

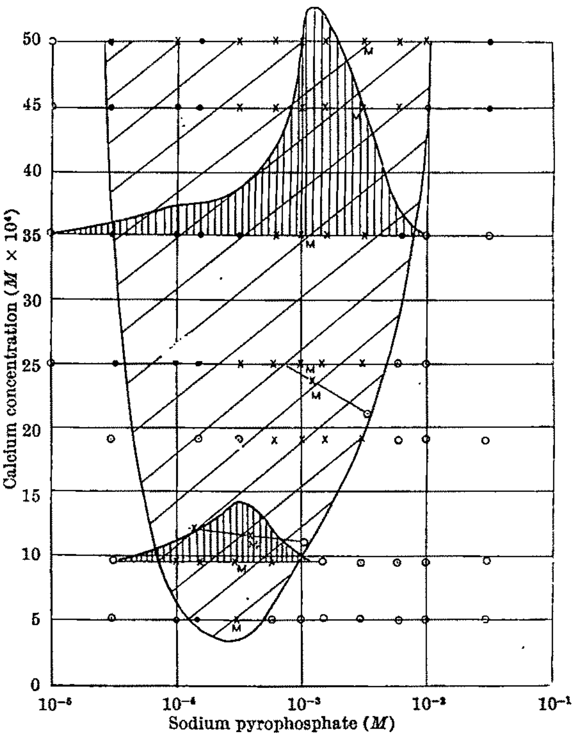

Fig. 1. Phase diagram of calcium pyrophosphate at 23°C. ×, Precipitate ; ○, clear solutions; ●, doubtful; ///, intensity of phase ; M, maximum in any series

Fluctuations in the concentrations of divalent metal ions in the various parts of the cell could arise through precipitation of an insoluble salt (for example, CaADPCa) rather than formation of the soluble complex (as in the chemistry of Ca^{2+} and $P_2O_7{}^{4-}$; see above[6]). Calcium ions removed by the former method would be virtually lost to the aqueous phase because of the time-factor involved in bringing them back into solution. This would also hold for calcium ions precipitated on walls of cell, mitochondria and nucleus. In effect, the system would have lost a homogeneous catalyst and possibly gained a heterogeneous one.

Table 1. EFFECT OF FLUCTUATION OF CALCIUM CONCENTRATION ON THE METABOLISM AND MUTATION-RATE OF THE CELL

Ca^{2+} concentration in the cell	Metabolic-rate	Rate of mutation	Mitosis rate of reproduction	State of cell
$\pm Ca^{2+}$	Normal rate of ATP breakdown	Normal number of chromosomal aberrations	Normal rate of mitosis	Normal integrated cell
$- Ca^{2+}$	Decrease in ATP breakdown	Increase in chromosome* aberrations (including mutant which allows cell to operate at lower Ca^{2+} concentration)	Mitosis retarded	Differentiation of the integrated cell (benign)
$+ Ca^{2+}$	Increase in ATP* breakdown (further increase for mutant which allows all to operate at lower Ca^{2+} concentration)	Decrease in chromosome aberrations (that is, holding on to any mutant produced in $- Ca^{2+}$ phase)	Mitosis increased (particularly for mutant) * Frequency of sensitive stage in cell division increased, that is, particularly sensitive to X-rays	Rapid (malignant) growth of non-integrated cell

—— show the effect of cycle in Ca^{2+} concentration.

* shows points where X-rays have an effect. They will also increase the permeability of the membranes.

If the cell, in order to carry on with its metabolism (transphosphorylation) removes calcium or magnesium out of the nucleus into the cytoplasm, it will increase the number of chromosomal aberrations. One of these may be a mutation which allows the cell to live with a metabolism at the new, lower calcium-ion concentration. When the original calcium-ion concentration is restored (by complexed calcium entering from outside the cell, or the calcium precipitate eventually redissolving into the aqueous phase) the cells with the 'low calcium metabolic' mutant would show a much higher metabolism and rate of mitosis. As increased calcium-ion concentration reduces chromosomal aberration, there would be no possibility of reversing the mutation or adapting it as before. This sequence of events is shown in Table 1.

Neoplastic growth induced by other agents would exert its effect at some point in the calcium control. For example, some carcinogens may induce this calcium cycle by interfering with the respiratory (electron and proton transfer) system, for example, phenanthrene. On the other hand, such compounds may cause precipitation of the calcium ions on the cell walls. X-rays will produce interactions at a number of points in the cell and these are shown (by an asterisk) in Table 1.

It should be possible to encourage the formation of 'rapidly metabolizing' mutants by submitting the cells to a diet which is alternatively low and high in calcium content. Conversely, mutation of normal cells should be suppressed by the introduction of a calcium 'buffer'[13] (for example, complex of calcium) into the cell. The way to decelerate the high rate of mitosis of mutant cells would be withdrawal of Ca^{2+} (for example, by means of EDTA). 'Synkavit' (tetrasodium 2-methyl-1, 4-methyl-1, 4-naphthohydroquinone diphosphate) which has been used by Mitchell[14] for the treatment of skin cancer and rectal adenocarcinoma probably acts in the same way. However, application of Ca^{2+} or Mg^{2+} depressants would induce chromosomal aberrations in normal neighbouring cells and, therefore, would have to be carried out locally. (From a medical point of view, the agent for removing calcium would have to be injected into the tumour or, if possible, the tumour would have to be bathed with such a solution.) There are obviously a great number of biological and medical experiments which could be based on the theory expressed above.

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REVERSAL OF DIURNAL TEMPERATURE RHYTHMS IN MAN

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RECENTLY, while working with the University of Nottingham Department of Pharmacy Expedition to Spitsbergen, the opportunity arose to study the diurnal temperature variations in a group of 6 men and 2 women, who were subjected to sudden reversal of their activity-sleep and light-darkness schedule. The subjects lived a controlled life of activity, diet, time of meals and they went to bed and awoke at set times each day. Spitsbergen provides an ideal environment for this type of work during the summer months because of the 24-hr. daylight and even temperatures. It was necessary for the subjects to sleep with blindfolds on to ensure the alternation of light and dark. They retired at 22.30 hr. each night and arose at 07.00 hr. under control conditions. On any test day, body temperatures were recorded every 3 hr. from rising until 22.00 hr. and again on rising the following morning.

Temperatures were taken using clinical thermometers placed in the armpit. It would have been desirable, but was impossible in the circumstances, to record rectal temperatures. Oral temperatures were found to be unreliable in an environment where facial cooling was appreciable so that skin temperature was the only possibility, and the armpit a very convenient site. After 4 test days on the control routine (periods 1–4) the subjects reversed their routine exactly on a 12 hr. basis. Thus they arose at 19.00 hr., worked through the night and retired at 10.30 hr. By sleeping with blindfolds on, the day became an artificial 'night' and the night became 'day'. Due to the 24 hr. daylight, there was little difference between night and day. In order to minimize the stress during reversal the subjects slept all night on the last control period, had breakfast, and then retired to sleep again, from 10.30 to 19.00 hr. From this point they arose, had breakfast again and worked through the night. Temperature records were made as before on test days, 2, 4, 6 and 8 days after reversal (periods 5–8). After 14 days on this routine, a reversal back to normal was made and the adaptation of temperature rhythms noted. Tests were performed again on four alternate days after the change (periods 9–12).

Daily routine

Periods 1–4 and 9–12 (hr.)	Periods 5–8 (hr.)	
06.50	18.50	Reveille, blindfolds lifted.
07.00	19.00	Body temperature taken, weather records made.
07.30	19.30	Breakfast.
10.00	22.00	Body temperature taken.
13.00	01.00	Body temperature taken. Lunch.
16.00	04.00	Body temperature taken. Tea.
19.00	07.00	Body temperature taken, weather records made. Dinner.
22.00	10.00	Body temperature taken.
22.30	10.30	Subjects to bed, blindfolds on.
07.00	19.00	Body temperature taken.

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Table 1. Diurnal Body Temperature Rhythms expressed in Degrees Fahrenheit and Standard Deviation

Time Period	07.00	10.00	13.00	16.00	19.00	22.00	07.00
1–4	96·6 ± 0·9	97·3 ± 0·9	97·0 ± 0·9	97·3 ± 0·7	97·2 ± 0·8	97·4 ± 0·8	96·7 ± 1·0
	19.00	22.00	01.00	04.00	07.00	10.00	19.00
5	97·1 ± 0·9	97·1 ± 0·8	97·2 ± 0·6	96·9 ± 0·9	97·0 ± 0·9	96·8 ± 1·0	96·9 ± 0·9
6	96·5 ± 1·0	97·3 ± 0·9	96·9 ± 0·8	97·2 ± 1·0	97·1 ± 1·0	97·1 ± 1·0	96·9 ± 0·9
7	96·6 ± 0·9	97·8 ± 0·7	97·4 ± 0·8	97·4 ± 0·8	97·3 ± 0·9	97·7 ± 0·9	96·7 ± 0·9
8	97·3 ± 0·7	98·0 ± 1·0	97·4 ± 1·0	97·7 ± 0·9	97·2 ± 0·8	97·5 ± 0·6	97·1 ± 0·8
	07.00	10.00	13.00	16.00	19.00	22.00	07.00
9	97·2 ± 0·6	97·5 ± 0·8	96·9 ± 1·1	97·2 ± 1·1	97·0 ± 1·0	97·1 ± 1·0	96·8 ± 0·9
10	97·1 ± 0·7	97·9 ± 1·1	97·7 ± 0·9	97·7 ± 0·5	97·1 ± 0·9	97·6 ± 1·0	96·7 ± 1·0
11	96·9 ± 0·7	97·5 ± 1·1	97·5 ± 0·9	97·6 ± 0·9	97·5 ± 0·7	97·4 ± 0·7	97·0 ± 0·5
12	97·0 ± 0·9	97·9 ± 0·9	98·0 ± 0·8	97·8 ± 0·9	97·6 ± 0·7	97·6 ± 0·7	97·3 ± 0·5

The results are shown in Table 1, where the average temperature during each period is recorded.

The diurnal variation exhibited by subjects, under these particular experimental conditions, was characterized by a rapid rise of temperature from waking to 10.00 hr. followed by a decrease, though temperatures remained high for the rest of the day. A slight rise of temperature occurred after each meal though one may not assume any significance in this correlation. During the night, temperatures fell to low values. In period 5, two days after the reversal of routine, the waking temperature was high and no rise occurred in the following three hours. A slight rise to lunch-time was followed by afternoon and evening temperatures which were below the waking value. It was clear that the temperature rhythm had not become adapted, by reversal, to the new time-scale, and that the temperatures recorded from lunch-time on were 'night values'. During the second 'night', however, the temperature rose slightly and to the normal waking value.

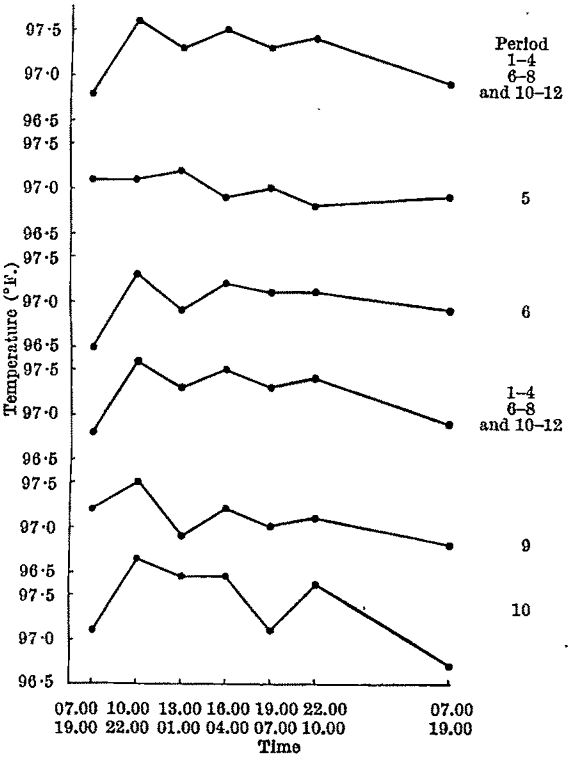

Fig. 1. Diurnal temperature variation

In period 6 a fairly typical diurnal variation was observed with a sharp rise after waking, a slight fall following, raised 'daytime' values and a fall at 'night'. Thus reversal of the diurnal temperature rhythm had occurred within 3–4 days of reversal of routine. After adaptation and two further test days a return to normal was accomplished by a further 12-hr. time shift. Period 9 represents the test day 2 days after the reversion to normal. The waking temperature was elevated, as in period 5, and only a slight rise occurred during the next 3 hr. The pattern from there onwards was similar to the control pattern except for lower temperatures and only a slight night fall. In period 10 a steep waking rise and night fall were both present. Reversion of rhythm to normal had again occupied 3–4 days. The pattern of diurnal temperature variations are shown in Fig. 1.

A diurnal variation of body-temperature was noted shortly after the introduction of thermometers into clinical practice. Warter[1], for example, recorded minimum temperatures in the early morning and maxima in the evening. Ogle[2] in the same year recorded afternoon maxima and also stated that the variations were greater in men than in women. A review of the earlier literature has been given by Woodhead and Varrier-Jones[3]. They pointed out the pitfalls of this type of work and emphasized the importance of sleep and light, the conditions and means of temperature measurement, and the fact that even when subjects are in bed for 24 hr. or more the diurnal temperature rhythm still persists.

Several investigations into the diurnal rhythm, and particularly its persistence, have been made. Kleitman and Doktorsky[4] have shown that the sleep-wakefulness cycle is an individually acquired phenomenon depending on the cerebral cortex for establishment and maintenance, and that the body temperature rhythm parallels the development of the sleep-wakefulness cycle. Observations on infants confirm the relationship between activity and temperature rhythms, though Metz and Andlauer[5] were unable to correlate temperature curves with variations of energy metabolism. That the temperature rhythms are developed by a process of acculturation is undoubted. Experiments on rabbits have shown that feeding habits play an important controlling part[6]. In man, fasting has been shown not to eliminate the rhythm. Kleitman found that diurnal temperature curves may differ in individual subjects with extremes of morning or early evening peaks[7], and also that after waking the morning rise in temperature may be followed by a 'plateau' effect for the remainder of the day and early evening[8]. In monkeys, the temperature rhythm has been reversed by reversal of lighting, while gradual extinction occurred after one week in darkness[9]. The diurnal rhythm has been reversed in man by reversal of daily routine[7], though resistance to change has been noted. Individuals may vary greatly in their adaptability, some failing to adapt after three weeks on night work, and others become adapted in a few days. Burton[10] found that his own temperature rhythm required 3–4 days to become accommodated with English time after a flight from Canada. This involves a time-shift of 5 hours in the daily routine.

In Fig. 1 selected periods are used to illustrate the diurnal variation, the intensity of the morning rise and the adaptation to a reversed routine. Reversal of the diurnal temperature curve occurred in 3–4 days and all the subjects showed the same general pattern. The normal temperature observed on waking on the third morning after reversal of routine is indicative that the rhythms were becoming stabilized on the new routine while the typical post-waking rise was present four days after reversal. Throughout the test periods no variations occurred in environmental temperatures sufficient to invalidate any comparison between periods. The temperature variations are clearly not directly dependent on physical activity or food intake because reversal did not occur immediately when the activity-sleep routine was reversed. They depend ultimately on habit and environment, with light as the most likely controlling stimulus. The time of adaptation reported here agrees well with Burton's observation of 3–4 days to become adjusted to a 5-hr. time-shift.

One further point of interest is that etiocholanolone causes a rise of body temperature after injection which may last for several hours[11]. In view of the diurnal variation of ketosteroid output which is greater by day than by night it was thought that this might be influencing the temperature rhythm. In 4 of the male subjects of this work, a simultaneous study of keto- and ketogenic-steroid excretory patterns was made. Reversal of the keto- and ketogenic-steroid pattern occurred in 2 and 6–8 days respectively. Because the temperature rhythm was reversed in 3–4 days it seems unlikely that these steroids play an important part in the control of body-temperature rhythms though this does not exclude the possibility that they exert some effect upon temperature-levels.

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TWO LINKED GENES SHOWING A SIMILAR TIMING OF EXPRESSION IN MICE

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TWO single gene mutations in the mouse show similar timing of expression during post-natal development. The two mutant alleles were found to produce nearly normal phenotypes until some critical time after birth following which normal differentiation is arrested. The first of these genes, originally reported by Morrow, Greenspan and Carrol[1] in *C3H* mice is expressed in the recessive state by a deficient production of the enzyme β-glucuronidase[2,3]. It has recently been demonstrated that homozygous mutant animals (*gg*) produce the enzyme in some tissues, notably liver, only until a certain post-natal stage, after which a progressive decline sets in and the enzyme ceases to accumulate further. Wild-type (*GG*) stocks produce the enzyme throughout growth[4]. A representative experiment is shown in Fig. 1.

The second mutation is that responsible for a recessive hereditary visual cell degeneration recently described from several laboratories[5–8]. At birth, the retinæ of mice homozygous for the recessive allele (*rd*) do not differ significantly from those of control animals, and post-natal differentiation at first proceeds normally. Beginning, however, at the onset of the most rapid phase of visual cell differentiation on the tenth day, and just after the visual cells have become minimally responsive to light stimulation, the cells die. By the fourteenth day, 50 per cent of the visual cells have disintegrated; at three weeks of age practically none remain, and the animals are blind. This process has been described in detail[8].

Both mutations are characteristic of *C3H/Ha* mice, and the experiments to be described show that in addition to sharing a similar pattern of expression, they are closely linked on the same chromosome. They do not, however, appear to control the same physiological process.

In these experiments β-glucuronidase was assayed by a modification of the phenolphthalein glucuronide procedure of Talalay *et al.*[9]. Activity is expressed as μgm. of phenolphthalein formed per hour when assayed at 56° C. Presence or absence of visual cell degeneration was ascertained by the electrical response to light stimulation (electroretinogram) and histological examination[8].

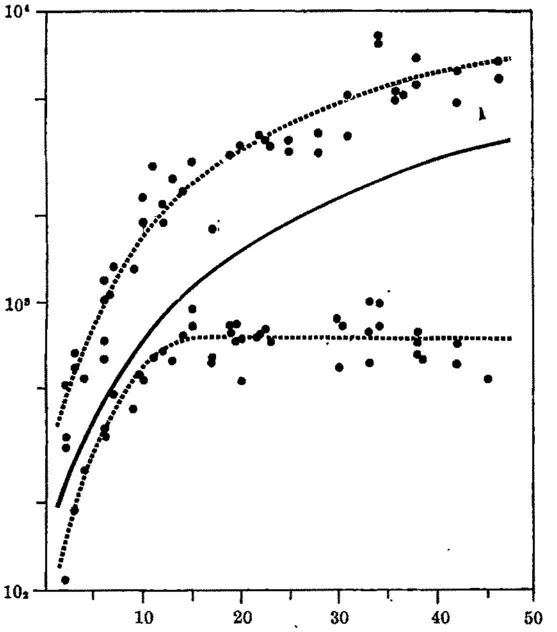

Fig. 1. The total enzyme content of liver plotted as a function of days of age in a population of mice segregating for the *g*-*G* alleles (that is, the progeny of a *gg* × *Gg* cross). Each point represents a single mouse and enzyme activity is expressed as total units per liver. The solid line is the growth curve of liver in this population expressed as mgm. × 10 dry weight. The dashed lines are drawn to indicate the growth curves of the enzyme in the two classes of progeny

It is apparent from Table 1, which lists the results obtained with a number of mouse strains, that *C3H/Ha* mice show both mutations. In *AKR*, *C3H/St* and *CFW* lines the *g*-*G* and *rd*-*Rd* allelic pairs are expressed independently of each other. The *C3H/St* and *CFW* are blind, but have high liver glucuronidase, whereas the *AKR* animals show no visual cell degeneration, but have low glucuronidase-levels. Evidence that the two loci do not affect a common function was also obtained from examination of *Ha/ICR* Swiss mice. The *Ha/ICR* Swiss are a deliberately non-inbred population. Among mice of this stock approximately 13 per cent (59/444) were blind and all (47/47) had high enzyme. That the genetic factor responsible for the visual defect in Swiss mice involves the same locus as that in the *C3H* is indicated by the failure (0/102) to observe sighted offspring of *C3H/Ha* × Swiss matings. Also, the behaviour of the *rd* allele in various other crosses was independent of its origin.

Table 1. GLUCURONIDASE CONTENT AND SIGHTEDNESS OF MOUSE STRAINS

Strain	No. of animals	Liver glucuronidase (units/mgm. wet wt.)	Sightedness
LaA	3	5·4	+
IPBR	3	6·7	+
I	3	6·7	+
5-*PRUNT*	3	6·8	+
H	3	7·0	+
CBA/St	6	7·4	+
CFW	3	7·6	0
C/St	3	7·8	+
B1+	3	7·9	+
CHI	3	8·0	+
C3H/St	9	8·3	0
JK	3	8·8	+
PBR	6	9·2	+
A	3	9·2	+
BrS	3	9·4	+
PIN	3	9·5	+
N	3	9·9	+
F	3	10·3	+
C57	3	10·3	+
2-*PRUNT*	3	10·5	+
DBA/2	10	11·6	+
LOW	3	12·2	+
AK	6	0·9	+
C3H/Ha	10	1·6	0

Table 2. LINKAGE OF GLUCURONIDASE AND HEREDITARY VISUAL CELL DEGENERATION

gG rd Rd × *gg rd rd*

	Blind	Sighted
High enzyme	1	33
Low enzyme	23	2

The crosses used were (*C3H/Ha* × *DBA/2*) × *C3H/Ha* and (*DBA/2* × *C3H/Ha*) × *C3H/Ha*. In crosses of the first type, the *C3H* carried the milk factor; in those of the second type, they did not.

The F_1 animals of the cross *C3H/Ha* × *DBA/2* are heterozygous at both loci and were indistinguishable from the dominant *DBA/2* parent. Examination of the offspring of a backcross of the F_1 to the *C3H* parent showed that the two loci are linked, being approximately 5 cross-over units apart (Table 2). Other linkages of the *Rd* or *G* genes are not known. In the present experiments neither factor was linked to sex, or to the coat colour genes *A*, *B* and *D*. In addition, these loci were not linked to the H-2 genic complex[10]. It has been reported that *Rd* is not linked to pigmy[11] or silver[12], and *G* is not linked to the colour genes, *A*, *B* and *C*[3]. These facts eliminate their location on linkage groups I, II, IV, V, VIII, IX or XX[13].

For the *rd* allele, although differentiation of the visual cells is blocked and cell death ensues, no effect is observed in the other retinal cells of the same ontogenetic origin, the pigment epithelium, the bipolar cells, and the ganglion cells. Similarly, for the *g* allele, although enzyme ceases to appear in the liver after the seventeenth day, enzyme production in the spleen is maintained throughout its growth. For both of these loci expression of the recessive alleles involves a nearly normal function until in some tissues, at a time characteristic for each, functional failure occurs. Despite these similarities, the two genes are separable by crossing-over and do not appear to involve a common function.

Although the genetic linkage observed may be purely fortuitous, in view of the similarities of the two loci the possibility must be considered that their linkage reflects the functional organization of the chromosomes.

A number of reports, stemming from the original observation of Beerman[14], have demonstrated that characteristic chromosomal regions swell and then shrink in the cells of particular tissues at certain stages in development, and the experiments of Briggs and King[15] suggest that differentiation may involve a concomitant loss of genetic totipotency among tissue nuclei. In the light of these findings several investigators have again proposed that the primary control mechanism in differentiation is a nuclear process involving differential gene activation or genetic segregation among the developing cells. Strong genetic support for a nuclear control comes from the experiments of McClintock[16–18], showing that a class of genetic elements exists that control the time during development at which secondary genetic events may occur.

The report of Callan and Lloyd[19] on the heterozygosity of puffing suggests that different alleles of the same gene can react differently to such control mechanisms during development, as appears to be

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the case with glucuronidase and hereditary visual cell degeneration. They found that in the oocytes of *Triturus* neither, one, or both members of a chromosome pair may show puffs at a given locus. The finding that although cells from different individuals differed widely, all oocytes from the same animal showed identical configurations at all sites, indicated that pairs of alleles were involved in which one member forms puffs and the other does not.

If the findings reported here are considered in relation to these cytogenetic phenomena, they suggest that particular chromosomal regions may be specialized to control the timing of expression of the loci within them, and thus provide a nuclear regulation of differentiation. More serious consideration of this hypothesis must await the finding of other cases of close genetic linkage between genes showing similar patterns of physiological expression.

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AMINOGLYCOLIPIDS AND GLYCOPROTEINS OF HUMAN BRAIN

I. Quantitative Fractionation of Brain Glycolipids by Silicic Acid Chromatography

WHILE there are a number of methods by which the glycolipids of mammalian brain may be individually isolated and purified, a method would be desirable whereby all the glycolipids of brain could be quantitatively separated and recovered, and their concentrations and other properties compared between individual brains. This is of interest not only with reference to the pathological lipodystrophies[1], but also with reference to the possibilities for chemical individuality of these substances in normal brain[2].

Since silicic acid chromatography has been found to be useful in the separation of both synthetic and native lipid mixtures[3], it was applied to the present problem. Whereas gradient elution of selected lipids from silicic acid columns had been found effective in the quantitative separation of a selected group of brain lipids[4], there was no evidence to indicate that a total hot alcoholic extract of brain lipids might be resolved quantitatively by these methods. With the desire to obtain quantitatively reproducible fractions which might then be compared in brain specimens from different individuals, a stepwise elution procedure was adopted, with quantitative exhaustion at each step of all the solids which could be removed from the column with a given solvent mixture.

Fresh whole bovine cerebral hemispheres, freed of investing meninges and blood vessels, were extracted with hot methanol as previously described for the isolation of brain ganglioside[5]. This procedure has been used by numerous investigators[6] for the preliminary extraction of cerebrosides and related glycolipids. In the course of preparation of brain ganglioside the filtered hot methanolic extract is allowed to stand at 4° C. for 48 hr., during which time a precipitate forms. The precipitate is filtered in the cold, then taken up at room temperature with chloroform – methanol (2 : 1), and partitioned twice with water. The upper phase contains the bulk of the brain ganglioside and other hexosamine-rich material present in the precipitate (and discussed in the next communication), and the resultant lower phase contains the other glycolipids and phospholipids. This lower phase material is taken to dryness by vacuum distillation at 60° C., taken up with minimal amounts of chloroform – methanol (2 : 1), allowed to dry at room temperature, and stored in a vacuum desiccator.

Silicic acid (Baker's reagent grade) was dried overnight at 105° C. To columns 40 mm. × 600 mm. with a fritted disk of coarse porosity was added a thoroughly mixed suspension of 250 gm. of silicic acid and 500 c.c. of chloroform – methanol (2 : 1) and additional chloroform – methanol added until a total of 1,250 c.c. were used. When the chloroform – methanol had run through to the level of the silicic acid, 1,000 c.c. of chloroform was added until the column was translucent and free of opacities[4]. The column was charged with 1,000 or 2,000 mgm. of lower-phase material in chloroform. The solvents utilized for elution were first chloroform, then mixtures of chloroform – methanol with increasing amounts of methanol, and finally pure methanol. The entire fractionation was done in a constant-temperature room at 25 ± 2° C. Fourteen fractions were thereby obtained (Fig. 1). In addition to the good general separation previously observed with more limited mixtures between glycolipids and phospholipids[4], excellent quantitative recoveries in terms of dry weight, hexose[2], phosphorus[2], and nitrogen[2] were achieved (Table 1). This quantitative result provides a possibility for a more reliable comparison of absolute amounts of these separate constituents in both human and animal brain preparations.

Fig. 1 shows that fractions III–VI are completely phosphorus-free, yet high in content of hexose. That these are distinct fractions is demonstrated: (1) by the fact that each fraction is exhaustively removed from the column by its particular solvent mixture

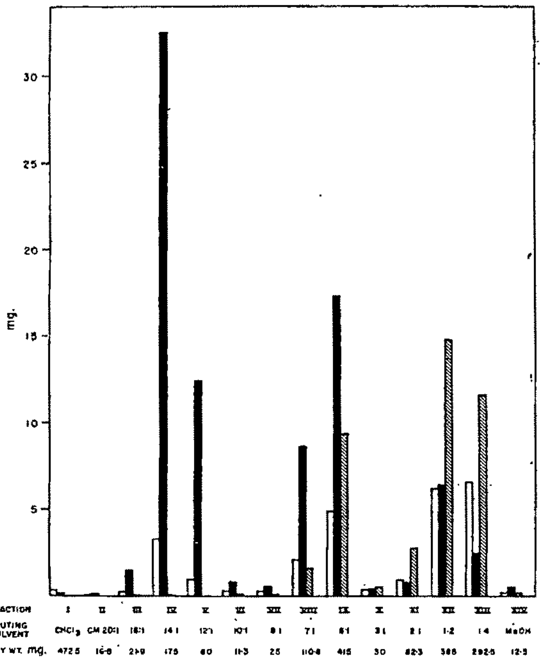

Fig. 1. Stepwise quantitative elution of brain glycolipids. Nitrogen, ☐ ; hexose, ■ ; phosphorus, shaded

until no further dry weight is obtained ; (2) the nitrogen percentage compositions as well as those of hexose differ appreciably in each of these fractions ; (3) these fractions can be distinguished by further partition in terms of their solubility in chloroform – methanol (2 : 1) at 4° C. Thus, for example, fractions IV and V under these conditions both yield precipitates with concentrations of nitrogen (1·73 per cent) and hexose (21·3 per cent) suggesting a cerebroside ; but the remaining supernatants differ markedly in fractions IV and V ; thus the material soluble in cold chloroform – methanol in fraction IV contains 1·8 per cent nitrogen, 22·4 per cent hexose, whereas the material soluble in fraction V contains 1·5 per cent nitrogen, 14·3 per cent hexose. Similar observations apply to each of the other fractions shown in Fig. 1 which contain hexose. Therefore, with respect to elution behaviour, total percentage composition, and composition of sub-fractions, the fourteen major fractions collected by the stepwise procedure described are distinctive.

When all the fourteen fractions obtained were examined for their content of neuraminic acid, this substance was found in only one of the fractions, fraction XII. That this represents brain ganglioside which has not (quantitatively) passed into the upper phase during the preliminary separation is suggested by the fact that purified brain ganglioside[6] was found to be eluted only at this stage when tested by itself on the identical silicic acid column. Thus, at chloroform – methanol ratios of 1 : 1, 1 : 1·5 and 1 : 2, respectively, brain ganglioside is obtained quantitatively. It is of interest to note that each of these three sub-fractions obtained from purified brain ganglioside shows varying concentrations of neuraminic acid and hexosamine. This variation would be in accord with the previous observation in this Laboratory that our preparation of brain ganglioside contains more than one immunochemically distinct substance[7], despite the fact that it demonstrates electrophoretic and ultracentrifugal homogeneity and homogeneity in several solvent systems. It must be considered, however, that despite the apparently non-traumatic nature of the silicic acid chromatography employed, partial degradation of a unitary brain ganglioside material might have occurred. In the case of more harsh solvent procedures utilized by others, in one case[8] acidic solvents known to promote degradation were utilized, and in another case[9] no fraction or sub-fraction of 'brain ganglioside' was obtained with concentration of carbohydrates equal to that of the purified brain ganglioside prepared in this laboratory. While there is no evidence that silicic acid chromatography as used here results in actual degradation in terms of neuraminic acid and hexosamine (recovery 104 and 105 per cent, respectively), the possibility that intramacromolecular disjunction occurred in such a way as to produce fragments with varying relative concentrations of neuraminic acid and hexosamine cannot as yet be ruled out.

Table 1. QUANTITATIVE RECOVERY OF DRY WEIGHT, NITROGEN, HEXOSE AND PHOSPHORUS IN STEPWISE ELUTION OF BRAIN GLYCOLIPIDS FROM SILICIC ACID COLUMNS

Constituent	Type of brain	Amount added to column (mgm.)	Amount recovered (mgm.)	Percentage recovery
Dry weight	Bovine	2000	2088·9	104·4
	Bovine	2000	2113·6	105·7
	Human	2000	2239·8	112·0
Nitrogen	Bovine	26·4	29·3	111·1
	Bovine	26·4	26·7	101·0
	Human	27·3	30·2	110·7
Hexose	Bovine	81·8	82·2	100·4
	Bovine	81·8	84·7	103·5
	Human	81·5	80·2	98·4
Phosphorus	Bovine	42·7	41·1	96·2
	Bovine	42·7	40·9	95·9
	Human	51·8	50·5	97·4

The presence of amino-acids has been tested for, both in the unhydrolysed and in the hydrolysed fractions obtained from the stepwise chromatography of lower-phase material. The combined precipitates obtained from fractions IV and V show the presence of amino-acids before hydrolysis to the extent of 0·17 per cent; this is increased by hydrolysis with 6 *N* hydrochloric acid for 36 hr. to 0·94 per cent. The filtrates show the presence of 0·13 and 0·80 per cent in the unhydrolysed fractions IV and V, and 1·5 and 1·74 per cent in the hydrolysed samples respectively. The presence of bound amino-acids in at least some of these glycolipid fractions of brain must therefore be considered as in the case of the *Drosophila* lipids[10], and is being further investigated.

The stepwise silicic acid chromatography method described therefore provides a quantitative means of separating, accounting for, and comparing individual glycolipid and phospholipid fractions from mammalian brain. The application of these quantitative methods to human brain specimens has been accomplished with the same yields (Table 1) and will be reported in detail elsewhere. These methods thus provide an opportunity of comparing quantitatively the presence of individual glycolipid fractions in individual brain specimens.

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2. Hot Methanol Extraction of Human Brain Aminoglycolipids

THE term 'aminoglycolipids' is suggested here to include the family of substances the basic constituents of which are glycolipid in nature, and the additional constituents of which include some amino-carrying components such as a hexosamine or neuraminic acid and its derivatives, or amino-acids, or any combination of these three classes of substances. This group of substances would therefore include cerebroside-like substances with extra hexose units per mole of sphingosine[1,2], cerebrosides with extra units of hexose and hexosamine[3] and the above substances with neuraminic acid and/or amino-acids attached[4,5]. The last members of this family, as first isolated[6], were named 'gangliosides'. The addition of these constituents bearing amino-groups is frequently associated with the fact that the complex which is formed bears macromolecular characteristics as determined by non-dialysability and sedimentation values in aqueous ultracentrifugal fields. Although very little is known of the biosynthesis of any of these[7,8], their structural similarities suggest a biosynthetic continuity. Furthermore, attempts to isolate these components by different solvent techniques frequently result in the extraction of protein or lipid substances apparently bound to the aminoglycolipid in either ionic or covalent form, but separable from the aminoglycolipid by a further purification procedure which is almost invariably rather harsh. Structural investigations carried out in this Laboratory[9,10] have led to some clarification of the structure of the macromolecular brain ganglioside of bovine origin and have demonstrated that cerebrosides are clearly structural sub-units of ganglioside[9].

The methods used for the extraction of bovine brain ganglioside have now been applied to human brain specimens obtained at autopsy in 36 mental hospital cases, most of whom were in the eighth and ninth decade of life. Quantitative results were obtained for the yield of 'crude brain ganglioside', neuraminic acid, hexose, hexosamine, nitrogen, and amino-acids, per gm. of whole fresh tissue.

Approximately 100-gm. aliquots of carefully dissected grey and white matter were homogenized for 2 min. with 5 volumes of methanol at room temperature. The homogenate was heated to the boiling point and quickly filtered through a pre-warmed Buchner funnel, the filtrate placed in the cold for 48 hr., the precipitate which formed separated by filtration in the cold and then taken up at room temperature with chloroform – methanol (2 : 1). This 'precipitate fraction' was analysed for dry weight and neuraminic acid, then partitioned as with the preparation of bovine brain ganglioside[10] with distilled water 12 per cent by volume, the upper phase removed and the lower phase again washed with 6 per cent water. The combined upper phases were designated 'crude brain ganglioside', taken to dryness by vacuum distillation at 55–60° C., taken up quantitatively with water and dialysed exhaustively at 4° C. in pre-washed 'Cellophane' bags against a double volume of water. After less than 0·5 mgm. was obtained in the outer water (usually by the tenth change) the dialysed material was collected quantitatively with water washings and analysed for its content of dry weight, nitrogen, neuraminic acid, hexosamine, and hexose[10]. Aliquots of this 'crude brain ganglioside' were hydrolysed with varying concentrations of acid and varying time to determine the maximum yield of hexosamine. This was found constantly to be obtained by 15 hr. of hydrolysis with *N* hydrochloric acid in a sealed tube at 100° C. in 6 hydrolysis curves (½, 1, 3, 5, 7, 9, 12 15, 24, 36, and 48 hr.) on five different brains. Then hexosamine was determined as a routine in this manner. Another aliquot of 'crude brain ganglioside' was hydrolysed with 6 *N* hydrochloric acid in sealed tubes at 100° C. for 1, 3, 6, 12, 24, and 36 hr. and the hydrolysate was tested quantitatively for free amino-nitrogen[11] and examined by paper chromatography[12]. Semi-quantitative paper chromatography of amino-acids present in this dialysed material after 36 hr. of hydrolysis demonstrated the presence of serine, glycine, glutamic acid, aspartic acid, cysteic acid, arginine, alanine, *iso*leucine, leucine, lysine, and one as yet unidentified amino-acid quantitatively present in approximately the order named. These findings are similar in some respects, but quite different in others from those described for a similar preparation from ox brain[5]. Thus threonine, phenylalanine, histidine, and valine were absent in our preparation, and serine was present in excess of glutamic acid. With short-term (1 and 3 hr.) hydrolyses, some indication was obtained of the order of release of these amino-acids. Glycine and serine were almost completely released in 1 hr., the amounts of arginine, glutamic acid, aspartic acid were present in approximately one-half their total, together with only traces of the other amino-acids mentioned here.

Table 1 shows some of the characteristic values for the carbohydrate constituents obtained to date. It may be noted that the crude brain ganglioside material obtained by identical extraction procedures in different brains analyses differently in each case, not only with regard to the absolute amounts of neuraminic acid, hexose, and hexosamine obtained per gram of whole tissue, but also with regard to their relative ratios. There is a higher content of hexosamine with reference to neuraminic acid in human brain and usually still higher content of hexose. It is therefore clear that this 'crude brain ganglioside fraction' derived from human brain by hot alcoholic extraction contains related non-dialysable aminoglycolipids other than bovine brain ganglioside as classically defined in terms of percentage constituents by Klenk[6] and structurally clarified in our own investigations[10]. Thus, our bovine brain ganglioside contains, in ash-free form, 30 per cent neuraminic acid, 24 per cent hexose and 10 per cent hexosamine, and the salt form of bovine brain ganglioside contains approximately

Table 1. Yield of 'Crude Brain Ganglioside' and Constituent Neuraminic Acid, Hexosamine and Hexose in 36 Individual Human Brain Specimens

	'Crude brain ganglio-side' (mgm./gm. whole tissue)	Neur-aminic acid (μgm./gm. whole tissue)	Hexos-amine (μgm./gm. whole tissue)	Hexose (μgm./gm. whole tissue)	Nitrogen (per cent)
Grey matter					
Mean	2·20	248·3	143·1	247·9	1·54
Range	0·44–4·50	105–406	29–284	54–512	0·5–2·8
White matter					
Mean	2·10	66·0	48·0	179·0	2·2
Range	0·13–7·3	9·7–191	3·2–124	31–419	1·1–5·0

26, 20·8, and 8·7 per cent respectively of these three constituents. In no case so far analysed did these ratios hold for the material examined. There must therefore be either a different type of ganglioside in the human brain, or a ganglioside similar to that found in bovine brain is present, but considerably contaminated by substances richer in hexose and hexosamine which are non-dialysable and partitioned to this point along with the hypothetical human brain ganglioside.

In the purification of bovine brain ganglioside, the crude material is repartitioned four of five times between chloroform - methanol and water[10]. When crude brain ganglioside material, after isolation from brains Nos. 63, 65, 69, 70, 72, was combined and further repartitioned four times, the lower phase contained some 9 or 10 per cent of the dry weight of the fraction at each stage and the material in the lower phase contained increasing amounts of both neuraminic acid and hexose. Thus, the lower phase from the second, third, and fourth repartition contained respectively hexose 6·7, 8·9, 12·4 per cent and neuraminic acid 4·6, 10·8 and 13·0 per cent. The upper-phase material remaining after the fourth repartition now contained 16 per cent hexose and 22·3 per cent neuraminic acid thus approaching the theoretical concentrations and molar ratios of these substances observed in purified brain ganglioside. Further purification and structural investigations on larger quantities of this material are in progress.

It is clear from these results that the classical brain ganglioside is only one of a group of non-dialysable aminoglycolipids which can be extracted from human brain grey matter by means of the hot-methanol method described; that the other substances so extracted are quite rich in hexose, hexosamine, and neuraminic acid; and that these substances can be separated from each other. It would therefore appear that a large family of related substances exists in human brain with varying amounts of the structural constituents described here, in macromolecular association, which might be grouped under the term 'aminoglycolipids'. From a practical point of view, it is clear from our results that neither hexose, hexosamine, neuraminic acid, nor any combination of these three components isolated by the above techniques can be used to extrapolate the concentration of brain ganglioside[13]. The next communication demonstrates that, in addition to the heterogeneity of these materials isolated with alcoholic solvents, the yield in terms of both neuraminic acid and hexosamine is only one-third to one-fifth of the total which can be demonstrated by the emulsion-fractionation procedure developed.

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3. Quantitative Separation and Determination of Brain Glycoproteins, Neuraminic Acid, Hexosamine, and Hexoses by Means of Whole Tissue Emulsion-Fractionation

THE demonstration that there is a family of non-dialysable aminoglycolipids in brain related to classical brain ganglioside[1,2] but differing from it in terms of the relatively greater content of hexosamine and hexose (previous communication) has made it necessary to devise suitable methods whereby all these substances may be extracted quantitatively, further purified, and studied in terms of their relative concentrations in individual brain specimens. In addition, these observations have raised the question of the nature of the macromolecular organization of these constituents *in situ*. This organization would appear to be well worth studying because, on one hand, electron microscopic observations may approach visualization of these complexes and, on the other hand, better understanding of such biological problems as the properties of living cell membranes in terms of receptor and transport functions appear to require better structural definition of what might be called the molecular geometry of membrane components.

Thus, for example, the isolation of brain ganglioside, which has been shown in this Laboratory to have physiological properties suggesting membrane receptor and transport functions[3-6], has generally been accomplished by means of hot or cold organic solvent mixtures. In one method of extraction[1], this aminoglycolipid is obtained in crystalline form and without the presence of amino-acids; in a second method[2], essentially the same glycolipid is obtained with further macromolecular properties, but still without amino-acid constituents; and in a third method of extraction, the glycolipid is obtained with macromolecular characteristics and with the presence of attached amino-acid constituents[7]. These experiments suggest that it would be fruitful to approach these macromolecular aminoglycolipids from the 'protein point of view'. That is, where harsh organic solvents might dissociate protein – aminoglycolipid complexes, leaving only a few amino-acids tied to the aminoglycolipid (previous communication, and ref. 7), solvent methods which are directed towards isolation of the proteins of brain might accomplish the isolation of the intact protein and carry with the protein the glycolipid constituents.

A qualitative method for approaching this problem has been developed in recent work in this Laboratory and will be reported here briefly, and elsewhere more extensively. By means of the emulsion-fractionation method to be described, essentially all the proteins of the brain are separated into nine primary fractions in a manner which does not necessitate the discarding of large amounts of protein as insoluble[8], and which permits further fractionation to be undertaken. Furthermore, new results for the total concentrations of brain neuraminic acid, hexosamine, and hexose are thereby obtained.

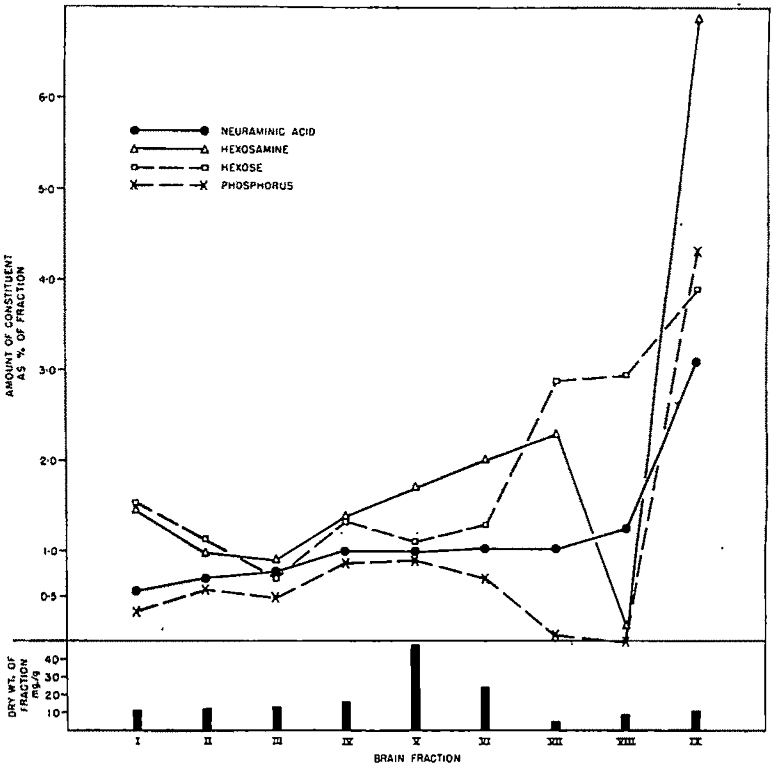

Fig. 1. Emulsion fractionation of human brain grey matter

Freshly autopsied human and bovine brains were immediately stripped of investing meninges and blood vessels and grey and white matter carefully dissected from particular anatomical areas. While the results reported will be on the basis of 5-gm. aliquots of dissected grey matter, all the procedure can be carried out with 1 gm. or less of whole tissue and has been done with white matter as well. 5 gm. of brain tissue is homogenized in a Waring blendor with 180 c.c. of distilled water for exactly 5 min. The temperature at the end of homogenization is 32-33° C. The balance of the procedure is carried out at room temperature (25° C.). (The entire procedure is also effectively carried out at 0°.) The rather stable emulsion which is formed is transferred with 3 c.c. water washing to centrifuge bottles and centrifuged at 1,300 r.p.m. (225 *g*) for exactly 10 min. The precipitate which results is denoted fraction I. The supernatant is carefully decanted, methanol is added to the extent of 50 per cent by volume, the solution is mixed and immediately centrifuged for exactly 10 min. The precipitate which results is denoted fraction II. To the supernatant, which has been carefully decanted, is added sufficient methanol to reach an equivalent of one volume relative to the original volume of emulsion. The solution is immediately centrifuged as above and the precipitate (fraction III) is obtained. By successive addition to the supernatant at each stage of aliquots of methanol so that the final solution contains 1·5, 2, 2·5, 3, and 3·5 volumes of methanol relative to the original volume of emulsion, precipitates are obtained at each stage which are successively denoted fractions IV – VIII. The supernatant remaining after precipitation of fraction VIII with 3·5 volumes of methanol, which is now water-clear and which no longer yields appreciable further precipitate on the addition of further volumes of methanol, is taken to dryness by vacuum distillation at 55–60° C. and is designated fraction IX. Each of these nine fractions is quantitatively taken up in distilled water to form a 1–10 mgm. per c.c. suspension. This suspension can then be analysed, taken up in buffers, or further partitioned into dialysable and non-dialysable constituents. For this further partition the suspension is transferred quantitatively with distilled water to pre-washed 'Cellophane' bags and dialysed at 4° C. against equal volumes of distilled water if collection and further study of the dialysable material is desirable, or against 10- to 20-fold volumes of distilled water for more rapid isolation of non-dialysable components when the dialysable components are not sought.

Fig. 1 summarizes a typical emulsion-fractionation procedure in terms of 5 gm. of grey matter. The dry weight is shown in the bar graph and the percentage of phosphorus, neuraminic acid, hexose, and hexosamine is shown for each of the nine dialysed fractions. The analytical methods used are as given in the previous two communications. It may be seen that each of the nine fractions is distinguished not only in terms of its precipitability at different concentrations of methanol, but also in terms of its percentage composition of the above constituents. Thus, the early fractions are poor in phospholipid, relatively rich in bound hexose, and relatively poor in bound neuraminic acid. With increasing concentration of methanol, non-dialysable complexes are precipitated with varying, but generally increasing concentrations of neuraminic acid, hexosamine, and hexose. The concentrations of nitrogen in fractions I–IX respectively are 4·6, 5·2, 4·8, 13·2, 8·1, 6·8, 6·1, 5·2 and 14·2 per cent. The concentration of bound hexose increases markedly in the last three fractions. Phosphorus is low in fractions VII and VIII, and the concentrations of nitrogen, hexose, hexosamine, and neuraminic acid in fractions

VII, VIII and IX suggest relatively purer glycoprotein preparations. Recovery in terms of dry weight of the unfractionated emulsion is 94–98 per cent.

Each of these fractions can be fractionated further by virtue of solubility in different buffer systems or, as illustrated here, by treatment with mixed solvent systems. Thus, for example, in one brain when fraction IX was partitioned between the three-solvent system of chloroform–water–methanol[6], whereas the lower phase contains lipid material with only 3·6 per cent nitrogen, 0·19 per cent neuraminic acid, and 1·6 per cent hexose, the first two upper phases which are obtained combined contain glycoprotein CIX*a* with a nitrogen content of 11·8 per cent, neuraminic acid content of 1·7 per cent and hexose content of 4·7 per cent. In other brain specimens CIX*a* contained as much as 4·1 per cent neuraminic acid and 8·0 per cent hexose.

The above emulsion-fractionation method thus permits the quantitative fractionation of all brain proteins into nine primary fractions in association with native lipids in the form of emulsions (not true solutions, with the exception of fraction IX), in which the protein components appear to be protected from gross denaturation by the presence of the associated lipids (possibly the same lipids as those *in situ*) and by the absence of harsh organic solvents at the preliminary separation stages. This procedure, in contrast to others[8], does not produce masses of insoluble denatured protein amounting to 50–90 per cent of the total protein content of brain.

The yield of grey matter neuraminic acid by emulsion-fractionation (1·7–1·8 mgm./gm. whole tissue) is in excess of that obtained with chloroform–methanol[10] and with hot methanol techniques (previous communication). The partition of fractions CIV, CV and CIX by chloroform–methanol–water demonstrates that much of the neuraminic acid of brain previously assumed to be part of brain ganglioside is in fact part of other aminoglycolipids and glycoproteins.

Similarly, the yield of hexosamine from human grey matter alone by emulsion-fractionation (2·3–2·7 mgm./gm. fresh tissue) is two or three times that previously reported[12] for whole bovine brain. In addition to higher total yield, the disadvantage of the multiple organic solvent and water extractions for hexosamine[12] (which left 62 per cent of the recovered brain hexosamine in the insoluble residue) is overcome.

Both hexosamine and neuraminic acid are seen here in association with several brain glycoprotein fractions, which vary in the ratios of their content of each of these constituents. The total concentration of grey matter hexosamine is found to be in excess of that of neuraminic acid (whereas in purified brain ganglioside the reverse is true, that is, neuraminic acid being present in threefold excess of hexosamine by weight). Therefore, attempts to estimate brain ganglioside on the basis of the amounts of total neuraminic acid and/or hexosamine which can be extracted[11,13] cannot be considered valid, and brain ganglioside can only be determined at present by isolation of the material which has the characteristic ratios of these constituents. The presence of this non-ganglioside brain hexosamine and neuraminic acid is partially accounted for by brain glycoproteins.

The yield of grey matter hexose by emulsion-fractionation is 2·2–6·0 mgm./gm. fresh tissue. Whereas essentially all the neuraminic acid and hexosamine is non-dialysable, 16–40 per cent of grey matter hexose is dialysable, that is, approximately 1 mgm./gm. fresh tissue, a figure in good agreement with those given previously for 'labile' brain glucose[9].

There is evidence of variation between specimens of brain from different individuals in the total amounts of each constituent in different fractions, a finding of interest in relation to the high degree of chemical individuality observed in this Laboratory in work on the same constituents in another compartment of the nervous system, namely, the glycoproteins of human cerebrospinal fluid[14–16].

The further isolation of individual brain glycoprotein components from each of the nine primary fractions is now in progress and these are denoted CI*a*, CI*b*, etc. for cortical grey matter glycoproteins, and WI*a*, WI*b*, etc. for white matter glycoproteins. Antisera have been prepared in rabbits to fractions CIV and CV and the histological localization of these substances observed in human brain sections by the fluorescent antibody technique, as done in the case of brain ganglioside[6]. Preliminary results suggest a predominantly glial localization of CV and a predominantly neuronal localization of CIV, and the method is being extended to the other seven fractions.

The emulsion fractionation method described provides a simpler, yet more quantitative means of estimating brain neuraminic acid, hexosamine, and hexose in both normal and pathological specimens of brain than methods previously available; permits the quantitative separation and study of nine primary individual brain glycoprotein fractions which differ in their chemical composition; and permits the further separation of sub-fractions from each of the primary ones. In addition, because of the relative mildness of the procedure, it offers an opportunity for enzymatic studies on one hand, and for histological studies on the other, and therefore may be useful in approaching structure-function problems with reference to glycoproteins and aminoglycolipids in the nervous system.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Terrestrial Accretion from the Solar Wind

IN order to account for the observed motions of comet tails, Biermann[1] conceived that the solar corona emits suprathermal particles at the rate of 10^{14}–10^{15} gm./sec. Since this material is evidently supplied by the Sun itself, this seems to imply that Unno's observation[2] of turbulent motions in the chromosphere and lower corona corresponds in reality to a systematic radial emission of particles from the Sun, the velocity v of which is related to the hydrogen ion concentration n by the relation $v = Bn^{-1/4}$, where $B = 8 \times 10^8$ (cm.)$^{1/4}$ sec.$^{-1}$. This empirical relation is of the same form as Parker's magneto-hydrodynamical velocity-amplitude relation[3,4], $v(r) = v_0(n_0/n_r)^{1/4}$, the applicability of which is certainly not restricted to the chromosphere. Thus the possibility of extrapolating Unno's result to interplanetary space deserves further consideration.

Application of the equation of continuity to this flux yields $nr^2v =$ constant, r being the radial distance from the centre of the Sun. Combining this with Unno's relation, one obtains $n \propto r^{-8/3}$ and $v = Kr^{2/3}$, where K is a constant. Writing $v = dr/dt$ and integrating the resulting equation, the transit time of a proton in the solar wind from the solar surface at r_0 to the orbit of the Earth at r_1 is $t = 3(r_1{}^{1/3} - r_0{}^{1/3})/K$, and since $K = v_1/r_1{}^{2/3}$, we obtain:

$$v_1 = \frac{3r_1}{t}\left[1 - \left(\frac{r_0}{r_1}\right)^{1/3}\right]$$

Transit time measurements show that t is about one day, hence $v_1 = 4{\cdot}33 \times 10^8$ cm./sec. and $n_1 = (B/v_1)^4 = 11{\cdot}65$ protons/cm.^{3} at the orbit of the Earth.

This result is compatible with the recent findings of Pope[5] (from a study of whistlers) and of Kupperian[5] (from Lyman α observations). It is also reasonably compatible with van de Hulst's estimate of 3×10^7 electrons/cm.^{3} at a distance $r_c = 10^{11}$ cm. from the centre of the Sun, for which the present theory gives a concentration $n_c = n_1(r_1/r_c)^{8/3} = 0{\cdot}74 \times 10^7$ protons/cm.^{3}. On the other hand, Biermann suggests that at the orbit of the Earth, $n_1 = 500$ protons/cm.^{3} on magnetically quiet days, and quotes the outward radial velocity v_1 as ranging from 500 to 1,500 km./sec. It would seem that Biermann's n_1 is too high, his v_1 too low; the same dynamical effects on comet tails, dependent on $n_1v_1{}^2$, would result from the extrapolation of Unno's relation. Denoting the proton mass by m_H, the rate of emission of hydrogen by the Sun is then $4\pi r_1{}^2 n_1 m_H v_1 = 2{\cdot}4 \times 10^{13}$ gm./sec.

Moreover, the energy of a proton travelling at $4{\cdot}3 \times 10^8$ cm./sec. is about 100 keV., and assuming a localized equipartition of proton and electron energy within the outer Van Allen radiation belt, the mean energy of the electrons in this region must be 50 keV., in accord with Van Allen's results from satellite data. Electrons of this energy have a velocity $v = 1{\cdot}24 \times 10^{10}$ cm./sec., yielding an electron flux $n_1v = 1{\cdot}44 \times 10^{11}$/cm.^{2} sec., again in accord with recent observations.

The rate of accretion of hydrogen by the Earth from the solar wind is of great interest. The Earth must have been exposed to this flux throughout its estimated age, $T = 3{\cdot}3 \times 10^9$ years or $1{\cdot}04 \times 10^{17}$ sec. Assuming that the magnetic field of the Earth remains effective in capturing protons and electrons up to a distance $r_m = 12{\cdot}3$ Earth radii from the Earth's centre, as suggested by the onset of field disturbances at this distance[5], the total mass of hydrogen accreted by the Earth throughout its existence is $\pi r_m{}^2 n_1 m_H v_1 T = 1{\cdot}70 \times 10^{23}$ gm. Complete oxidation of this hydrogen would yield $1{\cdot}53 \times 10^{24}$ gm. of water, in close accord with the known ocean mass[6] of $1{\cdot}42 \times 10^{24}$ gm. This remarkable result indicates that the seas must have been formed gradually by accretion of solar hydrogen at the rate of about 1·5 tons/sec., and that this process is still taking place.

Finally, the presence of about 1 part of deuterium in 6,700 of hydrogen in sea-water is in reasonable agreement with Severnyi's estimate[7] of the relative abundance of deuterium at the solar surface, especially when allowance is made for the possibility of isotopic fractionation during the transfer process. This implies in turn a rate of emission of deuterium of $3{\cdot}5 \times 10^{10}$ deuterium atoms/cm.^{2} sec. from the solar surface. The question of the origin of this deuterium remains one of the greatest unsolved problems of solar physics.

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GEOPHYSICS

Measurement of Positive-Ion Density in the Ionosphere by Sounding Rocket

Two sounding rockets, *Kappa*-VIII-3 and -4, a detailed account of which will be published elsewhere, were fired at 15h. 32m. 00s. September 22 and 20h. 25m. 00s. September 26, 1960, in order to observe the vertical profiles of positive ion density in the ionosphere. The launching site was the Akita Rocket Range in Japan, of which the geographical co-ordinates are lat. 39° 34′ N. and long. 140° 04′ E. Simultaneous observation of the ionosphere in vertical incidence was made at the Akita Radio Wave Observatory of the Radio Research Laboratories, geographical co-ordinates lat. 39° 44′ N. and long. 140° 08′ E.

Each rocket was provided with the spherical Langmuir probe in the nose cone, which was split vertically into two parts at an altitude of about 60 km. to expose the ion probe to space.

The probe installed in the rocket for the day-time flight was a meshed spherical cage of molybdenum

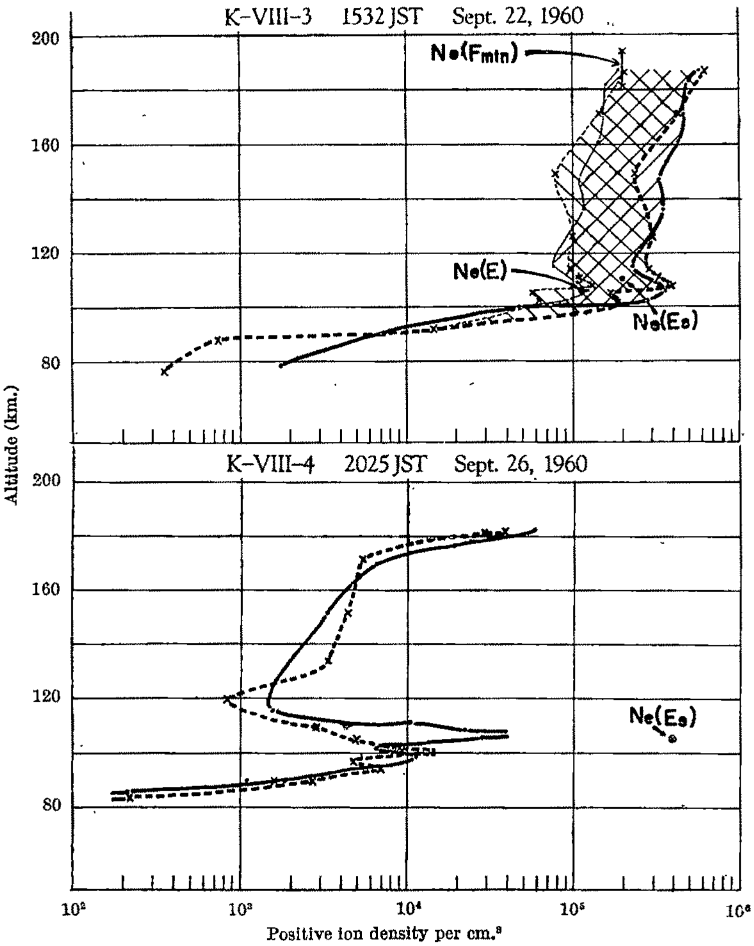

Fig. 1. Plots of ion density as a function of altitude. Observations during ascent, ●—●; during descent, ×---×. *Ne(E)* is the electron density of *E*-layer; *Ne(Es)* that of *Es*-layer and *Ne(Fmin.)* is that of lowest part of *F* layer. These values are calculated from the ionograms shown in Fig. 2

wire of 0·3 mm. diameter and welded electrically. This form of probe was used so as to eliminate the effect of the strong photoelectron emission from the surface of the probe excited by the ultraviolet component of solar radiation in the upper atmosphere. This effect has been found to cause serious disturbance in the measurement of ion current[1]. The spacing of the wires was such that the ion sheath formed around the probe was perfectly spherical, and observations in the region of low ion density in the ionosphere were reliable. The probe has a diameter of 2·0 cm. and is gold-plated. The probe for the night-time flight was the usual gold-plated spherical shell but of the same diameter.

Each probe was fastened at the top of a long tapered metal rod of 65 cm. to avoid deformation of the ion sheath around the probe due to the rocket body. The metal rod, made of duralumin, was coated with silicone resin to insulate the probe electrically from the environment. When the probe system was immersed in plasma, the ion current in the probe system would flow only from the spherical probe, and calculations of the ion density would be quite trustworthy.

The flights were successful and complete records were obtained throughout both ascent and descent. In this communication, we describe preliminary results analysed from measurements of ion densities by sounding rocket. A complete analysis of the observations will be reported elsewhere.

Fig. 1 shows the profiles of the positive ion density calculated from the observed probe currents at a probe potential of about 20 V. against the body of the rocket. The values of electron temperature and ion mass in the ionosphere, which are essential for calculation of the ion density from the probe current, were taken from Yonezawa's paper[2]. Depending on the behaviour of ions in the ion sheath, different methods of calculation were adopted for various altitudes of the rocket, namely, regions of many collisions, one collision and orbital motion in the sheath. In the orbital region, only a portion of the ions gathered on the probe can collide with the meshed spherical cage. The minimum correction factor, that is, collection probability, is determined here from the geometrical configuration of the probe; the maximum correction factor is believed to be

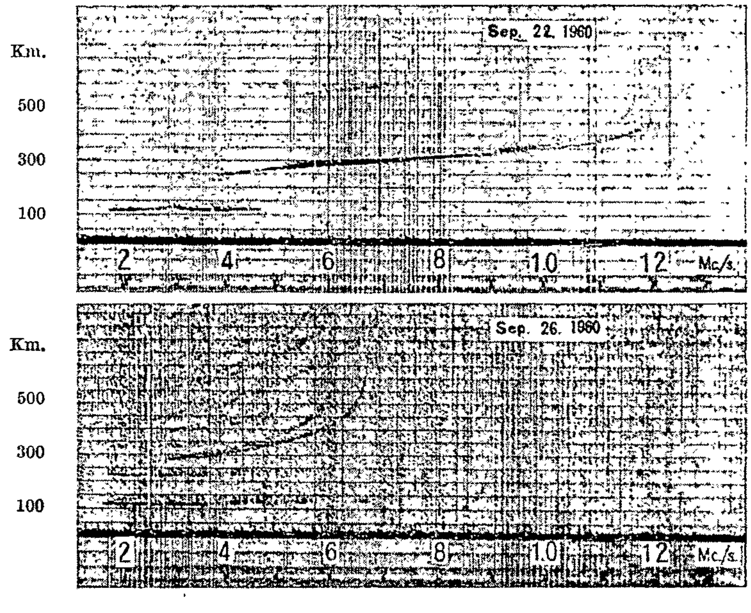

Fig. 2. Ionograms obtained at the Akita Radio Wave Observatory

about three times as great. Hence the results shown by the hatched area in Fig. 1 indicate the probable maximum values of the ion density.

The *h′–f* curves obtained by vertical incidence observation of the ionosphere are shown in Fig. 2. Numerical values of the electron density at the true height scaled from these *h′–f* curves are plotted in Fig. 1 in order to compare the ion and electron densities.

It is remarkable that the records both in the day-time and at night during the ascents show close agreement with those during the descents. Also, the numerical values of electron density scaled from *h′–f* records agree fairly well with those for ion density in the measurements by sounding rocket. It is interesting that the night measurements indicate remarkably thin layers of ion density in space of very low density, corresponding to the sporadic *E* layer observed in *h′–f* observations. Furthermore, it is noticeable that ions exist from the rather low altitude of 60 km. in the ionosphere during day-time.

We are indebted to the Institute of Industrial Science in the University of Tokyo for firing the sounding rocket of *Kappa* VIII, and we thank Prof. K. Maeda for his encouragement.

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Lavas from an Abyssal Hill on the Floor of the North Atlantic Ocean

IN May 1958, during a cruise of R.R.S. *Discovery II*, underwater photographs by Dr. A. S. Laughton revealed rock outcrops and talus slopes on a small abyssal hill which rises 200 m. from the Iberia Abyssal Plain to a summit depth of 4,960 m. and is situated 300 miles north-west of Lisbon at 41° 21′ N., 14° 28′ W. Dredging produced a large collection of rocks from the hill. This collection is significant because it is believed to be unique[1] and may represent material from an outcrop of Hill's "seismic Layer 2"[2]. The petrographic homogeneity of the collection indicates that the dredged stones were not erratics: four are bedded palagonitic vitric-tuffs and four are granitic erratics, but all the remaining three hundred and fifty-four are highly altered vesicular basalt. Some characteristically shaped fragments of pillow lavas contain relatively fresh material, including a few grams of a tachylitic margin which has escaped palagonitization. The magma appears to have been a porphyritic bytownite-rich augite-basalt without olivine. Extensive alteration, believed hydrothermal, has produced relatively soft rocks from the original lavas. In the course of alteration there was significant oxidation of iron; loss of silica, lime and magnesia; gain in potash; manganous oxide was redistributed as pelagite ('manganese') within the rocks. Autoradiographs show that this secondary pelagite is quite strongly radioactive and accounts for the unexpectedly high radioactivity (13 p.p.m. equivalent-U_3O_8, an amount that would be normal for a granite) measured in bulk samples of the altered lavas. The 'manganese' encrusting the stones (visible in Fig. 3) is also quite strongly radioactive (about 50 p.p.m. *e*–U_3O_8). Probably, both the pelagite within the stones and that in the encrustation were deposited after solution in seawater and acquired its radioactivity from the sea. The available evidence does not suggest that the original basaltic magma was unduly radioactive.

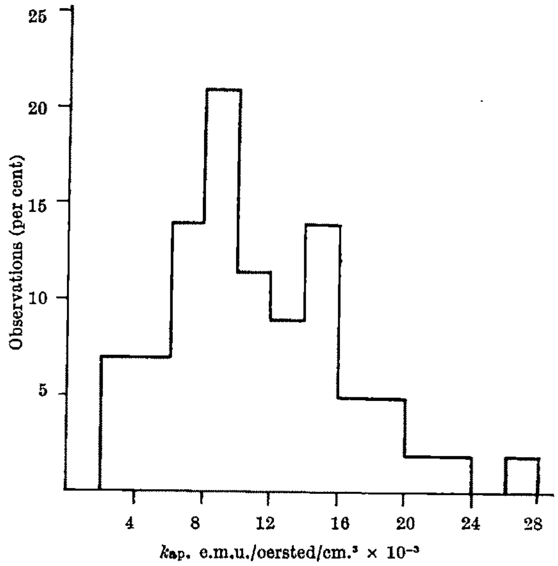

Fig. 1. Histogram showing distribution of apparent susceptibility, $k_{ap.} = \frac{I + kH}{H}$, in 43 specimens of lava. Number of observations (per cent) falling in intervals of 2 × 10^3. *I* is intensity of remanent magnetization, *k* the susceptibility, and *H* is 0·437 oersted

Data presented in Fig. 1 indicate that the altered lavas are slightly less magnetic than normal basalts although their susceptibility (median value 5 × 10^{-4} e.m.u./oersted/cm.³) and remanent magnetization (median 5 × 10^{-3} e.m.u./cm.³) lie within the known range of variation for basalts.

Fig. 2 shows results of determinations of compressional wave velocity in the altered lavas. Velocities were measured at room temperature and pressure, parallel to a uniaxial compressive stress of a few tens of atmospheres. Variation of velocity with hydrostatic compacting pressure was measured in six jacketed saturated specimens under differential confining pressures of 1–1,000 kgm./cm.² using apparatus similar to that of Wylie *et al.*[3]. At 1,000 kgm./cm.² the three softer specimens had become crushed and compaction was complete in the others. The maximum increase in velocity observed was 30 per cent. This result, and those of Fig. 2, indicate that the seismic velocity obtaining in these altered lavas under the sea bed would be in, or below, the lower part of the range of velocities (4–6 km./sec.) ascribed to Layer 2. It is suggested that lava flows

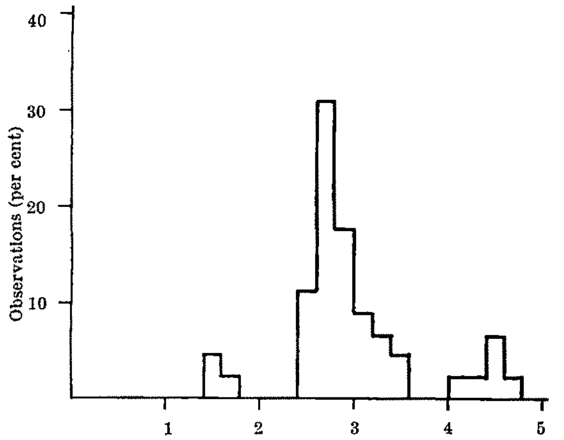

Fig. 2. Histogram showing distribution of compressional wave velocities measured in screw press in 45 water-saturated cylinders of lava. Number of observations (per cent) in intervals of 0·2 km./sec.

of *altered* basalt could be common in Layer 2 under the oceans.

The collection includes twenty large slabs thought to be pieces of the brecciated surface of an outcrop, held together by encrusting 'manganese'. Examination of these slabs (Fig. 3) has suggested details of a weathering process whereby the angular detritus frequently photographed on submarine talus slopes could have been detached from the outcrops. The breccia consists of closely interlocking angular fragments of lava, themselves riddled with cracks lined with dendritic films of pelagite. The wider cracks contain a 'soil' of the clay mineral alteration-products of palagonite and chlorophæite, with occasional calcite and zeolites. It is suggested that the change in volume associated with the formation of these minerals may serve to prise off angular fragments along the line of pre-existing shrinkage cracks.

Grateful acknowledgment is made to Dr. J. C. Swallow and the Director of the National Institute of Oceanography for facilities in R.R.S. *Discovery II* and to Dr. Bowie of the Geological Survey (Atomic Energy Section) for bulk radiometric assays. Part of this work was supported by a studentship from British Petroleum and part by the U.S. Office of Naval Research under a contract with the University of Cambridge.

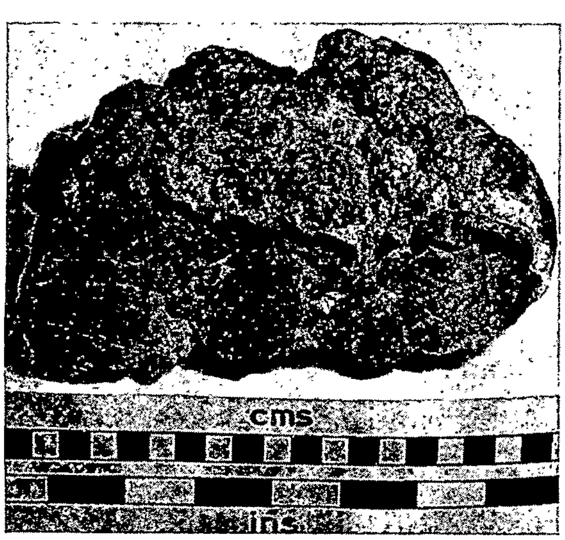

Fig. 3. Photograph of sawn section of a slab of 'manganese'-cemented breccia

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PHYSICS

An Apparent Paradox in Crystal Structure Analysis

A commonly used test for the correctness of a crystal structure deduced from X-ray diffraction data is to perform a Fourier synthesis using the observed structure amplitudes ($|F_o|$) and the calculated phases (α_c) in the Fourier coefficients (namely $|F_o| \exp i\alpha_c$) and to verify that this diagram gives peaks of the right magnitudes at the assumed positions of atoms and none elsewhere. It was thought worth while examining what would happen if the amplitudes and the phases that are fed in belong to different structures. The tests that were carried out are briefly described here, together with an explanation of the results observed and their relation to the methods of structure analysis.

A series of syntheses were first calculated using the phases (α_c) calculated from the atomic positions of tyrosine hydrochloride[1], but employing (*a*) structure amplitudes that were completely random, (*b*) structure amplitudes which were made equal for all reflexions except for a falling off with ($\sin\theta/\lambda$) corresponding to the unitary structure factor and (*c*) using a set of amplitudes which were made, so far as possible, anti-correlated to the correct values about the mean. In every case, the atomic peaks occurred at the same positions as in the assumed structure from which the phases were calculated.

This result, quantitative details of which will be published elsewhere, confirms the known fact that, particularly for a non-centro-symmetric structure, correct phases are of much greater importance than correct amplitudes in determining the positional co-ordinates of atoms[2]. For example, severe extinction can be tolerated provided that only atomic positions are required.

These tests suggested another possibility, namely, the use of the calculated phases of one crystal and the structure amplitudes of a completely different crystal and seeing what the resultant Fourier synthesis would look like. Fig. 1 (*a*) was obtained in this way. It is the *c* projection of a crystal (symmetry $P2_1$) for which the phases were obtained using the positions of the 13 light atoms in the molecule of tyrosine (as it occurs in tyrosine hydrochloride[1], $a = 11{\cdot}1$ Å., $b = 9{\cdot}0$ Å., $c = 5{\cdot}1$ Å., $\beta = 92°$, $Z = 2$, but without the two chlorine atoms) and the structure amplitudes $|F_o|$ of an imaginary crystal having 6 atoms in its asymmetric unit, but having the same unit cell and the same number of electrons in the unit cell. The positions of these atoms are marked by crosses in Fig. 1. They were taken to have a suitable scattering factor falling off with angle as for a carbon atom, so that the average intensity for both crystals would be exactly the same. Fig. 1 (*b*) is the electron-density diagram obtained by feeding back the data for tyrosine, that is, using both the amplitudes $|F_c|$ and the phases α_c of tyrosine itself. It will be noticed that there is a marked agreement between the two diagrams, and that all the atoms of tyrosine have come out in the first diagram even though the data for structure amplitudes used were of an entirely different crystal. The peak heights which are equal in Fig. 1 (*b*) are, however, very unequal in Fig. 1 (*a*) and traces of new peaks have appeared, one of which is about equal in electron density to one of the tyrosine atoms. Nevertheless, the absence of any semblance of a peak at three of the atomic positions of the structure amplitude data of the crystal used (marked by crosses) is very striking.

The use of a 'difference synthesis' cannot be expected to reveal the atomic positions of these three missing but 'correct' atoms, since for a difference synthesis to be meaningful, F_o and F_c must be very nearly equal in phase. However, Fig. 2 has been calculated using $(|F_o| - |F_c|) \exp i\alpha_c$ as the Fourier coefficients, where $|F_o|$ refers to the six-atom structure and $|F_c|$ to tyrosine. It will be noticed that although, at some of the tyrosine atoms (marked by dots), there is a small negative density, and although three of the X positions still indicate a very small positive density, the other three X atoms still fail to appear. In general, the positive and negative densities do not exceed that of a helium atom anywhere in this diagram.

It appears to be a logical conclusion to make from the above results that, if a Fourier synthesis is made using the amplitudes of the correct structure, but the phases calculated from assumed atomic positions different from these, then the resultant synthesis would contain peaks at the assumed positions, but not necessarily at the correct atomic positions. On the other hand, it is well known that the method of using a series of successive Fourier syntheses often gives the correct structure and in fact converges to it, which is in apparent contradiction with the above statement.

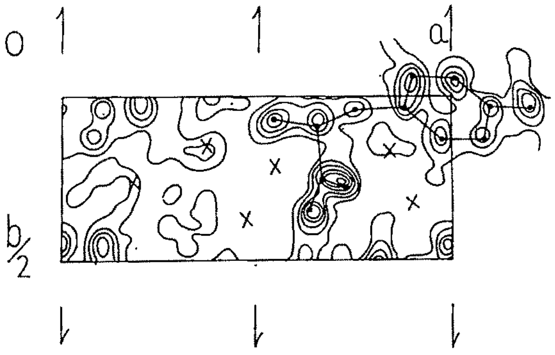

Fig. 1 (*a*)

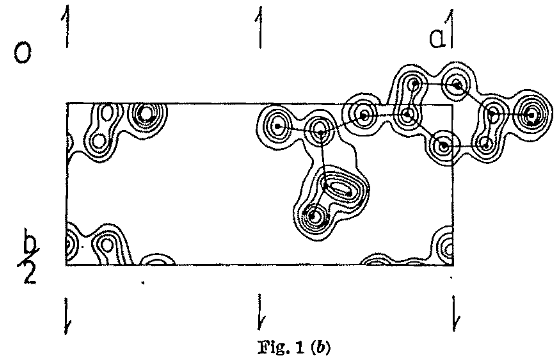

Fig. 1 (*b*)

Fig. 1. *c*-axis projection of the Fourier syntheses using the coefficients (*a*) $|F_o| \exp i\alpha_c$; (*b*) $|F_c| \exp i\alpha_c$, where *o* refers to a hypothetical six-atom structure marked by crosses and *c* refers to the structure of L-tyrosine. Contours are at intervals of 1 e./A.², starting from 1 e./A.²

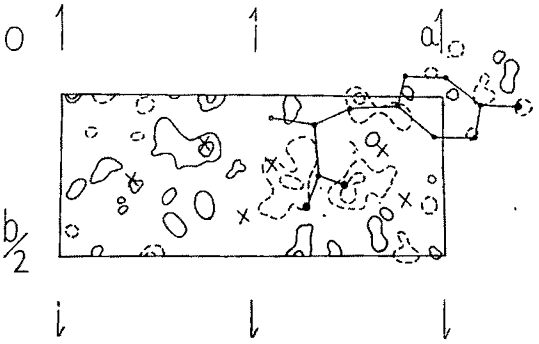

Fig. 2

Fig. 2. The difference Fourier synthesis $(|F_o|-|F_c|) \exp i\alpha_c$. Contours are at intervals of 1 e./A.². Positive contours are shown by continuous lines and negative by broken lines. The zero contour is omitted

There is, however, no real contradiction if it is remembered that in the present test the assumed atomic positions are *all* completely wrong; whereas, in structure analyses which converge, at least some of the atoms of the assumed structure would be at the correct positions (for example, the heavy atoms when such are present). The difference between the two cases is seen most strikingly by applying the general ideas of the de-convolution of the Patterson function and of the modulation of one structure by another developed in our laboratory[3,4]. Thus, the synthesis $|F_A| \exp i\alpha_B$ (where $|F_A|$ is the amplitude corresponding to a structure A and α_B is the phase corresponding to a structure B) is essentially equivalent to the modulations of the Patterson diagram of the structure A with the actual structure B. In this, it is not difficult to show that peaks occur only corresponding to the atomic positions of B and none of A, provided that A and B have no atoms in common. However, if the structures A and B have some atoms (P) in common, then not only would these atoms (P) and the remaining atoms of B come out prominently, but also there would be weaker peaks at the positions of the remaining atoms of A also, their strength being greater the larger the ratio $\sigma_P^2 = \sum_1^P f_j^2 \Big/ \sum_1^A f_j^2$. This last result shows that a necessary condition for peaks to occur at the unknown 'correct' atomic positions is that at least some of the atoms used for calculating the phases must be at correct or nearly correct positions. Since it is the mean contribution to the intensity of these correct atoms that determines the strength of the peaks at the unknown atomic positions, a small number of heavy atoms would serve this purpose equally well. Looked at from the phase point of view, convergence is usually fast in successive refinements in the heavy-atom technique because a large proportion of the phases is already nearly correct at the beginning. In fact, if the value of σ_P^2 is 50 per cent (or about half the phases are correct), a structure would refine rapidly even from very high R values.

As mentioned earlier, a peak has actually appeared at one of the crosses in Fig. 1 (*a*), contrary to the theoretical prediction. We believe this is fortuitous, and this is confirmed by the fact that no peaks at all have developed either in this figure or in the 'difference synthesis' at three other such positions.

It will also be noticed that in Fig. 1 (*a*), the peaks in the assumed positions have re-appeared with unequal peak heights, which itself would indicate that it is not correct, in addition to the large value that occurs for the residual factor (R value).

There has been some discussion in the literature (see, for example, ref. 5) as to whether the R value is really useful in judging the degree of correctness of an assumed structure, particularly since some structures with fairly low R values have turned out to be incorrect. The difficulty in the past has been that experimental errors were often greater than 10 per cent in $F_{\text{obs.}}$, and it was sometimes difficult to distinguish between errors arising from this cause and those arising from a part of the assumed structure being wrong. So also, in some cases, one projection would refine well whereas the others would not, because one set of positional co-ordinates was wrong or because of a wrong choice of origin out of two possibilities. The occurrence of disorder in one direction or of a very anisotropic temperature factor would also lead to difficulties. However, if three-dimensional data are used and an accuracy of better than 10 per cent in $F_{\text{obs.}}$ is attained, then the probability of the R value being low and yet the structure being wrong would be negligible.

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Electroluminescence of Gallium Phosphide Crystals

In the first papers on the electroluminescence of gallium phosphide[1], photographs of the emission spectra of undoped and zinc-doped crystals were observed to differ. As a working hypothesis, Wolff, Hebert and Broder[1] suggested that zinc might form a luminescent centre in gallium phosphide analogous to copper in zinc sulphide. Carrying this analogy further, they proposed that the zinc centres are ionized by impact with charge carriers injected at the negative electrode (the luminescence in most cases originated at the negative electrode). Since this work was reported, several workers[2-5] have observed electroluminescence at grain boundaries in gallium phosphide polycrystals and have proposed p–n junction recombination to be the dominant luminescent mechanism. Grimmeiss and Koelmans[3] report that undoped or weakly zinc-doped electroluminescing polycrystals can be shown to contain both p- and n-type regions (they were mainly n-type) and all give the same emission spectrum. Heavily zinc-doped samples were uniformly p-type and did not electroluminesce. I have investigated the electroluminescence of gallium phosphide single crystals of uniform conductivity type and find the emission spectrum from undoped n-type crystals to be similar to that reported by Grimmeiss and Koelmans. Weakly zinc-doped crystals, however, are p-type (10^{17} carriers/c.c., 50 cm.^{2}/V. sec. mobility, from Hall effect measurements) and give a unique electroluminescence emission which is probably the same as that observed by Wolff *et al.* (The 'zinc' electroluminescence has also been observed by M. Gershenzon (private communication).) Heavily zinc-doped crystals are darker in colour and do not electroluminesce, in accord with the observations of Grimmeiss and Koelmans.

The crystals studied were grown from solutions of 10–20 atomic per cent of phosphorus in gallium by slow cooling from about 1,200° C. The silica vial was in a constant-temperature zone of the furnace throughout the crystal-growth operation. Zinc was introduced by mixing metallic zinc with the gallium and phosphorus before heating. The crystals obtained from these procedures are usually platelets with <111> faces and dimensions of about 5 × 2 × 0·5 mm. They are single crystals although in some cases X-ray diffraction data and etching experiments indicate 180° twinning. Single-crystal whiskers have also been obtained from sublimation of gallium phosphide crystals or powder and are also observed to electroluminesce. Undoped crystals are spectrographically pure except for traces of silicon. The effect of the silicon impurity on the luminescent properties is not yet known. (Some silicon-free samples have been prepared. Hence, it is not known whether silicon is actually present in the gallium phosphide lattice or is merely a surface contaminant.)

The electroluminescence emission spectra were obtained with a Bausch and Lomb grating monochromator and a 1P21 photomultiplier detector, the output of which was fed to a Moseley X–Y recorder. A potentiometer attached to the wave-length drive of the monochromator provided a linear wave-length scale on the X axis of the recorder. All spectra were corrected later on an equal energy basis for the spectral response of the detector. Silver paint electrodes were used for all the measurements reported here. They are rectifying with breakdown potentials of a few volts. In all cases the light emission originated at the negative electrode.

A typical electroluminescence emission spectrum of an undoped crystal is shown in Fig. 1. This resembles the spectrum reported by Grimmeiss and Koelmans except for the relative peak heights, which are markedly different. In their spectrum the height of the longer wave-length peak is roughly twice the height of the shorter wave-length peak. Their measurements, however, were made at low temperature, where internally excited photoluminescence could enhance the longer wave-length emission.

Emission spectra taken at room temperature and at about − 100° C. from a weakly zinc-doped crystal (approximately 0·001 per cent zinc) are shown in Fig. 2. The unique 'zinc' emission is clearly seen. Its peak at 7000 Å. is in contrast to the 5700 Å. and 6500 Å. peaks obtained from undoped crystals. It is more than an order of magnitude greater in energy than the 'undoped' emission which can be seen in Fig. 2 as a small peak near 6000 Å. The structure in the 'undoped' emission is masked by the overlap of the 7000 Å. band. The ordinates of the two spectra in Fig. 2 have been normalized to percentage of maximum brightness. The two spectra coincide,

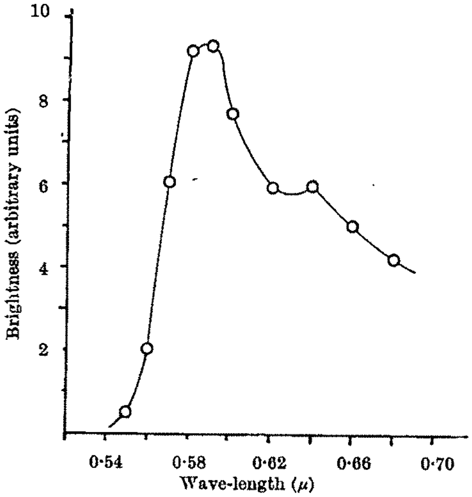

Fig. 1. Electroluminescence emission spectrum from undoped gallium phosphide crystal

approximately; the deviations have not yet been determined to be significant.

The results corroborate the observation of Wolff, Hebert and Broder, that a unique red electroluminescence is obtained from zinc-doped gallium phosphide. I have found, however, that the zinc concentration is critical; above about 0·01 per cent zinc only non-luminescent crystals are obtained. The lower limit on the zinc concentration is not known as yet. I am conducting experiments to correlate the amount of 'zinc' emission with the zinc concentration. It is possible that Grimmeiss and Koelmans did not observe the 7000 Å. emission because the concentration of zinc in their weakly doped crystals was too low, and in their heavily doped crystals too high.

The role of the zinc impurity in the luminescent process is not known. It is tempting to draw the analogy with copper in zinc sulphide. However, junction and impurity band formation have also been shown to play an important part in carrier injection electroluminescence[6], and it is conceivable that the zinc impurity is involved in this way.

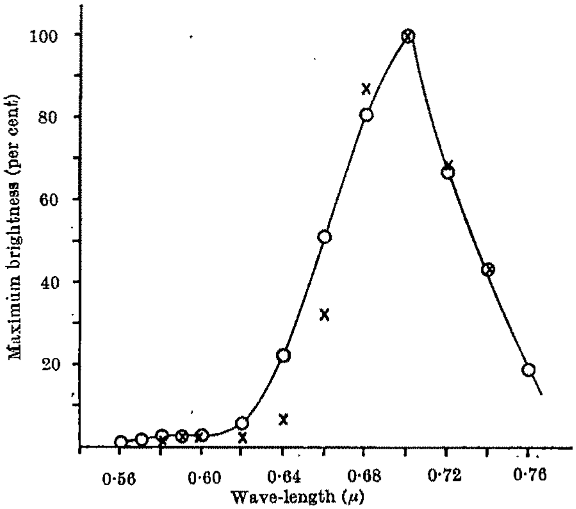

Fig. 2. Electroluminescence emission spectra from zinc-doped gallium phosphide crystal at room temperature and −100° C. (O, room temperature; ×, −100° C.

I wish to acknowledge the assistance of Messrs. E. L. Peirson and C. V. Osterday in the preparation of the crystals, and am particularly grateful to Prof. J. J. Dropkin for reading and commenting on this manuscript.

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The Unit of Neutron Flux

WE wish to support the suggestion[1] that a unit of neutron flux be defined. We feel, however, that it would be more practical to define the chad as follows:

$$1 \text{ chad} = 10^{12} \text{ n./cm.}^2 \text{ sec.}$$

The flux in most existing or contemplated power reactors lies in the range of 10^{12}–10^{13} n./cm.^{2} sec., the flux in zero energy reactors is generally about 10^9 n./cm.^{2} sec., and that encountered in sub-critical assemblies is approximately 10^6 n./cm.^{2} sec. High-flux research reactors operate at flux-levels ranging up to about 10^{16} n./cm.^{2} sec. The whole range of applied neutron physics could therefore be covered conveniently by the chad, as defined above, and the associated units microchad (10^6 n./cm.^{2} sec.), millichad (10^9 n./cm.^{2} sec.) and kilochad.

While having no objection in principle to the definition suggested earlier (1 chad = 1 n./cm.^{2} sec.), we feel that our definition is more practical and convenient in that, in addition to eliminating the clumsy compound nomenclature, it will remove the necessity for inclusion of large powers of 10.

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CRYSTALLOGRAPHY

Some Electron Microscopic Observations on Single Crystals of Polyethylene

SOME time ago it was found that in dilute solutions of polyethylene, particularly of 'Marlex 50', single crystals may be formed which can be made visible by means of electron microscopic techniques[1–3]. Morphologically, these crystals showed much resemblance to single crystals of paraffin, which were observed for the first time by Dawson and Vand[4]; the latter show a spiral terrace growth, the height of the terraces corresponding to the length of the chain molecules.

Keller[1] pointed out that the chains in the polyethylene crystal must necessarily be folded and that the height of the terraces corresponds to the distances between successive folds. He also noted that the crystals lose their crystallinity in the electron microscope, but that their morphological shape is probably preserved.

In an attempt to study undecomposed specimens, in which freshly prepared specimens were exposed

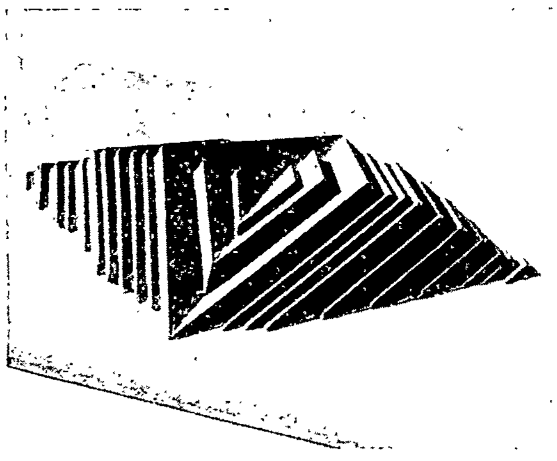

Fig. 1. Model of the striated structure of a polyethylene crystal

to a low-intensity electron beam, Keller and O'Connor[5,6] obtained completely different pictures. Although the crystal was still lozenge-shaped, it revealed a distinct division into four sectors, which were characterized by striations running non-parallel to the crystal boundaries, but which in our view were distinctly arranged in the (310) direction.

Some time ago we studied these types of crystals by electron microscopic stereo-techniques. From the micrographs we gained the impression that the crystals possess a pyramid-like structure. Using this technique, two micrographs are secured with the specimen set at different angles to the beam. As there was no visible difference between the two pictures, we concluded that the striations cannot possibly be due to extinction effects, as is sometimes stated in the literature.

Application of this technique requires that two pictures be taken of each crystal; owing to the change of the crystal in the electron beam, however, it is quite exceptional that two suitable micrographs will be obtained. To obviate this difficulty, we decided to use replica techniques. After some initial troubles had been overcome, we succeeded in securing pre-shadowed carbon replicas of polyethylene crystals.

We then took stereographs of these replicas; the first micrographs thus obtained completely confirmed our observation mentioned above with regard to the pyramidal structure. Some of the plates also revealed that the striations are straight. A model made of these structures is shown in Fig. 1. As can be seen from this model, the crystals do not have a true pyramidal structure, but can be better represented by a pyramid with a depressed apex. Mention ought to be made here of a statement by Bassett, Frank and Keller[7] that a pyramidal structure might be expected.

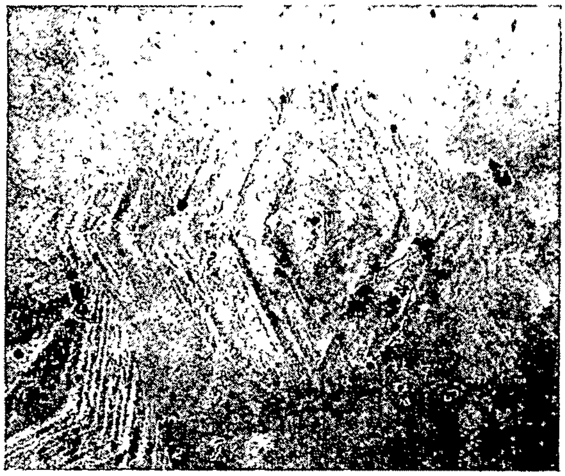

Fig. 2. Replica of a crystal of polyethylene. (× 15,000)

Although several attempts have been made in the past at explaining this striated structure, attention has never been directed to the relation between this structure and the spiral terraces observed in a later stage. In some of our replicas, both the pyramidal and the terraced structure can be seen (Fig. 2).

In view of the information provided by these micrographs, we expect that it will become possible to shed more light on the relation noted by us, and in general on the way in which polyethylene crystallizes from solution.

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CHEMISTRY

Localization of Ferrous Corrosion by Azide

Among the very large number of rust inhibitors, one of the simplest in structure, sodium azide, has received only scant attention. I have made some observations on the interaction between ferrous materials and neutral aqueous solutions of azide at ambient temperature.

Mild steel normally remains immune under azide solution. In sufficient concentration, this additive counteracts the corrosive effect even of chloride[1]. However, if mild steel is submerged in 1 *M* sodium azide containing 0·2 *M* sodium chloride, a scratch is made on the metal with a glass needle, and an anodic potential applied (for example, 2 V. vs. a stainless steel rod as cathode), a sharp red line appears instantly along the scratch, as if the steel were bleeding. This demonstration of highly localized attack is, however, only successful if no more than a few minutes elapse between the 'wounding' and the application of potential. Otherwise the scratch immunizes itself, obviously by growing an oxide film under the galvanic action of dissolved oxygen. If the scratch is applied in air, healing by dry oxidation is less rapid, and the lesion may be developed by submersion and anodic treatment after much longer intervals of time. The red ferric azide complex issuing from the scratch later diffuses away and hydrolyses, leaving a cloud of ferric hydrate.

A high-carbon steel, several low-alloy steels, a variety of cast irons, and 13 per cent chromium iron behaved quite similarly. A ferritic 17 per cent chromium iron also gave a 'bleeding' effect, but in this case the electrolysis had to be applied not later than a few seconds after the steel had been scratched under the azide/chloride solution. Austenitic 18–8 type stainless steels gave no effect, even if they were made anodic while being scratched. The effect may therefore be developed as a sorting test for steels and perhaps also for showing corrodible areas in welds.

Voltage and concentrations are not critical for the effect to occur. Chloride may be replaced by bromide or iodide, but in the absence of halide the scratch does not develop. Attempts were made to replace azide by thiocyanate, which is iso-electronic with azide and also forms a red ferric complex. Thiocyanate, however, showed no localizing action. It is an anodic stimulator of corrosion, not an inhibitor.

The role of azide in these experiments is dual: as an inhibitor of the attack, and as a reagent of the product of attack. Similar effects can be produced with pairs of additives in which these two functions are separated; for example, with benzoate as inhibitor and ferroin as reagent. Also oxidation need not be electrolytic but can be galvanic, using oxidants such as persulphate. Ferricyanide can be used as oxidant and reagent in one, as in the 'ferroxyl test' for showing up pores in tin coatings of steel. Conspicuous localized corrosion should, then, be generally favoured by four factors: (*a*) oxidizing conditions, (*b*) the presence of a stimulator, (*c*) the presence of an inhibitor and (*d*) the presence of a reagent. Table 1 gives examples of such combinations by which corrosion limited to a scratch could be produced.

Table 1. SETS OF CONDITIONS LEADING TO SCRATCH ATTACK OF MILD STEEL

Oxidant	mol./l.	Stimulator	mol./l.	Inhibitor	mol./l.	Reagent	mol./l.
·2 Volts		Chloride	0·2	Sodium azide	1·0	Sodium azide	1·0
Ferricyanide	0·2	,,	0·0025	*Cyclo* hexylamine	0·05	Ferricyanide	0·2
2,4-Dinitrosoresorcinol	0·001	,,	0·001	*n*-Decoic acid*	0·001	2,4-Dinitrosoresorcinol	0·001
·2 Volts		,,	0·001	*n*-Decoic acid*	0·01	*o*-Phenanthroline	0·005
Persulphate	0·02	,,	0·001	*n*-Decoic acid*	0·01	,,	0·005
·2 Volts		,,	0·001	Sodium picrate	0·01	,,	0·005
Persulphate	0·02	,,	0·001	Sodium picrate	0·005	,,	0·005
·2 Volts		,,	0·001	Maleic hydrazide*	0·05	,,	0·005
Persulphate	0·02	,,	0·001	Maleic hydrazide*	0·01	,,	0·005
Persulphate	0·02	,,	0·001	2-Methyl piperazine	0·02	,,	0·005

* Adjusted with sodium hydroxide to *p*H 7.

It is of interest to note that azide has none of the attributes normally held responsible for inhibition of rusting: in contrast to the structurally similar nitrite, and to chromate or picrate, it cannot act as an oxidant. It differs from the carboxylates in that it does not precipitate iron from solution, and from the amines by the absence of alkaline character. Unlike the proprietary rust preventives, it has a very simple structure. Further work on the azide/iron interaction may help to throw light on the still obscure mechanism of rust prevention by soluble additives.

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Hydrogen Bonding in Apatitic Calcium Phosphates

IT is well known that the formula of naturally occurring and of precipitated synthetic hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$: the molar Ca/P ratio of these compounds is 1·667. The compound mentioned can adsorb a consistent amount of water. For other synthetic calcium phosphates, with lower Ca/P ratios, the X-ray diffraction spectrum remains the same.

By combining data principally obtained from infrared spectrometry, X-ray diffraction and chemical methods of investigation, we have established that the general formula of the apatitic calcium phosphate series is: $Ca_{10-x}(H)_x(PO_4)_6(OH)_{2-x}$, the value of x varying from 0 to 2. Detailed results concerning this problem have been presented in a preliminary report with some restrictions appropriate to such an over-simplified view[1]. According to our conception, some $PO_4^{\equiv}$ groups of these compounds are in fact $HPO_4^{=}$ groups which are linked by hydrogen bonds: (O_3PO—H- - -OPO_3). In our work, special attention has been paid to hydrated tricalcium phosphate (Ca/P ratio = 1·5), or $Ca_9(O_3PO$—H- - -$OPO_3)(PO_4)_4(OH)$.

Others have reported similar findings in this field[2-4]: they point out that pyrophosphate, derived from these acidic groups by heating below 600° C., may be determined by chemical means and are also present in bone and teeth.

A paper published recently[5] gives us the opportunity to present some complementary information. According to these authors, infra-red spectra obtained from apatitic calcium phosphates with a Ca/P ratio between 1·667 and 1·5 show a band located at 3,400 cm.$^{-1}$ which is attributed to the O—H valence vibration of the (O_3PO—H- - -OPO_3) groups. The intensity of this absorption increases when the Ca/P ratio of the compounds considered decreases: apparently, this observation agrees with the conception of Posner *et al.*[5], the amount of $HPO_4^{=}$ groups being inversely proportional to the Ca/P ratio. However, it does not agree with the findings of other workers[6-8], who have shown that this vibration absorbs at about 2,850 and 2,400 cm.$^{-1}$. Moreover, when the frequency 3,400 cm.$^{-1}$ is plotted on the graph obtained by Lord and Merrifield[8], the distance O—O corresponds to 2·85 Å.; on the other hand, the distance observed for acidic phosphates is only 2·5 Å.[9,10], and this value agrees with the location of an absorption band at 2,400 cm.$^{-1}$. In our records, obtained with apatitic calcium phosphates with a Ca/P ratio lower than that of hydroxyapatite, we have actually observed a weak band located at 2,400 cm.$^{-1}$ and another one, with a high intensity, at 3,400 cm.$^{-1}$. The latter is also present on the spectrum of synthetic hydroxyapatite and consequently is not specific to the compounds with a Ca/P ratio lower than 1·667: as is well known, it corresponds to adsorbed water.

Probably, Posner *et al.*[5] have compared synthetic apatitic phosphates to natural hydroxyapatite, which does not contain adsorbed water and shows no band at 3,400 cm.$^{-1}$ as it has been verified by us. The inverse proportionality observed by them between the Ca/P ratio and the intensity of the 3,400 cm.$^{-1}$ wave may be explained as follows: the lower the Ca/P ratio of the synthetic calcium phosphates, the smaller are the crystals and the greater is the total surface of these crystals: in this way, the amount of adsorbed water is greater, as can be shown experimentally. Like Posner *et al.*[5], we have observed on the infra-red spectra obtained with natural hydroxyapatite a band (3,560 cm.$^{-1}$) corresponding to the vibration of free OH groups. The same band appears when larger crystals of hydrated tricalcium phosphate, obtained by treating in the hydrothermal bomb, are studied: in this way, the amount of adsorbed water is much smaller than in the untreated compound, and the band corresponding to free OH groups clearly appears.

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Calcium Exchange of Bone Salts considered as a Measure of the Crystal Surface

COMPARING the *in vitro* calcium-45 uptake by bone salts (ashed by a method slightly modified from that of Gabriel) from alcoholic and aqueous calcium-45 labelled calcium chloride solutions, we have been able to demonstrate the occurrence of remodelling phenomena (chemical reaction between the solid and the ions dispersed in the liquid phase, recrystallization *sensu stricto*) and of an isoionic exchange, which reaches an equilibrium when 8 per cent of the calcium ions of the solid are involved. All remodelling processes being discarded, we believe that this figure corresponds to a pure exchange value. In our conditions, the intracrystalline ion exchange by thermal vibration is too slow to interfere with the surface exchange process[1].

In a recent paper[2] Engström, correlating X-ray diffraction data and electron microscopy investigations, states that the needle-like crystal of bone salt has an average diameter of about 50 Å. This figure is also given by Molnar[3].

If the needle-like crystal is considered as a cylinder of the same length, the base surfaces not being taken into account, one may calculate, as a percentage, the ratio—plane volume of the surface/volume of the crystal—in the following way:

$$\frac{\pi h(2Rd - d^2)}{\pi R^2 h} \times 100 \text{ (per cent)}$$

where h is the length of the crystal; R is the radius of the crystal base; d is the third dimension of the surface plane volume. After simplifying, it becomes:

$$\frac{2Rd - d^2}{R^2} \times 100 \text{ (per cent)}$$

which in the particular case of bone crystals gives:

$$\frac{4{,}900}{625} = 7\cdot85 \text{ per cent}$$

when d is equal to the unit (1 Å.).

It is interesting to point out that the length of the crystal is of no importance for this calculation. This calculated figure corresponds closely to the experimental exchange value in such a way that the latter may be considered a real measure of the crystal surface. This assumption implies that the composition of the surface is not different from the composition of the bulk of the crystal. If the ratio is expressed in m.^{2}/gm. we obtain a value 250 m.^{2}/gm., which also fits the published figures[4].

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Lifetime of the Pyrene Dimer

THE appearance of a new fluorescence emission band in concentrated solutions of pyrene was attributed by Förster and Kasper[1] to an excited dimer formed by combination of an excited singlet molecule with an unexcited molecule. Stevens and Hutton[2] have recently reported the lifetime of the dimer emission to be $1\cdot8 \times 10^{-3}$ sec. During tests of a new photoelectric spectrophosphorimeter an attempt was made to record this long-lived emission. The instrument was a modification of a spectrofluorimeter previously described[3], but in which two 800 c./s. choppers were used, one being situated in the beam of the exciting light and one in the beam of the fluorescence light. The choppers were driven by synchronous motors, and by rotation of one of the motors the choppers could be put into or out of phase so that the fluorescence monochromator could be made to record either the sum of the fluorescence and phosphorescence spectra or the phosphorescence spectrum alone. By adjustment of the photomultiplier voltage and by variation of the amplifier gain, the overall sensitivity could be increased by a factor of 10^4.

The operation of the instrument was checked by measurement of the visible phosphorescence from a 0·005 per cent solution of eosin in anhydrous glycerol which was reported by Mlle. Boudin[4] to have a lifetime of about 10^{-3} sec. We found the ratio of the intensities of fluorescence and phosphorescence to be, within a factor of two, the same as the value reported by Mlle. Boudin.

The efficiency of the choppers was tested by measurements on aerated solutions of fluorescein and anthracene, and it was found that the recorded intensity of the fluorescence was reduced by a factor of more than 10^5 when the choppers were moved to the out-of-phase position.

A 10^{-3} *M* solution of Light's pure pyrene in B.D.H. spectroscopically pure cyclohexane was thoroughly de-aerated and sealed in a silica optical cell. The total luminescence spectrum was first measured using two different wave-lengths for excitation, namely, 366 mμ with viewing at right angles to the beam of exciting light, and 313 mμ with viewing in the same direction as the beam of exciting light. The spectrum was the same in each case and was similar to that reported by Forster and Kasper, and by Stevens and Hutton. The measurements were then repeated, but with the choppers out of phase and with the sensitivity increased by a factor of 10^4. The apparent phosphorescence emission from the dimer, measured in this way, had an intensity less than 10^{-5} times that of the total luminescence from the dimer. It was thus estimated that a component of the dimer emission having the lifetime reported by Stevens and Hutton must have an intensity less than 10^{-5} times the total dimer emission and that the greater part of the dimer emission must have a lifetime less than 5×10^{-5} sec.

The measurements with our spectrophosphorimeter set an upper limit to the lifetime of the pyrene dimer. A lower limit can be determined by measurement of the oxygen-quenching of its luminescence. Saturation of the 10^{-3} *M* solution with air was found to reduce the intensity of the monomer luminescence by a factor of about 7 and that of the dimer luminescence by a factor of approximately 19. In a 10^{-5} *M* solution, where dimer formation can be neglected, saturation with air reduced the intensity of the monomer luminescence by a factor of about 22. Assuming that the dimer is formed by combination of an excited singlet molecule with a normal molecule, and that oxygen quenching of both the monomer and dimer is collisional, it can be shown that:

$$F_D{}^0 \Big/ F_D = F_M{}^0 \Big/ F_M \, (1 + k_d \, \tau_d[\mathrm{O}_2])$$

where F_D is the intensity of dimer luminescence in 10^{-3} *M* solution containing oxygen, $F_D{}^0$ is the corresponding value in the absence of oxygen, F_M and $F_M{}^0$ are the corresponding intensities of monomer luminescence in the same solution, k_d is the bimolecular oxygen quenching constant for the dimer and τ_d the lifetime of the dimer in the absence of oxygen. The encounter frequency in *cyclo*hexane at 20° C. (that is, the maximum possible value for k_d) is $6 \cdot 8 \times 10^9$ litre mole^{-1} sec.$^{-1}$, and the oxygen concentration in an air-saturated solution is $2 \cdot 5 \times 10^{-3}$ *M* (ref. 5). Hence $\tau_d \geqslant 10^{-7}$ sec. The corresponding values for the monomer lifetime are $\tau_M \geqslant 3 \cdot 5 \times 10^{-7}$ sec. in 10^{-3} *M* solution and $\tau_M \geqslant 1 \cdot 2 \times 10^{-6}$ sec. in 10^{-5} *M* solution. The latter value is in approximate agreement with the value of $0 \cdot 5$–$1 \cdot 0 \times 10^{-6}$ sec. which we estimated from the intensity of the long wave-length absorption band system.

The evidence from our experiments gives a value for the lifetime of the dimer which is at least thirty times less than that found by Stevens and Hutton, a discrepancy for which it is difficult to find an explanation. One possibility which we considered was that the long-lived emission was due to solute or photochemical decomposition products, adsorbed on the wall of the vessel (it being assumed that any luminescence from the vessel wall would have been included in the total phosphorescence spectrum measured by Stevens and Hutton). However, no evidence of a 'wall-adsorption' effect was found in our experiments with 10^{-3} *M* pyrene solution in the silica cell, the ratio of 'phosphorescence' to fluorescence being the same, within experimental error, whether right-angle illumination (366 mμ) or frontal illumination (313 mμ) was used (in the latter arrangement the illuminated cell face, and hence also the luminescence from any adsorbed matter, was viewed by the photomultiplier). Whatever the cause of the discrepancy it is sufficiently important to merit further investigation.

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WE previously reported[1] a value of $1 \cdot 8 \times 10^{-3}$ sec. for the lifetime of the pyrene dimer in de-aerated *cyclo*hexane solutions. This value was obtained from the exponential decay of the emission observed from a flashed solution, the order of magnitude being confirmed by photographing the emission excited by a constant intensity source through a Becquerel phosphoroscope in which the time interval between excitation and observation was $\sim 5 \times 10^{-4}$ sec. Although it was not actually stated, it was implied that this lifetime referred to the total dimer emission.

In an attempt to repeat this observation Parker and Hatchard (preceding communication) report that they were unable to record an emission component of this duration for a similar solution and conclude that if such a component is present it must have an intensity less than one part in 10^5 of the total dimer emission which has the much shorter lifetime of 5×10^{-5}–10^{-7} sec.

We have again measured the lifetime of the long-lived component by recording the pyrene emission from the phosphoroscope on a Mazda 27*M*3 photomultiplier tube and photographing its signal on a Solartron oscilloscope type *CD* 711*S*.2; the apparatus was tested by measuring the emission lifetime of uranyl sulphate crystals for which the observed value of $2 \cdot 53 \pm 0 \cdot 03 \times 10^{-4}$ sec. is in excellent agreement with the published[2] lifetime of $2 \cdot 55 \times 10^{-4}$ sec. For a 10^{-3} *M* solution of pyrene a component of lifetime $1 \cdot 75 \pm 0 \cdot 1 \times 10^{-3}$ sec. was observed which confirms the value previously obtained.

In order to measure the ratio of slow to fast components of the dimer emission, the apparatus was modified as described by Williams[3] and the intensity of the slow component was found to be 0·2 per cent of the total dimer emission. Clearly our previous implication that the two different emission spectra observed from pyrene solutions are respectively associated with slow and fast emission components was unjustified and the system of electronic states must be more complex than we at first visualized.

At lower temperatures (− 10° C.) in liquid paraffin we have observed a value of 60×10^{-3} sec. for the long-lived pyrene component; this could be ascribed to the suppression of an energy-dependent first-order radiationless decay of the dimer which should lead to overall self-quenching of fluorescence in pyrene solutions at room temperature. It is possible, however, that the residual oxygen in the outgassed solutions partially quenches the long-lived component at room temperature but is much less effective in the more viscous paraffin at − 10° C. The radiative lifetime of the slow component may therefore exceed 60×10^{-3} sec. and in the complete absence of oxygen this component may contribute appreciably to the total intensity of dimer emission.

The problems associated with the outgassing of solutions are not simplified by the observation that the pressure in a vessel sealed-off at 10^{-7} mm. may be increased to as much as 10^{-2} mm. by the act of sealing-off (Heavens, O. S., private communication); since quartz requires a higher seal-off temperature it is possible that the failure of Parker and Hatchard to observe the slow component is due to a comparatively high pressure of gas released from their quartz vessels during seal-off.

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BIOCHEMISTRY

Origin of Urinary Pyrocatechol

The formation of pyrocatechol in rats fed shikimic and quinic acids has recently been reported by Booth *et al.*[1] They proposed that human urinary pyrocatechol might have a similar origin since these acids are present in some edible plants. However, pyrocatechol also could arise from the oxidation of phenol, a compound formed in the gut by the action of bacteria on protein[2]. If pyrocatechol excretion-rates were shown to be dependent on gut bacteria, support for the proposed relationship would be obtained. An examination was therefore made of the pyrocatechol excretion-rates in several normal persons who later were treated with large doses of neomycin, a drug which can virtually sterilize the gut.

Pyrocatechol was determined fluorometrically by an adaptation of the method of Weil-Malherbe and Bone[3]. An aliquot of the urine, adjusted to *p*H 1 with hydrochloric acid, was heated to 100° C. for 10 min. and then made neutral by the addition of dilute base. An alumina adsorbate was prepared, and an aliquot allowed to react with the ethylenediamine reagent. The neutral fluorescent derivative which formed was extracted into *iso*butanol and its fluorescence compared with that of pyrocatechol using a Farrand photofluorometer. The specificity of the method for urinary pyrocatechol was determined by paper chromatography of the concentrated *iso*butanol extract. Under ultra-violet illumination only one major fluorescent spot was seen which had the same R_F and blue-green colour as similarly prepared pyrocatechol or norepinephrine. It has been shown that identical ethylenediamine derivatives are formed from both pyrocatechol and norepinephrine[4]. Since only trace amounts exist in urine no correction need be made for the norepinephrine.

While pyrocatechol excretion-rates in the normal person exhibit a large diurnal variation, daily excretion-rates remain rather constant[5]. When neomycin is ingested in large doses, however, the daily excretion-rates of pyrocatechol decline rapidly as shown in a typical result listed in Table 1. From these results a major fraction of urinary pyrocatechol appears to be dependent upon the activity of gut bacteria. Since phenol can be converted to pyrocatechol by the animal organism[6], the formation of the major urinary catechol may proceed as follows:

Table 1. Pyrocatechol Excretion-Rates before and during the Administration of Neomycin in a Daily Oral Dose of 8 gm.

Pre-treatment period		During neomycin treatment	
Days	Pyrocatechol excretion (mgm./day)	Days	Pyrocatechol excretion (mgm./day)
1	6·0	4	3·2
2	5·2	5	1·2
3	5·8	6	1·6

$$\text{tyrosine} \longrightarrow \text{phenol} \longrightarrow \text{pyrocatechol}$$

Although the conversion of tyrosine to phenol appears to be mediated by an enzyme present in coliform bacilli[7], further oxidation may be non-enzymatic. The site of this oxidation could be within the gut since pyrocatechol has been reported present in fæces[2], but oxidation of unconjugated phenol by other body organs probably can occur.

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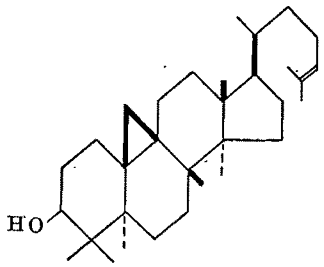
Cyclo-Arthenol : a Minor Constituent of Linseed Oil

Cyclo-Arthenol has been isolated by Barton[1] in addition to the corresponding ketone, *cyclo*-arthenone, from the latex of the *Artocarpus integrifolia* fruit and afterwards by Spring and co-workers from the unsaponifiable part of *Strychnos nux vomica*[2,3]. These authors have studied its structure and have demonstrated that *cyclo*-arthenol may be correctly described as 9 : 19-*cyclo*lanost-24-en-3β-ole :



Other compounds of similar nature are *cyclo*-laudenol[4], *cyclo*-eucalenol[5] and 24-methylen*cyclo*-artanol[6], all of which are characterized by the presence of a *cyclo*-propane ring at 9–19.

In the course of work on the constituents of the unsaponifiable fraction of vegetable oils I have

Table 1

	Alcohol, $C_{30}H_{50}O$, from linseed oil		Data quoted by:									
			Barton (ref. 1)		Spring (refs. 2, 3)		Gonzales (ref. 10)		Chapon (ref. 11)		Shimizu (ref. 6)	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
Alcohol	115–116	+51·4	85–92	+48	115	+54	97–112	+47	80–105	+48·5	114–115	+52
Acetate	121–122	+60·2	122·5 123·5	+58	122–124	+59·5	118·2	+54	119–120	+58	122–124	+59
Benzoate	129–130	+68	129–130	+65	130	+76	129	+54	130	+67	129–130	+75

isolated, by chromatography of the neutral[7] unsaponifiable of a linseed oil of Italian production on silicic acid, a group of materials characterized by a yellow-brown Liebermann–Burchard reaction. Paper chromatography[8] shows the presence in this fraction of three different substances at least, probably terpenic alcohols. Subsequent chromatography of the fraction on aluminium oxide made it possible to isolate, by elution with hexane–benzol, a substance which after crystallization from methanol melted at 115–116° C., $[\alpha]_D = +51 \cdot 4°$. This substance gave a yellow-brown colour with the Liebermann reagent and a distinct yellow colour with nitromethane; paper chromatography showed the presence of only one component.

The compound isolated has been characterized through the acetate and the benzoate; in Table 1 are recorded the values of specific rotations and of the melting points, which agree satisfactorily with those obtained for the *cyclo*-arthenol by the authors mentioned.

Identification has been confirmed through the infra-red spectrum of the compound isolated from linseed oil, which was identical with that reported by Cole[9] for the *cyclo*-arthenol. Finally, the mixed melting point of the acetate of the compound, with a sample of genuine acetyl-*cyclo*-arthenol, kindly supplied by Dr. Spring, did not show any decrease of melting point.

This investigation suggests that triterpenic alcohols of the same group may be present in other animal and vegetable oils, such as olive oil, maize germ oil, peanut oil and tallow oil.

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A Bilatriene Pigment in the Green Terebellid Polychæte *Eupolymnia heterobranchia* (Johnson)

Eupolymnia heterobranchia (Johnson) is a relatively large terebellid found in mud and under boulders on the Pacific coast of North America. All the specimens that I have found near Friday Harbor, Washington, had a deep green colour. This is due to granules of green pigment deposited in the cœlomic cells and in the epidermis. The pigment was readily extracted with acidified methanol, gave a good Gmelin reaction, and reacted positively to the zinc bilipurpurin test, demonstrating that it was a bilatriene. The red-fluorescent compound obtained in the latter test had a sharp maximum at 625 mμ (Unicam), indicating the presence of meso- rather than proto- side chains[1]. The great resistance of the pigment to concentrated sulphuric acid also distinguished it from biliverdin. In a 5 per cent hydrochloric acid : methanol mixture the absorption spectrum showed peaks at 360 and 675 mμ, resembling mesobiliverdin hydrochloride (363 and 670 mμ; ref. 1).

Mesobiliverdin has been found to occur in only a few animals, and these are all phytophagous insects[2]. The occurrence of a similar pigment in *Eupolymnia* is interesting in view of the report by Berkeley and Berkeley[3] that orange-red individuals are sometimes found. It would be most interesting to know whether the appearance of these orange worms is due merely to a lack of the green pigment or, as is just possible, to mesobilirubin or related pigment.

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Occurrence of *Cyclo*-Propene Acids in Some Plants of the Order Malvales

STERCULIC and malvalic acids are the only fatty acids known to contain a *cyclo*-propene ring in their structures. From the seed oil of *Sterculia foetida* (Sterculiaceae), Nunn[1] isolated sterculic acid (C_{19}) and defined its structure. Malvalic acid (C_{18}) isolated from Malvaceae by Shenstone and Vickery[2] has been structurally identified by Macfarlane, Shenstone and Vickery[3] and Craven and Jeffrey[4]. Afterwards we reported[5] the occurrence of minor amounts of sterculic acid associated with malvalic acid in the oils from some malvaceous plants. The occurrence of both acids in the seeds of *Sterculia foetida* and two species of Malvaceae has also been observed by C. R. Smith, jun., T. L. Wilson and K. L. Mikolajczak (private communication).

We have measured the concentrations of malvalic and sterculic acids in the seed and leaf oils of three species from each of the families, Malvaceae and Sterculiaceae. The solvent-extracted oils were saponified under mild conditions, and the total fatty acids were subjected to low-temperature crystallization. The filtrate contained almost all the *cyclo*-propene acids, and saturated acids were absent. Malvalic and sterculic acids were cleanly separated by the method of reversed-phase column chromatography[6]. They were closely preceded by, and sometimes merged with, linoleic and oleic acids, respectively.

The Halphen colour test, given by both *cyclo*-propene acids[3] but not by other unsaturated acids, was used to estimate the concentrations of malvalic and sterculic acids in the isolated chromatograph 'peaks'. The colour reaction given by each fraction was adjusted in a series of increasing dilutions to a point near visual extinction. The colours were then compared with a similar series obtained from standard concentrations of pure acids. The ratio of dilutions at similar colour intensities gave the concentration of *cyclo*-propene acid present in each impure fraction.

In the seed-oil of *Sterculia foetida* the colorimetric method was checked by an estimation of the products of partial hydrogenation of the fatty acids. In the hydrogenation, ethanol was used as a solvent with 5 per cent palladium on calcium carbonate as a catalyst. The *cyclo*-propane acids, dihydromalvalic and dihydrosterculic acids, together with small amounts of their methyl-branched derivatives, were separated from the straight-chain saturated acids by crystallization and afterwards isolated after reversed-phase and gas-liquid chromatography. The concentrations of the *cyclo*-propene acids estimated by the colorimetric and hydrogenation methods agreed within ± 2 per cent.

Malvalic and sterculic acids in the chromatographic fractions were identified after direct isolation by means of fractional crystallization. In some samples, however, identification was effected more readily by conversion of the *cyclo*-propene acids to the stable dihydromalvalic and dihydrosterculic acids.

Table 1. CONCENTRATIONS OF MALVALIC AND STERCULIC ACIDS IN PLANT OILS*

Plant family and species	Source of oil	Malvalic acid (per cent)	Sterculic acid (per cent)
Malvaceae			
Malva parviflora†	leaves	6·9–13·9	3·1–6·1
Malva verticillata‡	leaves	1·5–3·7	0·3–0·8
Gossypium hirsutum	seeds	0·7–1·5	0·3–0·5
Sterculiaceae			
Brachychiton acerifolium	leaves	nil	nil
	seeds	6·1–8·1	1·4–1·9
Brachychiton populneum	leaves	nil	nil
	seeds	8·9	1·1
Sterculia foetida	seeds	4·4	45·6

* When expressed as percentages of the total fatty acids, these values will be increased by factors of 1·14–1·25 for the seed oils and 1·49–1·82 for the leaf oils.

† Total *cyclo*-propene acids in seed oil = 5·0 per cent.

‡ Total *cyclo*-propene acids in seed oil = 1–2 per cent.

The concentrations of malvalic and sterculic acids in the oils from several plant species are given in Table 1. In the oils containing *cyclo*-propene acids both occur together, and the concentration of malvalic acid exceeds that of sterculic acid in five of the six species examined. The seed oil of *Sterculia foetida* is exceptional in its high concentration of *cyclo*-propene acids and in the preponderance of sterculic acid. In oils where malvalic acid predominates, the highest concentrations of *cyclo*-propene acids occur in *Malva parviflora* (up to 20 per cent of the oil or 35 per cent of the total acids) and in *Hibiscus syriacus* (25 per cent of the total acids). The concentrations in freshly extracted, unrefined oils of cotton seeds (*Gossypium hirsutum*) were always low but in most samples fairly constant, while in *Malva* spp. the concentrations in the leaf oils were very variable. There is evidence from the chromatograms of the presence of other *cyclo*-propene acids, but so far it has been impossible to isolate sufficient amounts for identification.

We are indebted to Mr. K. E. Murray for isolation of the *cyclo*-propane acids by gas-liquid chromatography.

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Identification of Œstradiol-17_β Testosterone and its Precursors from *Scylliorhinus stellaris* Testes

SINCE its first isolation by Laqueur *et al.*[1] in 1935 from tissue of steer testis, testosterone has been obtained from some species of mammals only, namely, horse and man, by Ruzicka *et al.*[2,3]. Furthermore, Brady[4], by *in vitro* experiments on the biosynthesis of testosterone from ^{14}C-acetate, indicated that testosterone is probably the androgenic testicular hormone in two additional species, rabbit and hog. Androgenic substances of non-mammalian vertebrates are still unknown. On the other hand, œstradiol-17_β has been found to be the œstrogenic hormone not only in mammals but also in the elasmobranch *Squalus suckleyi*[5], in the lungfish *Protopterus annectens*[6], in birds[7] and in the starfish *Pisaster ochraceus*[8]. The investigation of the androgenic material in non-mammalian vertebrates seems therefore necessary to fill this gap, and is of obvious evolutionary importance.

We have started an investigation of the androgenic steroids by their direct determination in the testicular tissue of the elasmobranch *Scylliorhinus stellaris*. 110 gm. of material were obtained from six sexually mature animals and extracted according to the method of Ruzicka *et al.*[9]. The acetonic residue was refluxed with chloroform in order to eliminate urea, which is present in large amounts in elasmobranch tissue. The steroid fraction was separated by counter-current distribution. The neutral fraction (25 mgm.) was then resolved by paper chromatography in systems developed by Bush[10]. The phenolic fraction (81 mgm.) was chromatographed according to the system developed by Heftmann[11].

Neutral fraction. First chromatography in system 'Bush *C*' showed the presence at the starting line of blue tetrazolium-positive material, which probably corresponds to corticosteroids. Five spots were identified in successive paper chromatographies by short-wave ultra-violet light and rechromatographed. Three of these spots corresponded to testosterone, androstenedione and progesterone on the basis of the constant R_F values and absorption maxima. The spots corresponding to androstenedione and progesterone showed the characteristic colour blue-violet and blue, respectively, with the Zimmermann reaction. The spot corresponding to testosterone, after elution and oxidation with CrO_3, was rechromatographed in system Bush *A*. The oxidation product showed the same R_F as androstenedione.

According to semiquantitative estimation these steroids were present in the amounts of approximately

50 μgm./kgm. of testosterone, 70 μgm./kgm. of androstenedione and 100 μgm./kgm. of progesterone.

The two additional unidentified spots both showed absorption maxima at 240 mμ and the positive Zimmermann reaction. One of these gave an R_F value of 0·50 in system Bush B_3 (25 ± 1° C.); the other one gave an R_F value of 0·79 in system Bush A (25 ± 1° C.).

Phenolic fraction. The phenolic fraction resolved by paper chromatography and the ferric chloride plus $K_3[Fe(CN)_6]$ colour reaction showed the presence of œstradiol-17_β amounting to about 20 μgm./kgm.

The present investigation has demonstrated that the androgenic substances secreted by the testis of the elasmobranch *Scylliorhinus stellaris* are the same as those of mammals. Furthermore, as in mammals, œstradiol-17_β is present as well[3,12,13].

Especially interesting is the identification of progesterone, which has not been isolated before from testis tissue, but has been considered as a probable precursor of testosterone. In fact Slaunwhite and Samuels[14] demonstrated, by *in vitro* work, the biosynthesis of 17_α-hydroxyprogesterone, androstenedione and testosterone from isotopically labelled progesterone.

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Occurrence of Catecholamines in Acrania and Invertebrates

The occurrence of catecholamines in vertebrates has been extensively studied in recent years after the development of simple and accurate methods for the quantitative assay of such compounds. Relatively few investigations have been carried out with regard to their presence in invertebrates, however.

Biedl[1] and Gaskell[2] concluded from biological assay of extracts of leeches and the earthworm that these contained adrenaline. Later Erspamer and Boretti[3] showed the presence of *p*-hydroxyphenylethanolamine (octopamine) in the posterior salivary glands of *Octopus vulgaris*. These glands were also found to contain noradrenaline[4].

In an extensive investigation Östlund[5] found 0·13 μgm. of adrenaline and 0·48 μgm. of noradrenaline/gm. in the ventral ganglionic chain of *Lumbricus terrestris*, thus confirming the presence of catecholamines in this annelid. Similar amounts were found in *Arenicola marina* (lugworm). In a variety of other invertebrates Östlund[5] found only traces of catecholamines, however (less than 0·01 μgm./gm.). In insects, on the other hand, he observed relatively large amounts of adrenaline, noradrenaline and dopamine.

In the present experiments various invertebrates were collected fresh and extracted with 3–5 volumes of 10 per cent trichloroacetic acid. The catecholamines were adsorbed on an alumina column and eluted with acetic acid. Adrenaline and noradrenaline were estimated by a fluorimetric technique according to Euler and Lishajko[6].

The results are presented in Table 1. It will be seen that adrenaline and noradrenaline could not be demonstrated with certainty in invertebrates except in the annelid *Lumbricus terrestris* and in the larvæ of *Pieris brassicae*, the common cabbage white butterfly. This is in agreement with the previous findings of Östlund[5], who found in larvæ from various insects from 0·01 μgm. to 0·075 μgm. of adrenaline and from 0·1 to 1·3 μgm. noradrenaline per gm. In the present work the adrenaline content in the *Pieris* larvæ was negligible, while the noradrenaline content was 0·33 μgm./gm. From these results it is obvious that noradrenaline not only occurs in remarkably high amounts in insects' larvæ but also that it occurs greatly in excess of adrenaline.

Table 1. Adrenaline and Noradrenaline in Invertebrates and Acrania (Whole Animals, in Mesothuria Visceral Organs)

Phylum	Species	Adrenaline (μgm./gm.)	Noradrenaline (μgm./gm.)	Total weight (gm.)
Aschelminthes	*Priapulus caudatus*	< 0·005	< 0·005	15
Mollusca	*Buccinum undatum*	< 0·005	< 0·005	23
	Mytilus edulis	< 0·005	< 0·005	250
Annelida	*Lumbricus terrestris*	0·003	0·015	80
	Eumenia crassa	< 0·005	< 0·005	125
Crustacea	*Carcinus maenas*	< 0·005	< 0·005	44
Insecta	*Pieris brassicae* (larvæ)	< 0·005	0·33	47
Echinodermata	*Mesothuria intestinalis*	< 0·005	< 0·005	20
Urochordata	*Ciona intestinalis*	< 0·003	< 0·005	30
Acrania	*Branchiostoma lanceolatus**	< 0·005	0·16	5·6

* Syn. *Amphioxus.*

The presence of definite quantities of noradrenaline in *Branchiostoma*, 0·16 μgm./gm. in the whole animal, is of some interest since it indicates that this primitive organism is capable of synthesizing noradrenaline. On the other hand, there was no evidence of adrenaline.

The results presented have thus confirmed, by fluorimetric technique, the presence of considerable amounts of noradrenaline in *Lumbricus terrestris*, larvæ of *Pieris* and in *Branchiostoma lanceolatus*. Adrenaline was also found, as previously noted[5], in small amounts in *Lumbricus*. In the other invertebrates studied, the amounts of adrenaline and noradrenaline, if present at all, were extremely small.

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Effects of Speciation on Serum Proteins in the Genus *Macaca* with Special Reference to the Polymorphic State of Transferrin

The effects of speciation on the serum proteins of macaque monkeys and other primates are being studied in our laboratory by a combination of starch-gel electrophoretic and immunological techniques[1,2]. In an initial survey of the sera from 19 cynomolgus monkeys (*Macaca irus*) and 17 rhesus monkeys (*Macaca mulatta*) we found that the cynomolgus sera contained a larger proportion of γ-globulin than the rhesus sera and also that all the cynomolgus sera showed only one transferrin whereas 10 of the 17 rhesus sera showed more than one transferrin. At the time, we observed that one of the ten rhesus sera showed three transferrin components and that in the other nine sera, each of which showed two components, the relative positions of the transferrins in starch-gel varied in some cases between the samples. In order further to define the polymorphic state of transferrin in the species *Macaca mulatta* and the apparent absence of this state in the species *Macaca irus*, the sera from 40 more rhesus monkeys, newly arrived from India, and from 40 more cynomolgus monkeys, newly arrived from the Philippines, were kindly provided for study by Parke, Davis and Co. The rhesus monkeys in this shipment were captured in an area the size of a township near the Nepal border. It was not possible to trace exactly where in the Philippines the cynomolgus monkeys were captured. We have now detected fourteen transferrin patterns in the rhesus monkeys and still only one pattern in the cynomolgus monkeys. Thirteen of the transferrin patterns were represented in the last shipment. Our work, then, not only independently confirms but also considerably extends the report by Blumberg[3] of eight transferrin patterns in rhesus monkeys. Our results are particularly interesting in indicating that while the environment of one species selects for a polymorphic state, the environment of a closely related species selects against this state.

Although two-dimensional starch-gel electrophoresis[4] in a discontinuous buffer system[5,6] followed by immunological examination of the separated, fast migrating β proteins clearly demonstrated whether one, two or three transferrin components were present in a serum sample, comparative one-dimensional starch-gel electrophoresis revealed the subtler differences in the mobilities of the transferrins. Fig. 1*A* presents a typical result gathered by this latter type of screening in which a number of sera are run side by side in a large gel. Note the uniformity of the transferrin band in the 5 cynomolgus sera in contrast to the variability of the transferrin bands in the five rhesus sera. The fourteen transferrin patterns, of which type I is identical with the cynomolgus transferrin band, are shown in Fig. 1*B*. The actual homology of the cynomolgus and rhesus transferrins to human transferrin was established by two-dimensional starch-gel immunoelectrophoresis[6]. Fig. 2 presents the results of an immunoelectrophoretic experiment in which we employed one of our unispecific, chicken antisera[7] to human transferrin.

The transferrin types represented in Fig. 1 can be subdivided into those showing one component, types I, II, III and IV ; type V showing two components which almost merge ; those showing two closely adjacent components, types VI, VII and VIII ; those showing two components increasingly separated from each other, types IX and X, then types XI and XII, and finally type XIII; and the last showing three components, type XIV. Fig. 1 also lists the distribution of sera among these types. In this connexion the breakdown of sera among types I, II and V is still being checked, for a number of repeated determinations are needed to differentiate a large single band of transferrin from two which touch each other.

The genetics of the rhesus transferrins has still to be investigated. Yet by analogy to the human transferrins[8] we postulate that certain of these types (particularly those in the first sub-division) are con-

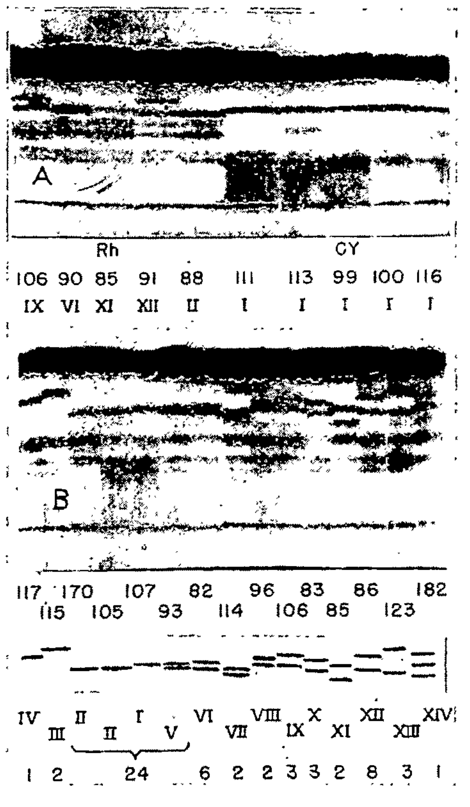

Fig. 1. *A*, Comparative one-dimensional starch-gel electrophoresis of five rhesus and five cynomolgus sera. The sample designations and the transferrin types of these samples are printed below the photograph of the gel. *B*, Comparative one-dimensional starch-gel electrophoresis of 15 rhesus sera representing the 14 transferrin types. Below the photograph of the gel and the sample designations, that portion of the photograph showing just the transferrin bands is presented diagrammatically. The numerical breakdown of the rhesus sera among the various transferrin types is presented below

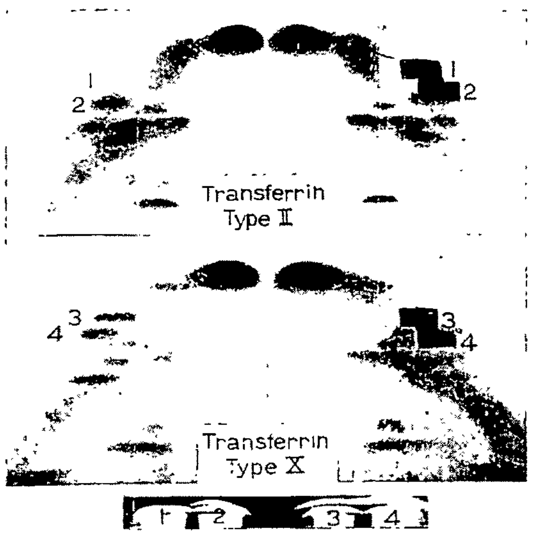

Fig. 2. Two-dimensional starch-gel immunoelectrophoretic identification of transferrin in type II and type X rhesus sera. One-half of each gel was first stained with amido black to show the distribution of protein components (photographs on the left). Then from the unstained halves pieces of gel, estimated to contain the transferrin or to serve as a control, are cut out. Then these halves are stained (photographs on the right). The pieces of gel are placed at the bottom of an immunoelectrophoretic chamber, overlaid first with agar, then with unispecific antiserum to human transferrin. After 12 days incubation the reactions were photographed (photograph on the bottom). Positive precipitin lines, demonstrating the presence of transferrin, were afforded by gel pieces 2, 3 and 4 but not by 1, the control piece. The lateral non-specific band which always forms just above the starch-gel may be discounted

trolled by homozygous combinations of genes and the other types by heterozygous combinations of genes. Although it would be necessary to test further specimens and other species of macaques to relate the frequencies of transferrins to the taxonomy of the genus, nevertheless it is clear that there are sharp species differences in the degree of polymorphism of transferrin.

In another approach to the detection of species differences in the state of the proteins, we found that our unispecific immune reagents could be used to measure as well as identify protein components in the macaque sera. For example, the relative γ-globulin concentration of each cynomolgus and rhesus sera was determined by immunochemical assay with a uni-specific anti-human γ-globulin reagent[9]. By this convenient means we clearly show that we are measuring the monkey protein equivalent to human γ-globulin. Though there is some overlapping of high rhesus and low cynomolgus values, the cynomolgus sera have a much larger mean γ-globulin concentration than the rhesus sera, 17·2 per cent as compared to 10·7 per cent. The difference in the means by the *t* test is significant at the 0·1 per cent level of confidence.

A more detailed account of the application of our experimental procedures to the study of primate proteins will be prepared later. It should be emphasized at this point, however, that the equivalent proteins in man and monkey are antigenically related, but not identical.

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PHYSIOLOGY

Polypnœa evoked by heating the Scrotum of the Ram

THERMAL or electrical stimulation of discrete regions in the brain of several species can cause specific and vigorous thermo-regulatory responses[1-3]. It is equally clear that in sheep and calves, the polypnœa in response to raised environmental temperatures may start before the temperature of the blood supply to the brain changes[4,5]. It was concluded that in these ruminants the thermal stimulus to panting can be entirely peripheral in origin. Whether the receptors concerned were concentrated in special areas of skin or were uniformly distributed over the surface of the body remained unresolved.

During experiments designed to examine the thermo-regulatory properties of the Merino ram's scrotum, a reflex polypnœa was observed to be initiated and sustained when this region was specifically heated. In 12 experiments on 5 rams, this was examined by enclosing the closely clipped scrotum in an insulated chamber through which dried air was circulated continuously. Temperatures were recorded at least once every minute from thermo-couples in the air outflow from the chamber, in the rectum at a depth of 10 cm., and in some experiments on the mid-posterior surface of the scrotum. The air of the room was controlled at 17 ± 2° C. throughout all experiments. Records were obtained for 1 hr. with the ram standing quietly and with air at 23 ± 4° C. passing over the scrotum. Scrotal air temperature was then rapidly raised to either 45 ± 1° C. (in 9 experiments) or 40 ± 1° C. (in 3) for 1–1½ hr. A typical response is shown in Fig. 1.

The mean respiratory-rates during the control periods ranged from 34 to 66/min.; in most experiments the respirations were quiet and almost imperceptible. The respirations became more definite and their frequency increased while scrotal air temperature was rising. The mean respiratory-rates varied from 61 to 171/min. in the first 30 min. of heating, they increased to 114–185/min. during the next 30 min. and were 117–163/min. in the final 30 min. Maximum respiratory rates of more than 200/min. were recorded during 3 experiments involving 3 of the rams. The typical response was not open-mouthed

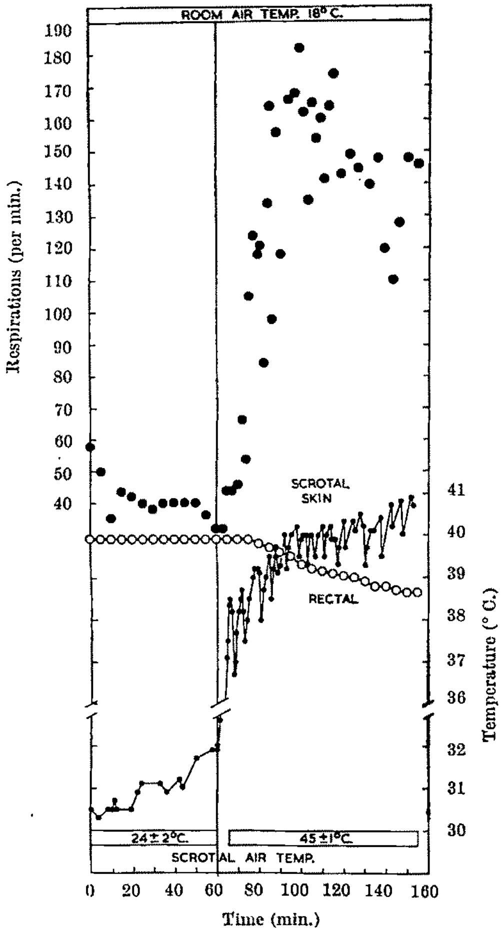

Fig. 1. The changes of respiratory-rate (●), rectal temperature (O–O) and scrotal skin temperature (●–●) induced by heating the scrotum of a ram with air at 45° C.

The falls of skin temperature occurring during the heating coincide with mass discharges from the sweat glands of the scrotum (Waites, unpublished observations)

panting, but a rapid polypnœa associated with a moist muzzle.

To confirm that the scrotal skin of each ram was specially sensitive to temperature, an area of mid-side skin equal to the area of the scrotum was close-clipped and heated with air at 45 ± 1° C. The respiratory response was negligible: mean frequencies were 45–65/min. during the control period, and 43–71/min. during the heating. It must therefore be concluded that the skin of the scrotum of the ram is particularly well endowed with peripheral thermal receptors.

No change in rectal temperature was detected before the start of the polypnœa, but thereafter rectal temperatures fell by 0·3–1·25 deg. C. in all experiments. In 7 of the 12 experiments, rectal temperature dropped at the rate of 0·5–1·0 deg. C./hr. and in these the fall started sharply within 8–18 min. after the onset of the polypnœa (Fig. 1). If the thermal inertia of the rectum of the ram is the same as that of the calf[6], then changes in rectal temperature would lag behind changes in blood temperature by 5–10 min. This would mean that in these experiments the temperature of the blood supplying the brain starts to fall almost immediately after the onset of the polypnœa. Furthermore, the polypnœa was sustained in spite of the continued rapid fall in deep-body temperature, a response which is paradoxical since the air ambient to the rest of the body was cool.

Thus, these results support the view that a thermally induced polypnœa can be evoked in rams without a previous rise in central temperature. Moreover, they show that the fall in deep-body temperature which followed the increase in respiratory-rate did not extinguish the reflex while the scrotal skin was being heated. The nature of the receptors and their pathways have yet to be described. However, it is now apparent that in the ram the scrotal cutaneous receptors alone are capable of influencing general body temperature, giving a broader thermo-regulatory role to the scrotum than the purely local one previously considered[7,8].

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Polypnœa evoked by heating the Udder of the Goat

At the suggestion of Dr. Waites (preceding communication), we have similarly tested the udder of the female goat, because the sensory innervation of the udder and the scrotum are essentially the same[1].

In seven tests on 2 non-lactating pregnant Saanen goats, the immersion of the udder in a stream of air at 45° C. caused a rise in respiratory frequency from 20–40/min. to 100–120/min. after a latency of 5–60 min. Neither passing air at udder-skin temperature over the udder nor heating an equivalent area of the abdomen in front of the udder had such an effect. In two tests on a third dry, non-pregnant goat with one mammary gland transplanted on to the neck[2], the heating of this denervated gland was without effect on respiration, but when the control gland in the normal position was heated, the onset of panting occurred after 20 min. Although the rectal temperature tended to rise during the periods in the laboratory, the onset of polypnœa could not be related to this rise, which occurred equally in control experiments.

These preliminary observations confirm the finding of Waites that the inguinal region appears to be more sensitive to temperature than the general body surface of ruminants.

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Recovery of Fertility after Protracted Steroid-induced Sterility in Mice

PROGESTERONE is known to prevent luteinization in experiments with guinea pigs[1]. The anti-luteinizing property of synthetic 19-norprogesterone and halogenated progesterone derivatives as absorbed from subcutaneously implanted pellets is greatly superior to that of natural progesterone[2,3]. The anti-luteinizing effect of these compounds can be made evident in experiments both with œstrogen-induced luteinization in ovarian grafts[4] and with spontaneous luteinization in the ovary *in situ* (guinea pigs[5], mice and rats). The anti-luteinizing effect of progestational compounds is not due to a direct action on the ovary but to a mechanism in which most probably the hypophysis and the hypothalamus are involved[6,7]. The physiological interest of these phenomena lies in the fact that the quantities absorbed per day from a subcutaneous pellet of progesterone and sufficient to antagonize luteinization are apparently not greater than those produced by the normal ovary *in situ*[4,8]. However, the findings are also of clinical interest; the progestational compound is also anti-ovulatory[9], in such a way that the anti-luteinizing action is implicitly also an anti-ovulatory one. Ample use of the anti-ovulatory action of progestational compounds has been made by Pincus *et al.* in experimental[10] and clinical[11] work.

The question arose whether the anti-ovulatory and anti-luteinizing faculty of progestational compounds can be made use of for the purpose of inducing protracted sterility and whether fertility can be recovered after a steroid-induced protracted sterility.

Mice, the life-cycle of which is such a short one and the reproductive activity of which deteriorates several months before death[12], are most suitable for such investigations. Our experiments were made with the strain *Balb-A* in use in this laboratory for several years; reproductive activity in this strain begins at the early age of two months, and is, under routine laboratory conditions, a stable one. But it starts to deteriorate 11–12 months later. Pellets of the highly active 19-norprogesterone were implanted subcutaneously; the pellets contained 20–40 per cent of this compound mixed with 80 and 60 per cent of cholesterol respectively.

20 per cent pellets were implanted into 10 mice 65 days old. The animals were killed 15 days later. Absorption was at the rate of 4–9 μgm. a day (for calculation of absorption from pellets containing a mixture of the specific steroid with cholesterol, see ref. 13). Corpora lutea were absent in 9 of these animals; the corpus luteum in the remaining animal was in a state of degeneration. In a group of 9 normal females 79 days old corpora lutea were present in 7 animals.

20 per cent pellets were implanted into 7 females 51–54 days old; 40 per cent pellets were implanted into another group of 7 females 40–51 days old. The experimental animals were maintained for the following 108 days with males in the same cage. None of these 14 animals became pregnant though they had reached the age of 5 months. In another group of 8 females 40 per cent pellets were implanted. The animals have now been sterile for 176 days and have reached the age of more than 7 months; that their sterility is not due to a failure of the male companion is shown by the fact that 2 other animals of the same group which lost implanted pellets had in the course of the same time 6 litters.

Table 1

No.	Age at implant. of pellets (days)	19-nor-progest. per day (μgm.)	First litter after removal of sterilizing pellets (days)	Size of first litter after removal of pellets
		20 per cent pellets		
5102	51	2	30	8
5094	54	2	30	11
97	51	2·6	no litter†	—
92	54	4·4	45	7
96	51	4·4	32	2
91	54	?*	31	10
93	54	?*	18(?)	9
		40 per cent pellets		
5103	51	12	37	8
12	42	12	22	13
07	49	13	35	9
13	42	13	42	9
21	40	13	22	8
11	42	15	22	7
04	51	16	36	4

14 females *Balb-A* aged 40 to 54 days, implanted subcutaneously with two pellets of 19-norprogesterone. Pellets were removed 108 days later, when the animals were 5 months old. Males were present in the same cage from the beginning of steroid treatment. No pregnancy occurred before removal of pellets.

* Only 1 pellet found 108 days after implantation.

† *Added in proof.* First delivery 86 days after removal of pellets. Size of first litter, 5; subsequent deliveries of 11, 5 and 7.

Pellets were removed after having remained in the above 14 animals for 108 days. Absorption was at the rate of 2–4 μgm. a day with 20 per cent pellets, and of 12–16 μgm. with 40 per cent pellets. The animals were left with the same male as before. As seen from Table 1, 13 out of 14 animals became pregnant at 1–24 days after the removal of the pellets. The average number of young in the litter was 8, that is, no less than in the first litter of normal females of this strain which is of 6·7 (average of 15 normal animals). In one case (No. 5112) the size of the first litter after protracted sterility was even larger, reaching the maximum as observed in our normal animals.

In other words, after protracted steroid-induced sterility, full recovery of fertility occurred. The young were suckled as with normal litters; this was the case even with twelve young out of a litter of thirteen (No. 5112). The animals belonging to these litters began their reproductive activity at the normal age of about two months.

As mentioned above, the reproductive activity of females in *Balb-A* begins deteriorating at about the age of 13–14 months. It may be assumed that an experimentally induced sterility of three months in *Balb-A* mice corresponds to no less than five years in women.

Added in proof. The experiment in which 8 females with 19-norprogesterone remained sterile during 176 days has now also been concluded. All the animals remaining with the same male as during the sterility period became pregnant after the removal of the pellets and produced litters 20–42 days later. The average size of the litters was 6·25, 6·25 and 5·9 at the first, second and third delivery; that is, these females behaved as regards fertility like normal females. Six months of the reproductive life-span in our mice are assumed to be equivalent to not less than ten years in women.

We acknowledge financial help from the Population Council, New York, given to one of us (R. I.). Thanks are due to Dr. A. Zaffaroni, director of Syntex, Mexico, for a gift of 19-norprogesterone.

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Arginine and 'Neurosecretory' Material in the Pituitary Gland of the Pig

IN a previous publication[1], the distribution of arginine as demonstrated histochemically by an improved Sakaguchi reaction[2] was examined in sections of rat pituitary gland. The results were discussed in relation to present knowledge of the origin and chemical nature of the hormones of the pituitary gland, with particular reference to the correlation between the stainable 'neurosecretory' material of the pars nervosa and the octapeptide antidiuretic-vasopressor hormone (ADH-vasopressin). It was further suggested that a similar examination of the neurohypophysis of the pig would be of interest since the ADH-vasopressin extracted from the posterior lobe of the pituitary of this animal contains lysine in place of arginine. Unfortunately, there does not appear to be a specific histochemical method for lysine; nevertheless, some information may be derived by applying the reaction for arginine, for if the stainable 'neurosecretory' material represents the hormones oxytocin and ADH-vasopressin, as postulated by the neurosecretory theory, then it might be expected to give no reaction for arginine in the case of the pig.

This communication describes results of such a preliminary investigation; pituitary glands were fixed in Susa or Lewitsky's fluid and the methods used were as described previously[1].

By comparison of adjacent sections it was found that the 'neurosecretory' material of the pars nervosa of the pig, as stained by the chrome alum-hæmatoxylin method, showed no reaction for arginine. However, other structures within the same section, such as cells of the pars intermedia, acidophils of the pars anterior, blood vessels and connective tissue fibres, gave a positive reaction for arginine, as did also 'neurosecretory' material in sections of rat pituitary stained under exactly the same conditions and used as a 'control'. It is therefore concluded that arginine is either very low in concentration or absent from 'neurosecretory' material in the pars nervosa of the pig, as predicted from the known chemical structure of pig ADH-vasopressin and the theory of neurosecretion. The investigation is being extended to other species.

I wish to thank Dr. F. R. Johnson, of the Department of Anatomy, for his help and advice on the photomicrography, and Mr. L. G. Cooper, of Messrs. T. Wall and Sons, Ltd., London, N.W.10, for providing the pig pituitaries, which were obtained within 30 min. of death.

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Renal Proliferation in the Unilaterally Nephrectomized Rat

RENAL growth following unilateral nephrectomy has been referred to as 'compensatory renal hypertrophy'[1]. The work of Rollason emphasizes the importance of hyperplasia in this process[2]. Utilizing thymidine as a specific precursor of deoxyribonucleic acid (DNA), and assuming the constancy of DNA in the absence of dividing cells, the incorporation of thymidine serves as an index of cellular proliferation[3]. We now describe the effect of uninephrectomy on the incorporation of tritium-labelled thymidine into renal DNA.

Male Wistar rats, approximately 4 weeks of age and weighing 40 gm., were housed in colony cages and maintained on 'Purina Laboratory Chow' and water *ad lib.* Experimental and control groups were chosen at random from a group of sixty rats. Left nephrectomies and sham operations were performed under pentobarbital sodium anæsthesia (29 mgm./kgm. body-weight intraperitoneally). 36 hr. following the operative procedure each animal received intraperitoneally three separate injections, 1 hr. apart, of tritium-labelled thymidine, 20 μc./kgm. body-weight: 24 hr. following the last injection, the animals were killed by decapitation, the kidneys removed and frozen. DNA protein was isolated from the kidneys according to a modification of the Schmidt–Tannhauser procedure as outlined by Friedkin[4]. The dried DNA protein was weighed into a counting vial and 1 ml. of methanolic 'Hyamine' (Packard Instrument Co., Chicago, Illinois) was added. The vials were sealed and heated in a water-bath at 55° C. for 18 hr.; 5 ml. of a toluene scintillation solvent system were added[5]. A homogeneous solution was obtained by swirling. The vials were cooled to 4° C. and counted in a liquid scintillation counter (Packard Instrument Co.). The final counts were corrected for quenching by use of an internal standard. In order to express the counts as counts per min. per milligram of DNA, an aliquot of each DNA protein sample was heated with 3 per cent perchloric acid for 15 min. at 70° C., and the optical density of the supernatant solutions was read at 265 and 295 mμ and compared

Table 1

	No. of Animals	Average weight of total renal tissue (mgm.)	Per cent restoration of renal tissue	Thymidine incorporation (c.p.m. per mgm. DNA)	*P*
Nephrectomy	8	634	45	970 ± 84*	< 0·0001
Sham	4	877		488 ± 31*	

*Replicate determinations upon pooled tissue.

to a DNA standard (Sperm DNA, California Biochemical Co.).

A representative experiment is shown in Table 1. The unilaterally nephrectomized animals incorporated 100 per cent more labelled thymidine than did the control, sham-operated rats. These results, obtained with tracer amounts of tritium-labelled thymidine, are, according to our present concepts, indicative of increased cellular proliferation in the remaining kidney of the unilaterally nephrectomized rat[6]. They support the morphological work previously cited and recent reports concerning DNA synthesis in the uni-nephrectomized rat utilizing other techniques[7,8].

The mechanism(s) regulating such growth is poorly understood; however, Ogawa and Nowinski have reported a specific renal mitosis-stimulating substance in the serum of uni-nephrectomized rats[9]. We have found that kidney slices in a balanced salt–glucose medium incorporate tritiated thymidine into DNA at a measurable rate. Work is being carried out utilizing this *in vitro* technique as an assay for the presence of growth-promoting factors in the serum of unilaterally nephrectomized animals.

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The Distribution of Light in an Image formed in the Cat's Eye

Flamant[1] has estimated the distribution of light in the image formed in the human eye of a brightly illuminated slit. By the kindness of Prof. Arnulf and Mlle. Flamant of the Institut d'Optique in Paris, we were allowed to use their apparatus and method to make similar measurements on the eyes of cats. Visual stimuli have been used for electrophysiological work on the visual system of the cat, and it is often important to know how accurate an image is formed.

The cat was anæsthetized with 'Nembutal'; the pupil was dilated and the accommodation fixed with atropine. The image of a narrow slit of light formed in the cat's eye was photographed by the method described by Flamant[1] for the human eye. The eye was placed in Maxwellian view, so that a small image of the source was formed near the centre of the pupil, and an image of the slit at the tapetum. The light reflected from the tapetum was observed after emerging through the pupil, and the correcting lens was adjusted to make the tapetal image, as observed, as sharp as possible.

The principle of the method is to measure the increase in width of the photographic image of the retinal image of a vertical slit the intensity of which varied from top to bottom. It was immediately obvious that the image was much less sharp than that formed in the human eye. Satisfactory photographs were obtained from one cat only, but two others were examined and appeared to have images of similar size. When the image could be formed on the tapetum the reflexion was very much greater than that from the human eye, so that much shorter exposures were required.

The light which was photographed had traversed the eye media twice, once entering through a small central area of the pupil, and once leaving, after dispersion by the tapetum, through the whole of the dilated pupil. It is assumed that scattering in the second transit is equivalent to that occurring when the eye forms an image with the pupil dilated, and that scattering in the first transit is negligible in comparison. Fig. 1 thus represents the distribution of light in the retinal image of a point source when the light enters the eye through the whole of the dilated pupil. At 12 min. of arc from the centre of the image the intensity has fallen to about 50 per cent of its original value, at 32 min. the intensity is down to about 10 per cent. The spread of the image is about 6–8 times greater in these experiments than in those on the human eye. The conditions of our measurements were, however, different: the eye was atropinized and the position of the image on the cat's retina was not known—it was not necessarily very close to the central area. Comparison with human

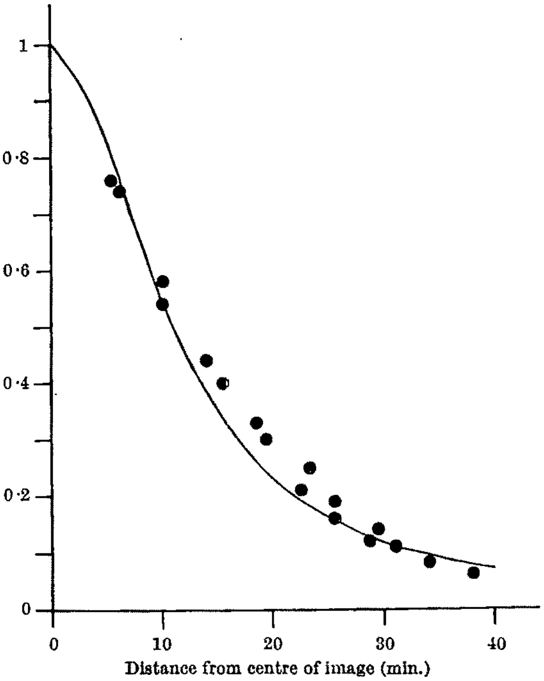

Fig. 1. Relative intensities at different angular distances from the centre of the image of a point source of light viewed by a cat's eye with pupil dilated

results may therefore be misleading. The experiments show that the images of small light sources formed by the cat's eye may be very blurred when light enters through the whole of the dilated pupil. They give no information about the scattering which takes place when light enters only through a small area in the centre of the pupil; this is likely to be much less.

We are grateful to Prof. Arnulf and Mlle. Flamant for allowing us to use their apparatus and for their assistance with the experiments. This work was supported by research grant *B*–1810 from the National Institute of Neurological Diseases and Blindness, United States Public Health Service, and both authors received Medical Research Council personal grants.

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A Neuronal Model for Conditioning

THE difficulty of explaining conditioning in terms of a simple neuronal model has been discussed by Burns[1]. The problem lies in the fact that the only relatively long-lasting type of facilitation that has so far been experimentally demonstrated involves presynaptic terminals so that activity of neurones in the unconditioned reflex pathway cannot be expected to facilitate synaptic connexions from neurones which converge on to them from another afferent pathway. Eccles has proposed a rather complex neuronal model to avoid this difficulty[2]. Even this is admitted to be inadequate since it requires that the conditioned and unconditioned stimuli be applied simultaneously, and even then could only provide a low-level bombardment of the synapses which must be facilitated unless additional reverberating circuits are included. There is evidence to suggest that reverberating circuits do play a part in the early stages of conditioning[3,4], but no complete model including them has been proposed.

The following simple scheme appears to fulfil many of the requirements: the neurones of the unconditioned reflex pathway are linked to the afferent neurones excited by the conditioning stimulus through a number of parallel reverberating circuits. Each unit (Fig. 1) is reciprocally linked through synapses with similar parallel units. The synaptic resistance must be such that impulses from *C.S.* alone result in the damped oscillation of only a few of the units. If impulses from *U.S.* impinge on the circuits while damped oscillations initiated by *C.S.* are still present, then spatial facilitation occurs and additional parallel circuits are recruited. Once a critical number of parallel units is activated the oscillations become self-maintaining because of reciprocal reinforcement. When the oscillations eventually die down the synapses linking *C.S.* to *E* through *A*, *B* and *C* remain facilitated.

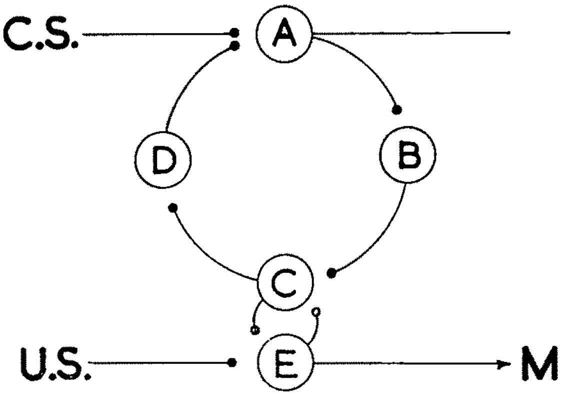

Fig. 1. *A*, *B*, *C*, *D* and *E* represent nerve cell bodies in a model unit. *C.S.* is the afferent pathway for conditioned stimuli and *U.S.* for the unconditioned stimulus. *M* represents the motor pathway for the unconditioned response

A difficulty arises. *E* is continuously bombarded with impulses during the initial stage of the conditioning process which may be expected to prolong the reflex greatly. It seems possible that activity in *E* may be cut off by negative feedback once the reflex has been fulfilled.

I thank Dr. B. D. Burns for his comments.

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Local Endotoxin Hypersensitivity and its Relation to the Shwartzman Phenomenon

ALTHOUGH there have been previous claims that lesions like the Shwartzman phenomenon can be produced by two large injections of endotoxin into the same site in the skin, in animals other than rabbits (for example, mice)[1], it has been stated that the classical Shwartzman phenomenon is immunologically non-specific in that it does not depend on antigen–antibody reaction[2,3], and it can be elicited only in rabbits[2–4]. Recently, however, I have succeeded in demonstrating that the first phase of the Shwartzman phenomenon, that is, preparation of the skin by endotoxin, is a specific reaction depending on a well-characterizable immunological mechanism, namely, the phenomenon of local endotoxin hypersensitivity, and that in appropriate circumstances (*a*–*e*) it may be elicited by endotoxins on the skin of guinea pigs and rats also.

These characteristics are:

(*a*) Guinea pigs and rats, 3–4 weeks previously sensitized with *S. typhosa* or *E. coli*, will produce hæmorrhagic skin lesions at sites prepared with 25–50 μgm. endotoxin, on intravenous injection of 50–100 μgm. endotoxin 24-hr. later. (The sensitization was carried out by the weekly subcutaneous injection of 0·5–1, 0–1, 0–2 × 10^{-9} bacilli. Typhoid and *coli* endotoxin prepared by the Boivin method were reprecipitated three times by a double volume of ethanol. Their Shwartzman-reactive unit was 3 μgm.) Animals not pretreated with the bacteria do not produce hæmorrhagic lesions. These experiments proved that the endotoxin must not be derived from the same bacterial species as was used for sensitization.

(*b*) 0·025–0·1 ml. rabbit antiserum, obtained by hyperimmunization with typhoid endotoxin, injected into the skin of guinea pigs or rats, followed after 24 hr. by intravenous endotoxin, results in skin hæmorrhage.

(*c*) Mixture of typhoid endotoxin (25–50 μgm.) with colloidal silver (50–100 μgm.) for intracutaneous injection and subsequent intravenous challenge (0·1–1·0 μgm. endotoxin and 10–20 mgm. colloidal silver) results in skin hæmorrhage in guinea pigs at 6–8 hr.

(*d*) Normal rabbit mononuclear peritoneal exudate cells (2–10 × 10^4) obtained 4 days after paraffin oil had been introduced in the peritoneal cavity[5], injected intracutaneously in guinea pigs, and followed by 50–100 μgm. endotoxin intravenously after 24 hr., result in skin hæmorrhage.

(*e*) Normal guinea pig or rat mononuclear peritoneal exudate cells (5–10 × 10^4) injected intracutaneously in guinea pigs or rabbits, and followed by 100 μgm. endotoxin intravenously after 24 hr., result in skin hæmorrhage.

The histology of the skin lesions (6–8 hr. after intravenous challenge) resembled that of the Shwartzman phenomenon in rabbits.

a–c could be explained by localization of endotoxin in relatively high concentrations in the skin by the antibody or by the effects of colloidal silver. Antibody which combines with endotoxin has been shown (in some instances at least) not to neutralize its toxic properties. A specific additional effect of antigen–antibody reaction or of colloidal silver might or might not be involved, but it does not seem proved.

d and *e* can scarcely be explained in the same way, since the peritoneal exudate cells do not produce secreted antibody in normal circumstances. The possibility is worth examining that they carry delayed type hypersensitivity to endotoxin, and that the combined result of a delayed type reaction and intravenous endotoxin is a Shwartzman phenomenon.

I wish to thank Dr. E. Kelemen for advice and generous help throughout this work.

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Lactic Acid Concentration in the Kidney of the Golden Hamster, *Mesocricetus auratus*, in Different Conditions of Diuresis

WORK by Zerahn on frog skin[1] and by Leaf on the bladder of the toad[2] have produced evidence that the active transport of sodium is accompanied by a proportional consumption of oxygen; glycolysis and lactic acid production also increase, according to Leaf, with the active transport of sodium in aerobic conditions.

It is known that the renal emunctory is also a site of intense transfer of sodium through the convoluted and collector tubes from the urine to the plasma; in addition, it seems that consumption of oxygen is related to such a process[3]. In the present communication we present the results obtained on lactic acid concentration in the cortex and papilla of the kidney in three different experimental conditions: (1) after 12 hr. fasting: (2) under water diuresis (samples of renal tissue taken 1½ hr. after the administration of distilled water to the amount of 5 per cent of the animal's weight by gastric tube); (3) under mannitol diuresis (samples of renal tissue taken 1½ hr. from the subcutaneous administration of 7·5 ml. per cent bodyweight of a 10 per cent mannitol solution).

Mesocricetus auratus was selected for experiments as the renal cortex of its kidney can be easily separated from the papillary tissue (Fig. 1). The lactic acid has been measured by Barker and Summerson's method[4] using as a standard lithium lactate purified according to Schatzkes[5]. Each examination was made on samples of approximately 40 mgm. of papilla tissue and 100 mgm. of cortical tissue, frozen in liquid nitrogen as soon as they were taken from the animal; the two samples of cortex and papilla which were compared came from the same kidneys.

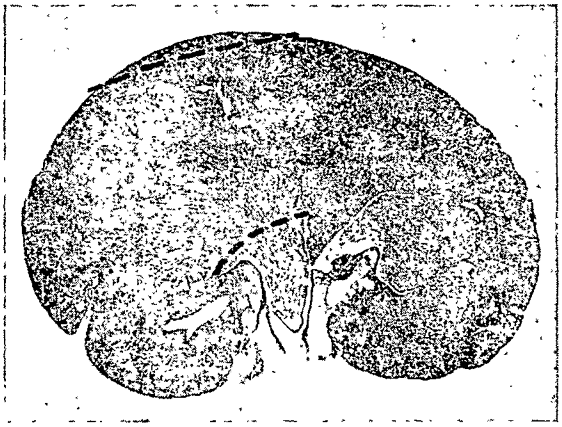

Fig. 1

The results are shown in Table 1. From these a constant and significant difference of the lactic acid content in the papilla and in the cortex appears in all conditions. If one excludes a state of relative hypoxia in the papillary zone, this difference could account for greater chemical work in the papilla than in the cortex, or it could simply be the expression of a greater concentration in the tissue of this part as there is for sodium chloride and urea[6].

Table 1. LACTIC ACID MGM. PER CENT GM. OF FRESH TISSUE

Treatment	Cortex mean value	Papilla mean value	Difference ±*S.E.*
$n = 7$	50·93	71·12	20·19 ± 3·55
Water diuresis $n = 7$	54·25	80·29	26·03 ± 3·48
Mannitol diuresis $n = 8$	54·00	108·88	54·88 ± 9·91

It is interesting that under mannitol diuresis the difference increases significantly, although it seems that the cortex–papilla gradient of total concentration tends to decrease in this condition[7]; the greater concentration of lactic acid in the papilla under mannitol diuresis seems therefore to exclude the second hypothesis.

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Pharmacological Actions of Some Methoxyindolealkylamines

The O-methylation of catecholamines has been shown to be an inactivation process[1], but the recent characterization of melatonin (N-acetyl-5-methoxytryptamine—a potent agent in lightening frog skin) as a methoxy indole[2] indicates that this may not be true with hydroxyindolealkylamines. To investigate this possibility, we have compared the actions of a number of methoxyindolealkylamines with their corresponding analogues on smooth muscle, blood pressure and behavioural patterns.

Oxytocic activity was measured on isolated œstrous rat uterus. Gramine and its methoxy derivative were found to possess no oxytocic activity at a dose-level as great as 0·1 mgm. per ml. (Values are given as final concentration in the muscle bath.) At the same dose-level, 5-hydroxyindole acetic acid and 5-methoxyindole acetic acid caused small but demonstrable contractions. This action could not be blocked with bromolysergic acid diethylamide (*BOL*). N-Acetyl-5-methoxytryptamine also exhibited slight oxytocic effect, but only at the high dose-level of 0·1 mgm. per ml.

The four other compounds tested were serotonin, bufotenine and their respective methoxy analogues. These exhibited marked oxytocic activity at dose-levels below 10 μgm. per ml. The relative activity of these compounds is given in Table 1, and it can be seen that the methoxy derivatives were only slightly less active than their hydroxy analogues. The results obtained for bufotenine and 5-methoxytryptamine are in general agreement with those of Erspamer[3] and of Barlow and Khan[4].

Whereas N : N-dimethylation of 5-methoxytryptamine only reduces the oxytocic activity slightly, N-acetylation reduces the potency a thousand-fold.

The effect on arterial blood pressure was measured on a smoked drum from a mercury manometer. Two cats and three dogs anæsthetized with pentobarbital were used. The action of compounds was noted before and after the nervous system had been destroyed in cats by pithing, or blocked in dogs by large doses of the ganglioplegic agents : tetraethyl ammonium chloride, 30–40 mgm./kgm., or chlorisondamine ('Ecolid' : 2 mgm./kgm.).

In doses of 1–2 mgm. N-acetyl-5-methoxytryptamine, 5-hydroxyindole acetic acid, 5-methoxyindole acetic acid and 5-methoxygramine had no effect on arterial blood pressure of dogs or cats.

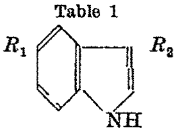
Serotonin, 5-methoxytryptamine, 5-methoxy-N : N-dimethyltryptamine in doses of 0·1 mgm. and bufotenine, 0·5 mgm., all caused depression of arterial pressure or an amphibaric response in both species while the nervous system was intact. After pithing or blocking, these compounds caused blood pressure to rise by 26–92 mm. mercury.

It has been reported that ganglioplegic agents cause powerful augmentation of a variety of pressor agents[5]. Serotonin and 5-methoxy-N : N-dimethyltryptamine were augmented to an almost identical degree, both being slightly exceeded by 5-methoxytryptamine. 5-Methoxytryptamine also exhibited a depressor component, after pithing in cats, usually following a strong pressor phase.

The relative vasopressor potency of these four compounds is : Serotonin > 5-methoxy-N : N-dimethyltryptamine > 5-methoxytryptamine > bufotenine. Thus, O-methylation reduces the vasopressor activity of serotonin but greatly increases that of bufotenine, and N-acetylation of 5-methoxytryptamine inactivates it.

To investigate behavioural disturbance, rats were conditioned to escape, in a shuttlebox, from a 535-V.

Table 1



Compound	R_1	R_2	Oxytocic*	*BOL*†	Effect on blood pressure‡ (unblocked)	(blocked)	Behavioural§ disturbance
Gramine	H	CH_2NH_2	0·0001	—	None	None	0
5-Methoxygramine	OCH_3	CH_2NH_2	0·0001	—	,,	,,	+
Serotonin	OH	$CH_2CH_2NH_2$	1	Inhib.	⇅	↑	(++)
5-Methoxytryptamine	OCH_3	$CH_2CH_2NH_2$	0·7	,,	⇅	↑	++
N-Acetyl-5-methoxytryptamine	OCH_3	$CH_2CH_2NHCOOH_3$	0·0001	—	None	None	0
5-Hydroxyindole acetic acid	OH	CH_2COOH	0·0001	No inhib.	,,	,,	—
5-Methoxyindole ,, ,,	OCH_3	CH_2COOH	0·0001	,,	,,	,,	—
Bufotenine	OH	$CH_2CH_2N(CH_3)_2$	0·49	Inhib.	↑	↑	++
5-Methoxy-N : N-dimethyltryptamine	OCH_3	$CH_2CH_2N(CH_3)_2$	0·08	,,	⇅	↑	+++++

* Potency of equimolar concentrations of the base. † Inhibitory effect of *BOL*. ‡ Blood pressure raised ↑, biphasic ⇅. § At 0·1 m*M*/kgm. dose-level each + represents 2 mistakes during 10 tests.

2-m.amp. shock in response to a warning auditory stimulus, individual trials being separated by 6-min. intervals. The criterion of completed conditioning was a correct response to the conditioning stimulus of not less than 9 out of 10 times on two consecutive days. The animals were then used for assay and the number of mistakes plotted against the dose-level. N-Acetyl-5-methoxytryptamine caused no behavioural disturbance at dose-levels of 0·2 m*M*/kgm. At dose-levels between 0·1 and 0·3 m*M*/kgm. 5-methoxygramine caused a somewhat greater degree of disturbance than did gramine. At 0·1 m*M*/kgm., 5-methoxytryptamine, bufotenine and serotonin all gave similar results, though in the case of the latter compound the paresis of the hind legs which developed introduced a physical handicap which modified the interpretation of this result. The most active compound of the series proved to be 5-methoxy-N : N-dimethyltryptamine, which caused 10 mistakes out of 10 trials at a dose-level of 0·05 m*M*/kgm. This was three times more effective than bufotenine, which has been reported to be psychotomimetic in man[6] and to affect the performance of trained rats[7]. At dose-levels exceeding 0·1 m*M*/kgm. the animals given 5-methoxy-N : N-dimethyltryptamine developed rhythmic beating of the forepaws, which tallied closely with the description given by Fabing and Hawkins[6] for the effects of bufotenine in rats which we have, however, not observed. 5-Methoxytryptamine also proved quite potent and in the same range as bufotenine. Finally, 5-methoxygramine appeared to cause more mistakes than did gramine itself.

Thus the ability of gramine, serotonin and bufotenine to cause behavioural mistakes was increased by O-methylation; and whereas N : N-dimethylation of 5-methoxytryptamine increases its potency, N-acetylation inactivated it.

It can therefore be concluded that O-methylation of hydroxyindoles is not an inactivation mechanism as is the O-methylation of catecholamines. The O-methylation of hydroxyindoles, however, changes their activity in the three assay procedures used in the following ways: oxytocic activity is still exhibited though slightly decreased; the vasopressor activity of serotonin is decreased but that of bufotenine is increased; ability to cause behavioural mistakes is increased. In addition, although O-methylation and N-acetylation are both necessary for the frog skin lightening activity of melatonin[2], it is the N-acetyl group which renders melatonin inactive towards smooth muscle, blood pressure and behaviour.

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HÆMATOLOGY

A Possible Specificity of Albumin Auto-antibodies

ANTI-*I* was the name given by Wiener *et al.*[1] in 1956 to a cold antibody found in the serum of a patient suffering from cold antibody hæmolytic anæmia. Further investigations into the *I* group system were made by Jenkins *et al.*[2], Tippett *et al.*[3], Weiner *et al.*[4] and Marsh[5]. These workers showed that cold auto-antibodies (previously called non-specific cold agglutinins) had *I* specificity and that occasional powerful examples of these antibodies may be found as auto-agglutinins in cases of cold antibody hæmolytic anæmia. The *I* antigen has an almost universal distribution, Wiener *et al.* finding only five *i* people from 22,000 blood donors, while Jenkins *et al.* failed to find an example of the *i* phenotype after testing 17,000 donors. Marsh afterwards described examples of cold auto-antibodies having *i* specificity and showed that normal infants at birth possessed the *i* phenotype, which changed within 18 months of birth to *I*. He concluded that the rare *i* phenotype in adults arose from the absence of a genetically determined factor essential for the proper development of *I*. The majority of cold auto-antibodies therefore appear to have *I* specificity, a minority may be anti-*i*.

In 1956, Weiner *et al.*[6] described some examples of a rare auto-agglutinin active against all cells, but only when they were suspended in bovine albumin. These auto-agglutinins were inactive by anti-globulin or enzyme techniques and could not be absorbed by saline-suspended red cells. We have encountered two examples of the albumin auto-agglutinin described by these workers and were able to test them for *I* or *i* specificity.

Both sera were active against the patient's own cells and also all cells of a large genotyped panel, but only when albumin suspended red cells were used. One serum contained an additional trace of anti-*D* which was successfully removed by absorption with saline-washed rhesus-positive red cells.

The sera were tested against the red cells of two examples of the i_1 phenotype, one i_2 and four cord blood samples, with appropriate adult controls. The tests were all positive with about the same strength of reaction. Repeated tests with one of the sera in titration (using *AB* serum as a diluent) showed that the i_1 and i_2 cells scored the same as the adult controls; the cord samples were slightly weaker.

It is apparent from these results that while cold auto-antibodies have *I* or *i* specificity, albumin auto-agglutinins are not aimed at antigens within the *Ii* system.

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Action of Proteolytic Enzymes on the Human Erythrocyte Surface

SEAMAN *et al.*[1] have recently described the liberation of a sialomucopeptide by the action of trypsin on the human erythrocyte surface. Similar results have been obtained by Mäkelä *et al.*[2]. My work concerned with the chemistry of *MN* blood group-active mucoids from human erythrocytes has revealed that N-acetylneuraminic acid-containing mucoids, which carry *MN*-active groupings, were liberated into the supernatant by the action of papain[3]. A review of the *MN* blood group active substances, their isolation and inactivation by several methods has been published elsewhere[4]. Further work led to the examination of other enzymes which are known to inactivate *M* and *N* : trypsin, ficin, bromelin, and stem-bromelain[4]. In all cases the isolation procedure to recover the non-dialysable split products was similar to that described previously[2] for papain-treated red cells, which involved phenol/extraction of the supernatant after incubation with enzyme and centrifugation. Some chemical data of the mucoids obtained are given in Table 1.

Table 1. QUANTITATIVE CARBOHYDRATE VALUES OF MUCOIDS RELEASED BY DIFFERENT ENZYMES

	Trypsin	Ficin	Bromelin	Papain
N-Acetylneuraminic acid (per cent)	19	19	13	14·7
Reducing sugar (galactose) (per cent)	29·4	39·9	73·5	47
Hexosamine (per cent)	9·9	13·9	8·2	18·1

In the case of bromelin and also stem-bromelain a glucose-containing polysaccharide from the crude enzyme is recovered in the final product so that chemical analysis gives carbohydrate values which are too high. Paper chromatography showed in all other samples the presence of galactose, glucosamine, galactosamine and small amounts of fucose and mannose. The same sugars have been detected in other *MN*-active preparations from erythrocyte stroma[3]. Although these mucoids were isolated from human *O* cells, no *H*-activity could be demonstrated by rabbit anti-*H* ; only slight inhibition was observed when tested with anti-*H* extract from *Ulex europaeus*.

The fact that all N-acetylneuraminic acid-containing and *MN* blood group-active mucoids prepared in this way give no inhibition of myxovirus hæmagglutination has been interpreted[4] and may be in accord with the findings that the enzyme-treated cells are equally well agglutinated by mumps- and influenza-virus as are the untreated cells. The *MN* activity of these substances is considerable but not so good as the activity of preparations isolated by a procedure where no enzyme is involved[4]. By the latter method large amounts of the N-acetylneuraminic acid-containing influenza-virus receptor are also obtained[4]. In the light of these observations the lowered electrophoretic mobility of enzyme-treated cells is understandable. On the other hand, it is worth noting that removal of either neuraminic acid or neuraminic acid-containing mucoids renders the cells agglutinable by incomplete antibodies[2,4].

The same method has been successfully used for investigating mucoids released by papain from animal erythrocytes. The substance from bovine erythrocytes has already been described[3,4]. The material from horse cells contains 15 per cent N-glycolylneuraminic acid, 21 per cent reducing sugar, 5·7 per cent hexosamine and inhibits slightly the agglutination of human *O*, *N* cells by anti-*N* extracts of *Vicia graminea* ; whereas that from hog cells has 9·5 per cent N-glycolylneuraminic acid, 33·5 per cent reducing sugar and 11·2 per cent hexosamine. Tests of the treated cells with mumps- and influenza-virus indicated that not all the neuraminic acid-containing mucoids had been removed[4].

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Blood Potassium and Sodium of Hereford and Brahman Cattle and some Breeds of Sheep maintained in Florida

INCIDENTAL to other comparative physiological investigations with taurine and zebu cattle[1], it was possible to determine the potassium and sodium content of the erythrocytes of these species by flame spectrophotometry. Although there have been several recent reports concerning potassium and sodium in the blood of sheep and their interesting genetic implications[2], there is a paucity of information regarding these elements in the red blood cells of cattle.

Abderhalden was one of the first to study blood electrolytes of cattle, and more recently Kerr reported findings for six *Bos taurus* cattle. The low potassium and relatively high sodium of the erythrocytes of cattle compared with other species as found by Kerr and Abderhalden have been widely cited in text-books, in spite of their meagre basis. Bernstein[3] has, however, confirmed the results of the earlier workers, but fails to state which types of cattle were used in his work.

Twelve Hereford and twelve Brahman heifers in the sixth or seventh month of gestation, and all approximately the same age, were used in the present work. All animals were under the same management and were kept under identical environmental conditions. All the animals were on an adequate plane of nutrition as described previously[1]. Blood was collected in heparinized tubes from the jugular vein of each of these twenty-four animals and centrifuged under standard conditions with a centrifugal force of 1,500*g*. The force was applied for 2 hr. rather than 30 min. in order to eliminate more of the plasma entrapped between the packed cells. Jennings *et al.*[4] have reported that bovine blood centrifuged under standard conditions for 30 min. still retains some 12·5 per cent of the plasma in the packed cell column ; but Chaplin and Mollinson[5] were able to remove most of the trapped plasma by increasing the time the centrifugal force was applied.

A 1-ml. sample of plasma was first taken from each tube of blood, then the remainder of the plasma and the upper layer of cells were removed and discarded. A small quantity of unwashed erythrocytes were then placed in a clean weighed volumetric flask which was reweighed, and sodium determinations were carried out using a Beckman model *B* spectrophotometer at wave-lengths of 770 and 585 mμ respectively. All results were corrected for dilution and those for the erythrocytes adjusted for weight

Table 1. Potassium and Sodium Content of Bovine Erythrocytes and Erythrocytes and Plasma expressed as m.equiv./l. for Hereford and Brahman Heifers

	Herefords				Brahmans			
	Erythrocytes		Plasma		Erythrocytes		Plasma	
	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+
1	16·5	118	9·0	146	14·1	115	6·4	146
2	18·0	107	6·4	141	15·7	127	6·4	141
3	16·8	104	7·7	141	11·2	91	6·4	137
4	13·9	124	10·2	146	11·9	102	8·3	146
5	15·7	112	7·7	141	17·2	130	6·4	137
6	20·4	113	10·2	146	12·9	202	6·4	141
7	15·7	118	5·1	130	15·1	95	9·0	141
8	25·7	119	9·0	141	11·3	73	7·7	141
9	14·6	103	9·0	141	20·7	125	7·7	135
10	17·0	108	7·7	137	14·7	154	6·4	135
11	18·6	109	9·0	137	11·7	129	7·0	137
12	27·6	116	11·5	141	8·5	134	6·4	135
×	18·4	113	8·5	141	13·8	123	7·0	140

$$\text{Hereford}\quad \frac{[K^+]_I}{[K^+]_E} = 2{\cdot}16 \qquad \frac{[Na^+]_I}{[Na^+]_E} = 0{\cdot}80$$

$$\text{Brahman}\quad \frac{[K^+]_I}{[K^+]_E} = 1{\cdot}96 \qquad \frac{[Na^+]_I}{[Na^+]_E} = 0{\cdot}88$$

Where $[K^+]_I$ = intracellular potassium and
$[Na^+]_E$ = extracellular sodium

of sample and red cell specific gravity (1·088). All the findings are presented in Table 1 as m.equiv./l.

The Herefords appear to have more potassium and less sodium in their erythrocytes compared with Brahmans, the differences for the potassium being significant at the 1 per cent level. Apart from this, these results further confirm the bovine characteristic of low potassium and high sodium content in red cells. The differences in the plasma electrolytes are small and not significant. The ratios of intracellular to extracellular sodium are similar to those reported by Bernstein[3], but the potassium ratios are slightly lower. Bernstein has shown that the potassium concentration ratio for red cells and blood plasma of the dog and cat is approximately 2, and implies that this small ratio may be explained on the basis of the Donnan equilibrium. The higher concentration ratios for potassium obtained by Bernstein for other species would, however, require extra energy for maintenance. The small differences in the potassium concentration ratios by Bernstein and those reported here could be explained by the differences in preparing the red cells for analysis. It has also been demonstrated that the potassium content of erythrocytes varies to a small degree with the level of dietary potassium[6].

Red cells from 35 ewes were similarly examined for potassium and sodium. Five of these animals were Hampshires, 20 Rambouillets and 10 Florida native ewes. The last-named represent a breed which has developed largely by natural selection in Florida since the time of early White settlement. Only a relatively small population of this breed exists, and it closely resembles a degenerate Cheviot, being much smaller and having an inferior coat when compared with that breed. Doubtless the Cheviot has figured in the background of this local breed; but it is also highly likely that other breeds have been intermingled at various times. Compared with imported breeds in the hot, humid environment of Central Florida these native sheep are much more hardy and more tolerant of the usual parasitic and disease conditions associated with sheep in moist places. The Florida native sheep is also characterized by a significantly higher blood hæmoglobin than imported breeds maintained under similar conditions[7]. Despite a well-defined seasonal pattern in blood hæmoglobin of sheep kept in Florida, the native always has a significantly higher concentration[7].

The blood of sheep has been classified into three different types by Kerr and Evans[2] on the basis of erythrocyte potassium. According to Evans, European sheep possess for the most part only two of these types, but Kerr found the third type well represented in Lebanese fat-tailed sheep. Evans has designated the two types common in European sheep as (*L K*), low potassium, and (*H K*) high red cell potassium. For the most part Evans has analysed whole blood to distinguish the potassium-levels, assuming that plasma potassium is fairly constant. While this is probably true, hæmatocrits vary, particularly in exotic livestock maintained in the lower latitudes. The chances of anæmia due to increased parasitic infestation, lower plane of nutrition or certain types of climatic stress are greatly increased, and it was therefore thought to be more reliable to conduct the analyses for red cell potassium directly on the erythrocytes.

Table 2. Incidence of the (*H K*) Blood Type in Three Breeds of Sheep maintained in Florida and a Comparison with Other Results

	5 Hampshires (per cent)	20 Rambouillets (per cent)	10 Florida natives (per cent)
H K	25	5	30
Evans (ref. 2)	28·7	0	(29·1 Cheviot)

The results for the few sheep examined are outlined in Table 2 and would not be expected necessarily to compare too favourably with other results, due to the small numbers. However, these results agree fairly well with those of Evans and co-workers, and it is interesting to note that the incidence of the *H K* blood type in Cheviots as found by Evans closely resembles that for Florida natives.

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PATHOLOGY

Mechanism of Increase of Endotoxin Susceptibility in Animals showing Delayed Hypersensitivity

In recent years considerable attention has been paid to the fact that an increase in susceptibility to the pyrogenic and shock-producing action of bacterial endotoxins could be demonstrated in animals showing delayed hypersensitivity[1].

Chinchilla rabbits were sensitized intravenously with live B.C.G. vaccine at a relatively high dose of 10–20 mgm. wet weight. Their condition was assessed repeatedly within 3–8 weeks after sensitization. We measured the variations of body-temperature during the day, skin hypersensitivity to 2, 0·4, 0·08, and 0·016 μgm. purified protein derivate and systemic hypersensitivity to 5 μgm. purified protein derivate or 20 μgm. heat-killed and twice washed B.C.G. bacilli injected intravenously. The increased degree of susceptibility to the pyrogenic effect of purified[2] *Salmonella paratyphi B* endotoxin was evaluated by determining the threshold doses capable of provoking standard fever reactions. In four independent experiments altogether 61 rabbits were used, and more than 30 normal unsensitized rabbits were used as controls. Only absolutely healthy animals were used, and allowance had to be made for the influence of the season of the year.

Controls generally gave the following results: no skin reactions to the tuberculin test doses, no fever reaction to 5–50 μgm. purified protein derivate and 20 μgm.–5 mgm. doses of killed B.C.G. given intravenously. Initial morning temperatures in the majority of the animals were 39° ± 0·2° C.; afternoon and evening temperatures remained unchanged or increased by less than 0·5 deg. C. Normal fever reaction was approximately + 1 deg. C. within the first 2 hr. after the injection of 1 ml. endotoxin in 10^{-8} dilution.

With test rabbits sensitized with the B.C.G. vaccine, the following results were obtained: different degree of skin hypersensitivity; positive systemic reaction in 62 per cent, negative in 38 per cent, of cases. In general, quite good correlation was observed between the results of the skin and systemic tuberculin reactions.

In 32 per cent of test rabbits the initial body-temperature rose by more than 0·5 deg. C., sometimes even by 1 deg. C., as compared with the average temperatures in controls. In approximately two-thirds of test animals the variations in day-temperature were greater than 0·5 deg. C.; in a quarter they exceeded 1 deg. C.

The pyrogenic endotoxin reaction in 45 per cent of test animals was similar to that in controls; a stronger reaction was noticed in 55 per cent of the animals. In these cases the standard fever reaction was produced by endotoxin doses 2–5 times lower than in control rabbits.

By comparison of all the results from individual animals, we were able to distinguish three well-defined groups among the test rabbits. The first group was characterized by a marked skin hypersensitivity, positive systemic reaction to tuberculin and killed B.C.G., normal initial body-temperature and daily temperature rhythm, and normal endotoxin reaction. A contrasting group consisted of animals exhibiting small skin hypersensitivity, negative systemic reaction, increased initial temperature as well as daily temperature variations, and increased endotoxin susceptibility. The third group gave intermediate results.

After B.C.G. sensitization, the development of specific hypersensitivity, accompanied by non-specific increase of endotoxin susceptibility, would be expected in all rabbits; yet this was not found. The increased susceptibility to endotoxins was not correlated with the degree of specific hypersensitivity, but quite otherwise. This excludes the hypothesis that the systemic tuberculin reaction is not a specific hypersensitivity reaction but that it should be considered as a reaction to traces of endotoxic substances present in tuberculin[3]. Moreover, this hypothesis is also refuted by the quantitative relations observed.

The increased endotoxin susceptibility might be attributed to the summation of irritations which arise in the reaction of hypersensitive cells with the sensitizing antigen persisting in the body. In the course of such a reaction, a partial desensitization of cells takes place and substances possessing pyrogenic and other effects are released[4]. In consequence, the state of unbalanced thermoregulation and increased susceptibility to endotoxins develops, together with a decrease in specific hypersensitivity. In animals where these reactions did not take place, a high degree of specific hypersensitivity is observed, without the non-specifically increased susceptibility. This hypothesis was confirmed in further experiments in which various sensitization doses were used[5]. Specific hypersensitivity and increased endotoxin susceptibility do not appear to be two parallel manifestations of delayed hypersensitivity.

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Absence of Thyroid Auto-antibody in Experimental Radiation Thyroiditis

THE association between thyroiditis and thyroid-specific autoantibody, both in man[1] and in animals[2], was established in 1956. There is still controversy as to whether the autoantibody is the cause or the consequence of the thyroiditis or whether, indeed, both thyroiditis and autoantibody are independent results of a single pathogenetic factor. However, it is widely believed that escape of thyroglobulin from the thyroid follicles initiates the development of autoantibody. It has been suggested that in experimental thyroiditis induced by radioactive iodine, the inflammation is a response to the liberation of colloid in the interfollicular stroma[3,4]. We have administered radioactive iodine to guinea pigs in order to determine whether autoantibody is produced in these circumstances. It is known that the guinea pig readily produces thyroid autoantibody when injected intradermally with thyroid extract[5].

Thirty-six adult female guinea pigs were divided into three groups, which were given single intraperitoneal injections of 0·2, 0·4 and 2·0 mc. of iodine-131 per kgm. body-weight, while three further animals received saline injections only. Eleven of the animals given the two lower doses received an additional injection of 8 mc. per kgm. 56 days later, in an attempt to stimulate a secondary antibody response when a primary response was undetectable by the technique used. Sera were tested for tanned cell agglutinating thyroid autoantibody[6] at intervals from one day to 229 days after injection. Animals were killed at intervals, and their thyroids examined histologically.

Those animals given the two lower doses showed no histological evidence of thyroiditis up to 28 days after injection. Animals given 2 mc. per kgm. showed no abnormality of the thyroid when examined from 1 to 5 days after injection, but thyroiditis was present from 7 days onwards. With a dosage of 8 mc. per kgm., there was mild thyroiditis when the animals were first examined at 4 days, progressing to extensive necrosis at 7 days. At this stage, the histological picture in those areas which were not necrotic was that of disruption of the follicles, with intermingling of epithelial cells, inflammatory cells and fibroblasts. With such disorganization of the gland there could be little doubt that colloid had been liberated from the follicles. In the later stages the inflammatory reaction subsided and there was considerable regeneration of thyroid epithelium. The high dose of iodine-131 required to produce thyroiditis is due to the low iodine uptake on diet *S.G.*1 (Oxoid).

In none of the 138 sera examined was there detectable autoantibody. Thus, in spite of the presumed escape of thyroglobulin from the follicles at an early stage in the experiment, the development and regression of thyroiditis occurred without production of autoantibody.

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BIOLOGY

Molecular Biology or Ultrastructural Biology?

IT is generally recognized that in the past decade or so we have seen great advances, which are of inestimable value for our understanding of fundamental biology, in the field which is concerned with proteins, nucleic acids, genes, viruses, antibodies, enzymes, etc. It is gratifying to note that several important bodies which are in a position to influence public policy in scientific matters have singled out this field as one requiring increased support.

It is unfortunate, however, that it seems to be becoming usual to refer to this connected series of topics by the name 'molecular biology'. This phrase is only marginally appropriate to any aspect of this area of biology, and is highly inappropriate to the greater part of it. Its most natural application would be to the biology of molecules of the conventional chemical kind, such as those involved in intermediate metabolism, and these are precisely not the types of entity with which the field under discussion deals. The smallest particles with which the new advances have to do may, indeed, be regarded as falling near the upper limit of size of the molecular, and, as for some proteins for example, are open to investigation by, more or less, conventional 'molecular' or chemical techniques. The greater part of the recent advances, however, have dealt with entities which are not defined in chemical terms; for example, genes, cistrons, mutational sites, ribosomes, antibodies, endoplasmic reticulum, and so on; and they have been studied predominantly by such non-chemical methods as those involved in viral and bacterial genetics, induced enzyme synthesis, complementation studies, immunology, ultra-centrifugation, electron microscopy, X-ray spectroscopy, and the like. An attempt to squeeze the whole of this wide front of advance under a title such as 'molecular biology', which places the emphasis on physical and chemical methods of investigation, brings with it the danger that the equally essential biological methods—which are in some respects even more penetrating than our existing chemical methods—will be unduly neglected.

The general characteristic of the field under discussion is, surely, that it is concerned with the analysis of entities, such as genes and ribosomes, which fall in the scale of magnitude lying between those visible with the light microscope and those accessible to conventional biochemical methods. A suitable name for it would be 'ultrastructural biology', which does not carry any particular implication as to the methods of investigation which should be employed. It is urged that this name should be used when the whole of this area of research is being referred to; the phrase 'molecular biology' could be retained, if desired, for that aspect of ultrastructural biology which deals with the chemical and physical study of the configuration of biologically important macromolecules.

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Influence of Uterine Position on the Response of the Mouse Embryo to the Teratogenic Effects of Hypervitaminosis-A

WHEN a teratogenic agent is administered to a number of pregnant mammals of a single species under identical conditions of timing and dosage, its effect on the development of the young is by no means uniform. In any individual litter, all, none or a proportion only of the young may be deformed. Even when a highly inbred strain is used, variations in the response to the teratogenic agent may be attributable to differences in the maternal genotype. Such differences cannot, however, be held responsible for the situation in which individual members of a litter develop normally while others are deformed. The differences in response of litter mates must derive either from individual variations in genetic make-up or from environmental factors as yet unanalysed. The present investigation was carried out in order to determine the influence of a single environmental factor, namely, the site of implantation, on the susceptibility of the mouse embryo to the teratogenic effects of hypervitaminosis-A.

Forty female mice of the Strong *A* line were used in these experiments. Brother–sister matings only were used and the onset of pregnancy was determined by the presence of a vaginal plug, the day on which

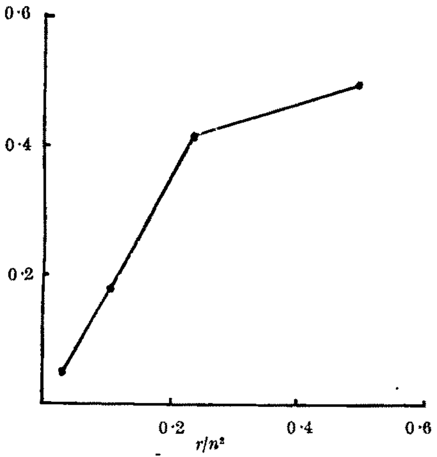

Fig. 1. P is probability of escaping deformity. r is ordinal position of a particular fœtus measured from the vaginal side. n is total number of young in horn. (Both r and n vary from 1 to 8)

the plug was detected being taken as day 1 of the pregnancy. Each animal received 8,000 i.u. vitamin A acetate dissolved in 'Tween 80' (ref. 1) by subcutaneous injection on day 11 of pregnancy. On day 18 of pregnancy all the animals were killed. Each fœtus was examined for the presence of cleft palate, and its position in the uterine horn was recorded.

Statistical analysis of the findings revealed two points of interest. First, fœtuses situated at the ovarian end of the uterine horn were found to be less subject to cleft palate than those at the vaginal end (Fig. 1). Secondly, the probability of cleft palate increased as the number of young in the horn increased.

There are a number of possible explanations for these findings. Since the critical period for the production of a deformity by hypervitaminosis-A is quite sharply defined, the stage of development of an embryo might be expected to have a considerable influence on its response to the teratogenic agent. It is interesting to note, therefore, the observations of Mossman[2] that in the mammalian bicornuate uterus the embryos implanted nearest the oviduct are always more advanced than those at the vaginal end. The weight of the embryo and the degree of development of the placenta at the time when vitamin A is administered are both factors which may play a part in determining whether a malformation does or does not occur. McLaren and Michie[3] found that in the mouse the embryos in the top half of the uterine horn were heavier than those in the lower half at 18½ days *post coitum*.

McLaren and Michie[4] suggested that the blood supply at the site of implantation might be an important factor in determining the occurrence of runting. It is generally agreed that fœtal weight in the mouse varies inversely with the number of young in the uterine horn. The more crowded the horn, the smaller are the individual fœtuses. This may have some bearing on the finding of the present investigation that the highest incidence of cleft palate was in the more crowded horns. It is hoped that estimations which are now being made of the levels of vitamin A in the placenta and liver in normal and malformed litter mates will throw light on the connexion established by the present series of experiments between uterine position and the degree of susceptibility to hypervitaminosis-A.

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Development of Eggs from a Hermaphroditic Sea Urchin

Reports of hermaphroditic sea urchins are not common. For *Arbacia punctulata* six examples are recorded over a period of twenty-five years by Harvey[1]. Since 1956, one report has been made[2].

In the course of work carried out in the summer of 1959, a hermaphroditic *A. punctulata* was found at Beaufort, North Carolina. On being subjected to an electric shock, two gonopores exuded sperm. Eggs were obtained from the remaining three pores. Each pore required individual stimulation in order for shedding to occur, and each gave viable gametes. The eggs were of normal size (80μ) for *Arbacia* collected in this area.

Since unfertilized eggs could be obtained, a study was made to compare the percentage fertilization and the rates of development of self-fertilized eggs and eggs fertilized by sperm from another male. As a control, additional eggs were reserved without sperm. All work was carried out at 23° C.

No indication of fertilization or cleavage was found in the controls. More than 85 per cent fertilization was obtained in both the self-fertilized and cross-fertilized egg batches. All stages of development up to the pluteus stage were directly comparable in both groups, and occurred at the normal rate for this temperature. No abnormal morphology was found.

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Disintegrated Spermatozoa from the Epididymis

In the course of an investigation of the response of spermatozoa in the epididymis of rabbits to the effects of artificial crytorchidism, it was noted that in the controls (that is, 12 normal epididymides on which no operative interference had been undertaken) a remarkable number of immotile decapitated spermatozoa was present in samples withdrawn from regions of the tubule proximal to the cauda epididymidis. Spermatozoa of this kind have been previously referred to as 'disintegrated spermatozoa'[1,2] and presumably indicate a process of degeneration.

In the present work the morphology of spermatozoa was examined in saline-diluted squash preparations under the phase-contrast microscope and in whole specimens. Quantitative estimates of particular characteristics were made from smears after staining with aqueous nigrosin eosin differential stain[3] in order that an estimate of the proportion of 'dead' (eosinophilic) spermatozoa also might be made. In view of this finding, spermatozoa from epididymides of some other species, namely the bull (6 specimens), the goat (2 specimens), the dog (4 specimens) and the cat (3 specimens) were also examined.

In all these cases a proportion of spermatozoa withdrawn from the vas deferens appeared to be degenerate; but it was possible to discern more evidence of disintegration in samples from the body of the epididymis. A variable percentage of dead and decapitated spermatozoa was observed in smears of samples taken from the head of the epididymis and from the initial segment[4]. These percentages were, however, generally lower than those in samples from the body of the epididymis. A relative paucity of degenerate forms from the tail of the epididymis in most specimens was typical. In bovine specimens the overall degree of disintegration of spermatozoa from all levels of the epididymis was comparatively small, but more eosinophilic spermatozoa were present in samples from the body of the epididymis than from any other level, which confirms an earlier report by Ortavant[5]. In both ruminants, differences between the incidence of disintegrated spermatozoa from the body of the epididymis and the cauda epididymidis were not conspicuous. Differences in mean values obtained for the incidence of decapitated spermatozoa from these two areas were, for the bull 8 per cent, and for the goat 16 per cent. In the dog, cat and rabbit, however, the contrast between samples from these two areas was striking, there being many more disintegrated spermatozoa from the body of the epididymis. Differences in mean values obtained between samples from the two parts in each of these species respectively were, 23, 66 and 49 per cent for decapitated spermatozoa, and 36, 63 and 61 per cent for eosinophilic spermatozoa. Despite considerable ranges of variation, differences between estimates from the two levels in these species were plainly significant.

From the observations it is not clear what proportion of disintegrated spermatozoa was due to artefact. The occurrence of disintegrated forms in whole samples where neither staining nor smearing was involved indicates that a number of spermatozoa were degenerate within the intact tubule of the epididymis, and thus some degree of wastage of spermatozoa in proximal regions of the epididymis is apparent. But in stained smears, it is conceivable that separation of the heads in several spermatozoa might have occurred *in vitro* and might thereby represent partly a variation in fragility of the cells at different levels of the tubule. In this connexion, it has been reported that decapitation of spermatozoa in ejaculated semen can be produced by certain methods adopted for the staining and clearing of smears[6]. Alternatively, differences in the viscosity of samples could interfere with the free movement of spermatozoa across the slide and give rise to an increased incidence of decapitated spermatozoa in smeared preparations. Subjectively, the milieu of spermatozoa from the tail of the epididymis appears to differ from that in the more proximal regions, especially in rabbits, and this might be related to possible differences in the effects of handling. Such variation in medium might also allow disintegrated spermatozoa to be obscured in smears of samples taken from the cauda epididymidis due to the effects of a specific distribution of degenerate forms. However, that the highest proportion of disintegrated spermatozoa was found throughout in samples from the body of the epididymis is of interest in that it suggests at least some characteristic quality of the tubular contents at this level. Experiments are now in progress to determine more precisely the inference to be drawn from this surprisingly regular pattern in the incidence of disintegrated spermatozoa from different areas of the epididymis.

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Tinsel-type Structure in Flagella of Zoospores associated with Cabbage Clubroot Tissue

PARTIALLY disintegrated clubbed roots of cabbage grown in the greenhouse in soil infested with *Plasmodiophora brassicae* Wor. were stored in a frozen condition for several weeks. They were then macerated in distilled water and much of the coarse debris was removed by filtration of the suspension through several layers of cheesecloth. This was followed by centrifugation at 4,000 r.p.m. for 15 min. and resuspension in sterile distilled water. Centrifugation was repeated three times and after the final one spores were suspended in the nutrient solution described by Macfarlane[1], or in autoclaved tap water in sterile 10 × 75 mm. culture tubes plugged with cotton. Tubes were incubated in the dark at 24° C. or at room temperature. Droplets were examined periodically under a Bausch and Lomb phase-contrast microscope for the presence of motile cells.

The occurrence of flagellated cells in such suspensions was unpredictable, but only one type of zoospore was encountered consistently in preparations from clubbed roots from three sources. Observations indicated that they were anteriorly biflagellate and markedly heterokont. The slightly recurved longer flagellum extended in front of the zoospore as it moved in droplets of the suspension. A much shorter flagellum was usually curved back along the body of the zoospore or extended forward at an angle with the longer flagellum. The zoospores rotated on a longitudinal axis as they moved forward freely and they varied from elongate to pyriform or globose in shape. Active zoospores measured $3 \times 3\mu$–$3{\cdot}9 \times 4{\cdot}8\mu$. A pulsating vacuole was observed in most zoospores. Emergence of zoospores from germinating resting spores was not observed.

Permanent mounts were made using a modified Löffler's stain[2]. Droplets containing motile zoospores were placed on clean glass slides, and the zoospores were killed and fixed by exposure to the fumes of an aqueous solution of 2 per cent osmium tetroxide for 30 sec. The droplets were allowed to dry in a desiccator. Several drops of mordant were filtered on to the dried preparation on the slide, which was heated gently for 45 sec. The mordant consisted of 20 per cent tannic acid, 10 parts; saturated aqueous ferrous sulphate solution, 5 parts; saturated alcoholic basic fuchsin solution, 1 part. The preparation was washed by dipping the slide in a large volume of distilled water. Several drops of stain were then added to the surface of the slide, which was heated gently for 1 min. The stain consisted of a 4 per cent solution of crystal violet in aniline water (2 parts aniline to 100 of water). Excess stain was removed by dipping the slide in several changes of distilled water. After drying in a desiccator, the preparation was mounted in Canada balsam with a No. 1 cover-slip.

Examination of stained preparations with a phase-contrast microscope indicated that the longer flagellum had a rather blunt end and was of the tinsel-type. The shorter flagellum was rounded at the end and bore no appendages. Stained zoospores measured 2·5–4 × 4μ. The long flagellum measured 6–8μ, while the short one measured approximately 2μ in length.

In the preparation of samples for the electron microscope, very small droplets of suspension containing active zoospores were placed on collodion-coated electron-microscope grids, exposed to fumes of 2 per cent osmium tetroxide for 30 sec., and allowed to dry in air. Grids were shadowed with uranium and examined with an *RCA*-type *EMU*2 electron microscope. Examination of such preparations confirmed the nature of the flagella indicated by observations of the stained preparations. A photograph of a typical zoospore taken of an electron microscope preparation is illustrated in Fig. 1 (× *c*. 15,000).

The resting spore suspensions in which these zoospores were observed contained bacteria in varying concentrations, and occasionally flagellate cells other than those described were found. None of these possessed blunt-ended flagella. In one preparation, zoospores were observed which possessed a long flagellum trailing behind the zoospore as it moved. This flagellum was of the whiplash type, and the tailpiece was observed to vary in length as the zoospore advanced. Ellison[3] reported that the two heterokont flagella of primary zoospores of *P. brassicae* appeared blunt-ended without whiplashes or tinsels when stained by the modified Löffler technique. Kole[4] reported that secondary zoospores of *P. brassicae* moved with the longer flagellum trailing behind the zoospore. Zoospores stained by the modified Löffler method showed an apparent whiplash structure of the longer flagellum. The zoospores observed in preparations described here are believed to have developed by germination of resting spores of *P. brassicae*. It would appear from this work that the longer flagellum of the primary zoospore is typically of the tinsel type.

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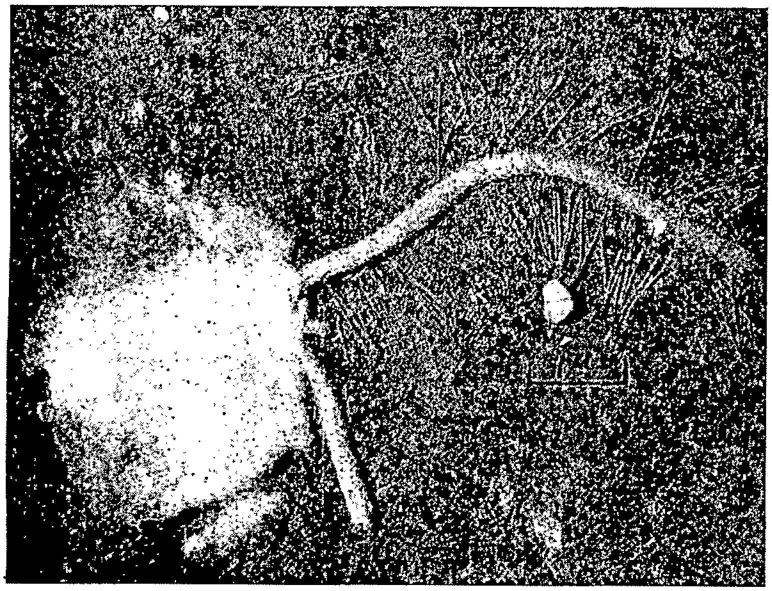

Fig. 1. Tinsel-type structure in flagella of zoospores associated with cabbage clubroot tissue

Substrate Specificity of the *Myrothecium* Ascorbic Acid Oxidase

THE substrate specificity of the copper-containing ascorbic acid oxidase of higher plants was studied principally by Zilva *et al.*[1,2]. More recently, Mandels[3,4] discovered an ascorbic acid oxidase in spores of *Myrothecium verrucaria* (Alb. and Schw.) Ditm. ex Fr. which was termed 'atypical' chiefly because it differed from the classical copper-enzyme in substrate specificity and in its resistance to metal enzyme inhibitors. It did not oxidize D-glucoascorbate or D-araboascorbate, and the latter inhibited oxidation of L-ascorbate. The mycelium of *M. verrucaria* also contains an atypical ascorbic acid oxidase, but with properties distinctly different from those of the spore-enzyme as well as of the copper-enzyme[5]. This communication describes the differences in substrate specificities.

The fungal oxidases were extracted from washed mycelial pellets or spores[6] by grinding in an ice bath with a Potter–Elvehjem homogenizer using 100-mesh glass and a *p*H 6·3, 0·01 *M* phosphate–0·005 *M* citrate buffer. This grinding was repeated twice following centrifugation at 0° C. for 10 min. at 1,000*g*. The pooled, cell-free extracts then were centrifuged at 0° C. for 30–40 min. at 20,000*g*. The resulting supernatant was diluted with buffer and stored frozen. Before use, the mycelial extract was acidified with 1 *N* hydrochloric acid to *p*H 4·5, the optimum for enzymatic activity. These fungal preparations showed no cytochrome oxidase or polyphenol oxidase activity. They had high catalase but little or no peroxidase activity. This evidence and the response to inhibitors of iron- and copper-enzymes[3,5] indicated that only ascorbic acid oxidase was involved in the activities measured. The copper-oxidase used was a crude cucumber juice prepared according to Johnson and Zilva[1].

Activity measurements were made at 30° C. in air by standard Warburg procedure at 2 ml. reaction volume, except with the rarer ascorbate analogues when 5-ml. flasks and 0·5-ml. volumes were used. The reaction buffers were 0·034 *M* phosphate–0·017 *M* citrate at *p*H 4·5 for the mycelial oxidase and *p*H 6·0 for the other two. The substrate concentrations used, 0·01–0·02 *M*, were sufficient for maximal velocity on the basis of previous reports[2,4] on the spore and cucumber enzymes and of work in this laboratory[5] on the mycelial enzyme. The concentration of the old samples of ascorbate analogues was based on amount of reduced form, determined by titration with 2,6-dichlorophenol indophenol or enzymatic oxidation with the copper-enzyme. Sufficient glutathione (30–60 μgm.) was added to all reaction mixtures to reduce autoxidation, and suitable autoxidation controls were run when necessary with certain analogues. Activities were based on initial reaction velocities since both fungal enzymes exhibited reaction inactivation[3,5], and rates of oxidation of some substrates also declined after 15–20 min. with the cucumber enzyme. It should be noted that the precision of the assay, particularly on the smaller scale and where autoxidation corrections were necessary, did not establish activity with analogues below perhaps 4–5 per cent of that with L-ascorbate. Thus 0 per cent in Table 1 may represent a low level of activity. The stoichiometry of analogue oxidation, like that of L-ascorbate, was one-half of oxygen uptake per mole of substrate.

Table 1. SUBSTRATE SPECIFICITY OF ASCORBIC ACID OXIDASE OF SPORES AND MYCELIUM OF *M. verrucaria* AND OF CUCUMBER

Substrate	Relative enzyme activity (per cent)* Spore	Mycelium	Cucumber
L-Ascorbate (I)	100	100	100
6-Deoxy-L-ascorbate (II)	0	50	113
D-Xyloascorbate (III)	0	0	65
D-Araboascorbate (IV)	0	38–88	107
L-Araboascorbate (V)	0	0	34
L-Glucoascorbate (VI)	0	90	91
D-Glucoascorbate (VII)	0	0	19
Imino-D-glucoascorbate (VIII)	0	0	0
L-Rhamnoascorbate (IX)	0	98	97
L-Galactoascorbate (X)	0	100	97
D-Galactoascorbate (XI)	0	0	27
Imino-D-galactoascorbate (XII)	0	0	0
L-Guloascorbate (XIII)	0	0	35
Imino-L-guloascorbate (XIV)	0	0	0
D-Erythroascorbate (XV)	0	36–90	100
5,6-Diacetyl-L-ascorbate (XVI)	0	50	83
L-Erythroascorbate (XVII)	0	23	46
Hydroxytetronate (XVIII)	0	88	104
Reductate (XIX)	0	84	62
Methyl reductate (XX)	0	77	46
Reductone (XXI)	0	55	52
Dihydroxyfumarate (XXII)	0	0	0

* Average values except for ranges in case of mycelial enzyme with IV and XV, where activity varied significantly among enzyme preparations.

Of the 22 compounds tested only L-ascorbate (L-xyloascorbate) was oxidized at a significant rate by the spore enzyme (Table 1). Not only are Mandels's results for D-araboascorbate and D-glucoascorbate confirmed, but also it is evident that this enzyme has a very high degree of specificity. Any alteration in the molecule represented in this collection of analogues eliminated activity: (1) change in C-4 configuration, (2) change in C-5 configuration, (3) change in chain length, (4) substitution of hydrogen for hydroxyl on C-6, (5) modification or elimination of the ring, or (6) acetylation of the C-5 and C-6 hydroxyls. The inhibition of L-ascorbate oxidation by D-araboascorbate reported by Mandels[4] also was confirmed. Limited materials permitted only a single test of the other analogues, but it appeared that only 6-deoxy-L-ascorbate and L-galactoascorbate were inhibitory.

The mycelial enzyme was clearly less specific for the L-ascorbate structure (Table 1). Like the spore enzyme, it did not oxidize any of the 6- or 7-carbon analogues (III, V, VII, XI, XIII) with the opposite C-4 (ring) configuration to L-ascorbate. However, it did have some activity toward the 5-carbon analogue (XVII) with the opposite configuration. Among analogues with the same ring configuration, the 7-carbon compounds (VI, IX, X) were oxidized at about the same rate as L-ascorbate, while the rates with the 6-carbon compound (IV) were lower and variable. Substitution of a hydrogen for the hydroxyl group on C-6 of ascorbate (II) or acetylation of the C-5 and C-6 hydroxyls reduced activity to one-half. Removing the side chain (XVIII) or replacing the ring oxygen with a methylene group (XIX, XX) caused only a small reduction in activity. Even the 3-carbon analogue (XXI), with perhaps the simplest possible structure, was oxidized.

Presence of significant amounts of the spore-type enzyme in the mycelial extracts is unlikely, since several of the analogues were oxidized at about the same rate as L-ascorbate. Furthermore, low concentrations (0·0007 *M*) of D-araboascorbate had no effect on the rate of L-ascorbate oxidation by the mycelial enzyme but completely inhibited the spore enzyme.

The results in Table 1 with the cucumber extract were in close agreement with published values[1,2] for the same substrates and include data for a number of compounds apparently not tested before with the copper-oxidase (II, VIII, IX, XII, XIII, XIV, XVII XVIII XX). Analogues with the same ring configuration as L-ascorbate (II, IV, VI, IX, X) were oxidized at about the same rate. Presence of side chain or chain length (VI, XVIII), hydroxyl configuration at C-5 or C-6 (IV, VI, X), and lack of primary hydroxyl at C-6 (II) or C-7 (IX) had no apparent influence on activity. All analogues with the opposite ring configuration (III, V, VII, XI, XIII, XVII) were oxidized, but at a slower rate than L-ascorbate. None of the three iminoascorbates was oxidized, adding support to Dodds's conclusion[7] that a free carbonyl group is essential for activity. Compounds without the lactone ring (XIX, XX, XXI) were oxidized at lower rates, and the transdienol (XXII)[8] was inactive.

Nothing is known of the nature of the active sites of the two fungal enzymes, beyond the fact that these enzymes apparently are not typical copper-oxidases[3–5]. Their specificity patterns, however, give some indication of the substrate groups involved and of the stereochemistry of attachment. The spore oxidase would seem to require a multipoint attachment involving the hydroxyl groups on C-5 and C-6, the dienol group (since it is involved in electron transfer), and possibly the carbonyl group. Lack of a suitable derivative, such as imino-L-ascorbate, has prevented a test of this possibility, but a free carbonyl is required for the copper-enzyme. Such an attachment would be consistent with the absolute stereochemical specificity observed, which involves asymmetric centres at both C-4 and C-5. The highly specific nature of the binding site is also evident from the loss of activity on addition to C-6 of a methyl (IX) or hydroxymethyl group (VI), presumably by steric hindrance. With the mycelial oxidase, substrate structural effects were more quantitative than qualitative. A side chain was not required for attachment or activation, but its configuration (C-4) with respect to the ring was critical. With the unfavourable configuration, only the structure (XVII) with the smallest side chain permitted any activity. Furthermore, with the favourable side chain configuration both the orientation of the C-5 hydroxyl and the presence of the C-6 hydroxyl influenced activity. Thus, the chain hydroxyls seem to participate in binding or to affect the orientation of the substrate at the reactive site.

A clear understanding of the difference in specificity between the spore and mycelial oxidases will require chemical characterization of the prosthetic group(s) and/or the reactive sites. This remarkable change in specificity of an enzyme between two morphological forms of the same organism provides an unusual opportunity to study the factors affecting enzyme specificity.

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'Antibiotic Effects' in Plant Communities

FAILURE of *Araucaria cunninghamii* Sweet. to regenerate in secondary rain-forest dominated by *Backhousia angustifolia* F. Muell. has been observed near Maryborough, Queensland. A plausible explanation of this observation appeared to lie in the widely discussed possibility[1] that a plant may interfere with the establishment of adjacent species in a natural community by releasing 'antibiotics', either from living roots or from decomposing surface debris.

On investigating this hypothesis it has been shown in laboratory tests that substrates, prepared from filter paper treated with *Backhousia* leaf oil, hinder the germination of cress and wheat. Further tests under similar conditions have revealed that dehydroangustione (in this case the major constituent of the oil[2]) hinders the germination of *Araucaria*, the effect varying with the concentration of dehydroangustione in the substrate. Thus, the number of radicles which emerged on substrates containing low (0·1–0·5 per cent by weight) and high (7 per cent) concentrations of dehydroangustione did not differ significantly from the control, but intermediate concentrations (1–5 per cent) caused a significant decrease ($P < 0{\cdot}05$). Normal germination occurred and healthy seedlings developed on substrates containing 0–0·2 per cent dehydroangustione, but toxic symptoms appeared in all other treatments either during germination (1–7 per cent) or after the emergence of the whole seedling (0·5 per cent).

In an attempt to test the ecological implications of this finding, a small area of secondary rain-forest containing a high proportion of *Backhousia* was chosen. Four plots were then selected at random within this area and germination tests (using 200 *Araucaria* seeds per replicate) were set out on both the natural litter and on a litter enriched with freshly gathered *Backhousia* leaves which contained 5·4 per cent of dehydroangustione. A watering treatment was also included in the field test, which was carried out when germination normally occurs in this locality.

If the dehydroangustione present in *Backhousia* litter normally has an adverse effect on the germination of *Araucaria* in the field, it would be expected that any such 'antibiotic effect' would be enhanced under these conditions.

However, germination was virtually complete 37 days after sowing and statistical analysis of the results (Table 1), using the 'arc sine$\sqrt{p}$' transformation, revealed that there was a highly significant *increase* ($P < 0{\cdot}001$) in germination on the litter containing fresh *Backhousia* leaves, compared with that on the natural litter. Moreover, germination on both watered treatments was significantly higher ($P < 0{\cdot}01$) than on the corresponding unwatered treatments. At the conclusion of the experiment, both the unwatered, and watered, enriched litters were recovered from the field. Chemical analysis then revealed that these contained 1·6 and 2·2 per cent by weight, respectively, of dehydroangustione; that is, concentrations within the range found to hinder germination of *Araucaria* in laboratory tests.

This evidence does not eliminate the possibility that in certain circumstances germination of *Araucaria* may be inhibited by the dehydroangustione present in *Backhousia* litter. However, these circumstances have yet to be defined, and an investigation of the microclimatic factors affecting germination and early survival of seedlings in the field appears to be a more profitable line of inquiry.

Table 1

Substrate	Watering treatment	Percentage germination of *Araucaria* after 37 days				
		Replicate				Mean
		(*a*)	(*b*)	(*c*)	(*d*)	
Normal litter	–	9	12	4	14	10*
	+	12	20	6	18	14
Enriched litter	–	20	18	10	19	17
	+	29	25	24	28	27

* In a nearby area the mean germination on an unwatered litter not derived from *Backhousia* was also 10 per cent.

In similar work, Went[3] suggested that the failure of some shrubs growing in the Southern Californian desert to harbour a population of certain annual species is due to the production of toxic substances by the shrubs concerned. This hypothesis was supported by Gray and Bonner[4], who isolated 3-acetyl-6-methoxybenzaldehyde from the leaves of one such shrub (*Encelia farinosa* Gray) and showed that this substance is a potent growth inhibitor in laboratory tests. However, Muller and Muller[5] criticized this interpretation of the field observations on the grounds that extracts of *Franseria dumosa* Gray, which does harbour these annuals, are even more toxic in laboratory tests than extracts of *Encelia*. These authors put forward several hypotheses to account for the failure of the toxic substances to reach an injurious concentration under natural conditions, and suggested that the distribution of these annual herbs may be correlated with the superior water-holding capacity of the mound of organic debris accumulated by *Franseria*, but not by *Encelia*.

The apparently contradictory results of laboratory and field tests in this type of investigation may be due to the fact that all the toxic substances present in the substrate are available to the test species during the laboratory experiment when extracts or milled plant material are used. In our opinion such substrates are not comparable with plant litter, for it has been found in a replicated experiment that *Araucaria* seeds fail to germinate on substrates of milled *Backhousia* leaves enclosed in Petri dishes, but under the same conditions germination is not significantly reduced (although it occurs more slowly than the controls) on substrates of the same intact leaves containing 4·1 per cent of dehydroangustione.

In our opinion, the experiments outlined above emphasize the dangers of arguing, without further experimentation in the field, that the 'antibiotic effects' demonstrated in simple laboratory tests operate in natural plant communities.

Full details of this work will be published elsewhere.

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Sorbitol Metabolism in Apple Fruits

Sorbitol is a polyhydric alcohol found commonly in the Rosaceae: in apples it can constitute a large proportion of the dry matter. Little is known, however, of its metabolism in higher plants. Edson[1] referred to a sorbitol dehydrogenase in animals and micro-organisms and mentioned a similar occurrence in plants.

I have been able to show that, when an aqueous extract of an acetone powder preparation from the fruit cortex of apples (var. Miller's Seedling) was treated with sorbitol in the presence of boiled yeast extract, an acid-labile non-reducing sugar was formed. The sugar was probably sucrose and the normal yield was 1–2 mgm. from 10 mgm. sorbitol (at about 20° C.), with due allowance for the sucrose in the yeast extract.

These results were repeatable up to two months after preparation of the acetone powder. It then became inactive, and more detailed examination of the enzyme system involved in this reaction will be made when a further crop of fruit is available.

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BACTERIOLOGY

Association of a Steroid and a Pigment with a Diffusible Fruiting Factor in *Myxococcus virescens*

A diffusible factor inducing fruiting-body formation in Myxobacteria has been demonstrated by Lev[1]. In an attempt to identify further such a factor in *Myxococcus virescens*, an aqueous extract of fruiting-bodies was concentrated by drying from the frozen state.

Using a medium which would permit the swarming of vegetative cells, but not the formation of fruiting-bodies, under normal conditions, consisting of 1/100 concentration routine meat-extract agar, with 3 per cent non-nutrient agar and 0·001 per cent sodium chloride, four circular wells were made on a plate supporting a film of vegetative cells, and this was incubated for a further 7 days to obviate any effects of mechanical disturbance. A few drops of extract were placed in two of the wells, with distilled water in the two control wells. After 8 days incubation at 30° C., small fruiting-bodies could be discerned around the wells containing the extract.

Comparison of descending chromatograms of the two extracts showed the existence of a spot on the chromatogram of the fruiting-body extract which was absent from that of the vegetative cells. This spot was associated with the pigment of the fruiting-bodies; it reacted with Liebermann's reagent and is therefore presumed to contain a steroid.

A further experiment in which actual portions of the chromatogram paper were laid on the plate showed that fruiting-body formation was induced in the vicinity of the pigmented spot.

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Accumulation of Indole Compounds in Cultures of *Chromobacterium violaceum*

Substances yielding a typical indole test with *p*-dimethylaminobenzaldehyde (Ehrlich reagent) were recently observed in peptone broth cultures of gelatinous strains of *Chromobacterium violaceum*[1]. The nature and the origin of the substances giving the pink-red colour with Ehrlich reagent was of some interest because the species has been reported to lack the enzyme tryptophanase[2] and the genus as a whole is generally considered to be indole-negative[3], which implies inability to degrade tryptophan to indole.

Employing the Ehrlich reagent and technique described by Skerman[4], several cultures of gelatinous *Chromobacteria* were tested for indoles after growth for 5 days at 30° C. in several media. The results shown in Table 1 indicate that the largest accumulation of indoles occurred in 'Bacto' peptone broth containing glucose and incubated under aerobic conditions. 'Bacto' tryptone, which contains more tryptophan than peptone[5], did not allow indole accumulation. When 0·5 per cent (w/v) tryptophan was added to peptone broth no increase in the amount of Ehrlich-positive substances was observed. Synthetic glucose-ammonium salts medium with or without added yeast extract (0·02 per cent) invariably failed

Table 1. FORMATION OF INDOLE COMPOUNDS BY GELATINOUS STRAINS OF *Chromobacterium violaceum*

	Relative intensity of the Ehrlich reaction					
	32		46		11	
Medium	Shaken	Static	Shaken	Static	Shaken	Static
1 per cent peptone	+ + +	+	+ +	+ +	+ + +	+
1 per cent peptone, 1 per cent glucose	+ + + +	+ +	+ + + +	+ +	+ + + +	+ +
1 per cent peptone, 0·1 per cent tryptophan	+ + +	+	+ + +	+	+ + +	+
1 per cent tryptone	–	–	–	–	–	–
1 per cent tryptone, 1 per cent glucose	–	–	–	–	–	–

The cultures were incubated at 30° C. for 5 days in flasks mounted on a rotary shaker (shaken) or in deep tubes (static). Qualitative tests for indoles with Ehrlich reagent produced various intensities of pink colour (+)which were estimated visually. A negative sign indicates that no pink colour was observed.

to support the accumulation of indoles though good growth and pigmentation were usually observed. These results suggest that an abundant supply of amino-acids is a requisite for indole compound accumulation.

Chromatographic examination of glucose–peptone cultures, from which cells had been removed, revealed two substances giving a pink-red colour with Ehrlich reagent. Table 2 shows the R_F values of the substances in three solvent systems and the colour reactions produced when developed chromatograms were treated with reagents commonly used for the identification of indoles. Unless otherwise indicated, the methods and reagents employed were those described by Jepson[6].

Table 2. CHROMATOGRAPHY OF GLUCOSE–PEPTONE CULTURES OF STRAIN 32

	R_F values in			Ultra-violet fluorescence	Colour reactions with				
	A	*B*	*C*		I	II	III	IV	V
Unknown 1	0·90	0·25	0·52	+	o—pk→b	v	r—bn	r	none
Unknown 2	0·72	0·19	0·25	+	pk→b	v	p	r	none
Indole-3-acetic acid	0·92	0·45	0·65	–	pk—p→vg	none	none	none	none
5 OH-indole acetic	0·82	0·25	0·34	+	pk→b	v	r—bn	r	none
Tryptophan	0·24	0·32	0·31	–	v—p	none	none	none	none
5 OH tryptophan	0·56	0·17	0·18	+	lt.b→b	v	r—bn	pk	none

Amounts of 10–20 μl. of cell-free culture fluid were spotted on Whatman No. 1 filter paper strips, and developed in the following solvents : *A*, butanol/acetic acid/water (4 : 1 : 5), *B*, *iso*propanol/ammonia (10 : 1), *C*, butanol/ethanol/water (4 : 1 : 1). Developed and dried strips were sprayed with I Ehrlich, II 1-nitroso-2-naphthol, III sulphanilic acid, IV anisidine, V 2,4-dinitrophenylhydrazine reagent. Colour reactions observed : orange (o), pink (pk), blue (b), purple (p), violet (v), grey (g), red (r), brown (bn).

Chromatography of the unknown substances with a number of known indole containing compounds indicated that the former were not indole, indole-3-aldehyde, indole-3-acetic acid, indole-3-propionic acid, indole-3-butyric acid, tryptophan, 5-hydroxytryptophan, 5-hydroxyindole-3-acetic acid or tryptamine.

Ehrlich reagent is not entirely specific for the indole nucleus, but because of the evidence obtained with other spray reagents, the known substances are believed to be indoles, more exactly hydroxyindoles. Udenfriend[7] has found that 1-nitroso-2-naphthol in the presence of weak acid and a trace of nitrite yields a violet colour with hydroxyindoles but not other indoles. The sulphanilic acid reagent described by Jepson[6] reacts not only with some phenolic acids but also with 4-, 5- or 6-hydroxyindole compounds. The latter also react strongly with anisidine reagent[8] to give an immediate, intense red-brown colour which is also true of the unknown indoles reported here. Phenolic acids do not apparently give the same immediate colour reactions with Ehrlich reagent under the conditions employed for indole detection. A negative test was obtained with 2,4-dinitrophenylhydrazine reagent which indicated the absence of keto- or aldehyde-groups.

Chromatography of peptone broth containing added tryptophan confirmed the observations described above, that is, it neither increased nor decreased the quantity of the two unknown indoles observed in peptone–glucose broth. Tryptophan did, however, give rise to several substances fluorescent in the ultra-violet which gave yellow colours with Ehrlich reagent. It seems clear that tryptophan is not the source of the unknown hydroxyindole compounds.

Chromobacterium species characteristically produce a purple pigment violacein, which has been shown to be a hydroxyindole-derivative[9]. DeMoss and Evans[10] have shown, using strain 553 (American Type Culture Collection), that pigmentation is dependent on L-tryptophan ; in fact they have demonstrated that violacein was produced from the amino-acid by non-proliferating cells, when used as a sole carbon compound in the reaction mixture. These workers and others[11] have shown the hydroxylation of tryptophan to yield 5-hydroxytryptophan to be a major reaction for the amino-acid in this organism and probably is the source of the precursors of violacein.

In the present work L-tryptophan seemed to stimulate neither indole accumulation nor formation of pigment under the conditions used. These observations suggest that the gelatinous strains possess a pathway for synthesis of hydroxy indole compounds and pigment precursors not observed in non-gelatinous strain 553 or other strains dependent on tryptophan for pigment synthesis.

Identification of the hydroxyindoles and their relationship to pigment formation in the gelatinous strains is being investigated further.

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VIROLOGY

Rous Sarcoma Virus associated with Parthenogenesis in Turkey Eggs

SPONTANEOUSLY occurring parthenogenesis in unfertilized eggs of the domestic turkey, *Meleagris gallopavo* Linnaeus, was first noted[1] at the Agricultural Research Center, Beltsville, Maryland, in 1952. Unselected Beltsville Small White turkey hens, as virgins, produced eggs of which 16 per cent showed parthenogenetic development following an incubation period of 9–10 days. This development consisted chiefly of a thickened sheet of unorganized and often multinucleated cells which covered varying areas of yolk surfaces. Usually, these cells did not attain the degree of organization necessary for blood formation and only on rare occasions were embryos noted. Through a system of selective breeding a strain of Beltsville Small White turkeys has been developed which regularly, as virgins, produce eggs of which 40 per cent can be expected to show some degree of parthenogenetic development. The greatest increase, by percentage, has been in the increased number of eggs showing a high degree of organized development. In 1959, embryos developed in 342 of the 2,926 eggs tested. This represented 11·7 per cent embryos, an increase amounting to 57 times that observed in 1952. Included were 12 poults which were helped from the shell and survived for some time (Olsen[2]).

In 1956, it was reported[3] that the incidence of parthenogenesis in turkey and chicken eggs could be significantly increased with an embryo-propagated live virus vaccine used for the control of the disease, fowl pox. Subsequent tests, at this Center, have confirmed these original observations. In 1958, Stolk[4] reported finding parthenogenesis in viviparous toothcarps following a local infection with the *Ichthyophonus* parasite, the phycomycete *Ichthyophonus hoferi* Plehn-Mulsow.

Work has since been extended to include other virus preparations, using unselected turkeys and observing the incidence of parthenogenesis in their eggs following inoculation. The present communication deals with the Rous sarcoma virus. Thirty unselected Beltsville Small White turkeys, housed in individual cages, were involved in the tests. Eighteen were inoculated subcutaneously in the wing webs with 0·1 gm. equivalent of partially purified Rous sarcoma virus (1 gm. equivalent represents the quantity of virus extracted from 1 gm. of tumour tissue). (I wish to thank Dr. Ray Bryan, U.S. Public Health Service, Bethesda, for the virus extract from chicken tumours.) The other 12 birds, full sisters of those of the treated group, served as uninoculated controls. Eggs from both groups of females were incubated for 10 days before being examined macroscopically for indications of parthenogenetic development. The percentage incidence of parthenogenesis observed over successive 6-day intervals in eggs laid by treated and control birds is presented in Table 1. During the 90-day test period following inoculation, 714 eggs were produced by the 18 treated turkeys, 527 by the 12 control birds. Of all eggs produced by the treated birds, 25·4 per cent showed parthenogenetic development, while 15·9 per cent of the eggs from the control group were so classified. The difference, when tested by the analysis of variance using weighted squares of means, was found to be highly significant at the 1 per cent level. (Thanks are due to Mrs. R. R. Taylor of this Center for this analysis of data.)

Table 1. Percentage Incidence of Parthenogenetic Development observed in Eggs of Beltsville Small White Turkeys following Inoculation with Rous Sarcoma Virus

No. of days following inoculation	Treated birds		Controls (untreated)	
	Eggs tested	Percentage parthenogenesis	Eggs tested	Percentage parthenogenesis
22–27	72	11·1	53	15·1
28–33	69	22·6	52	13·5
34–39	59	20·3	42	16·7
40–45	65	32·3	46	15·2
46–51	65	26·1	50	16·0
52–57	67	35·8	49	10·2
58–63	64	28·6	46	15·2
64–69	74	21·6	50	16·0
70–75	53	15·1	46	26·1
76–81	56	33·9	42	16·7
82–90	70	28·6	51	15·7
Totals and averages	714	25·4	527	15·9

The results presented clearly indicate that Rous sarcoma virus tends to increase the incidence of macroscopically observable development in unfertilized turkey eggs.

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Bromegrass Mosaic Virus: a Virus containing an Unusually Small Ribonucleic Acid

CHEMICAL and physical properties of viruses are being investigated in several laboratories in order to obtain structural information which relates to questions of virus synthesis and genetics. Bromegrass mosaic virus, a spherical plant virus, may qualify as a useful test object for such investigations. Data reported here show that this virus is particularly small, and furthermore that the molecular weight of its ribonucleic acid is much smaller than that of any other known virus.

The sedimentation coefficient of bromegrass mosaic virus was measured in a Spinco model *E* analytical ultracentrifuge at 23·3° C. in 0·1 ionic strength buffer (sodium phosphates + sodium chloride, *p*H 6·0) at 35,600 r.p.m. Runs were made at concentrations of 0·12, 0·25, 0·37, 0·49 and 0·99 per cent, and the resulting plot of sedimentation coefficient versus concentration was extrapolated to infinite dilution. The extrapolated value, 92·0 *S*, was corrected to the value expected in water at 20° C. Thus, $s^{\circ}_{20,w} = 86{\cdot}2\ S$. Diffusion measurements were carried out in a quartz Tiselius cell in a Spinco model *H* electrophoresis-diffusion apparatus at 2° C. in the same buffer as above. The virus concentration was 0·42 per cent. Apparent diffusion coefficients were calculated according to the method of Longsworth[1] from five photographs of the Rayleigh fringe patterns taken at selected time-intervals. From these results the diffusion coefficient was calculated to be $8{\cdot}62 \times 10^{-8}$ cm.^{2}/sec., and this value was corrected to that expected in water at 20° C. Thus, $D_{20,w} = 1{\cdot}55 \times 10^{-7}$ cm.^{2}/sec. From the sedimentation and diffusion coefficients at a virus concentration of 0·42 per cent and an assumed partial specific volume of 0·71 ml./gm., the molecular weight of bromegrass mosaic virus was calculated to be $4{\cdot}6 \times 10^6$. To our knowledge this is the lowest molecular weight reported for any virus.

The nucleic acid content of bromegrass mosaic virus was determined by means of the optical method of Englander and Epstein[2], in which the nucleoprotein concentration is determined by a measurement of the refractive index increment of a solution, and the nucleic acid concentration is determined by a corresponding measurement of the optical density at 260 mμ. The measured optical density of the virus solution was corrected for the effect of light scattering and for absorption due to protein. (The extinction coefficient for ribonucleic acid at 260 mμ was taken to be 22·3 cm.^{2}/mgm., the specific refractive increment to be 1·8 × 10^{-4} ml./mgm. and the protein extinction coefficient to be 0·5 cm.^{2}/mgm.) The ribonucleic acid content was found to be 21 per cent. By analogy with the reported characteristics of the ribonucleic acid of other small viruses, it is reasonable to assume that bromegrass mosaic virus ribonucleic acid is all in one piece. Its molecular weight is then 1 × 10^6. This is a lower molecular weight than that of any other known genetically functional nucleic acid[3,4].

Experiments were undertaken to show that the 4·6 million molecular weight particle described above is indeed the infectious virus. These experiments consisted of infectivity and particle assays of fractions obtained by density gradient centrifugation as follows: A 10–40 per cent w/v sucrose density gradient (*p*H 6·5 in 0·1 ionic strength phosphate buffer) was prepared according to the method of Bock and Ling[5]. A 0·25-ml. sample of a 0·53 per cent solution of bromegrass mosaic virus was layered on top of the gradient solution and this was centrifuged at 25,000 r.p.m. for 3 hr. in an *SW*-25 swinging bucket rotor of a Spinco model *L* centrifuge. A single, opalescent band corresponding to the 4·6 million molecular weight particle was observed in the centrifuge tube at the conclusion of the run. The tube bottom was punctured with a hypodermic needle, and 1-ml. fractions were collected and tested for infectivity on the local lesion host *Chenopodium hybridum*[6]. Fractions 1–5, 6–10, 11–15 and 25–29 were pooled. 0·1-ml. samples of the pooled fractions and the remaining fractions 16–24 were brushed on to alternate right and left half-leaves after dusting them with 'Carborundum'. The opposite half-leaves were brushed with a standard bromegrass mosaic virus solution. Adjustment of the local lesion count for difference in susceptibility of leaves was made. Results are shown in Fig. 1. It may be seen that the maximum infectivity coincides with the maximum particle concentration as measured by optical density, and moreover the entire set of infectivity and optical density values superpose very well. Since the separation into fractions was essentially on the basis of sedimentation velocity it may be concluded that the infectious agent sediments at the same rate as does the 4·6 million molecular weight particle.

A similar experiment was performed with a 35–38 per cent w/w rubidium chloride density gradient (same buffer as above). Bacteriophage $\varphi X174$ top and bottom components the particle densities of which in rubidium chloride are 1·32 and 1·40, respectively, were added to the virus solution as markers in order to establish the resolving power of the procedure. The density of the 4·6 million molecular weight particle is intermediate to these two values. After 12 hr. of centrifugation three bands were observed in the density-gradient tube. Infectivity analysis (carried out by Robert K. Fujimura) of appropriate fractions (by plaque counts on *E. coli C*) verified that the optical density in the first band was due to $\varphi X174$ bottom component. The second band contained the 4·6 million molecular weight particle, identified by its intermediate density and distinctive ultra-violet absorption spectrum. The third band contained $\varphi X174$ top component, verified by its distinctive ultra-violet absorption spectrum. Infectivity on *Chenopodium hybridum* showed that only the fractions corresponding to the second band contained infectious bromegrass mosaic virus. Since the particles were separated in this experiment according to their densities, it may be concluded that the infectious agent and the 4·6 million molecular weight particle have the same density.

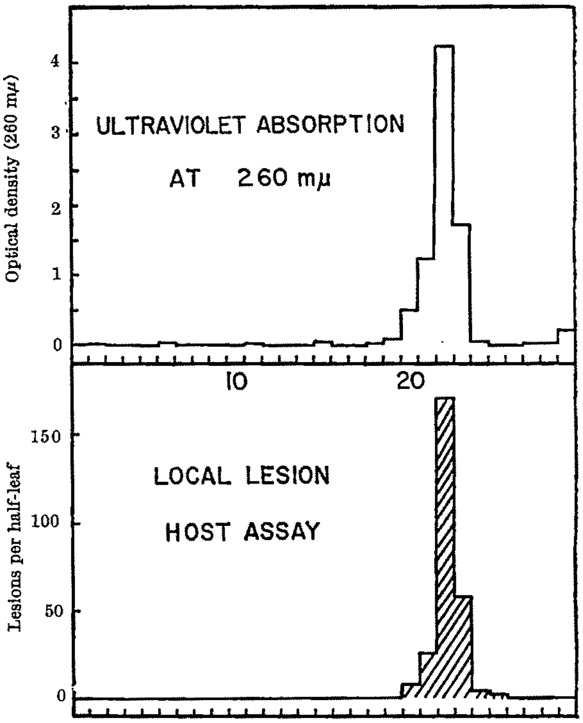

Fig. 1. Ultra-violet absorption at 260 mμ and infectivity assays of fractions obtained from sucrose density gradient (10–40 per cent w/v) centrifugation of bromegrass mosaic virus

Thus, we conclude on the basis of velocity and equilibrium sedimentation experiments that the particle, the characteristics of which we have described, is indeed the infectious virus.

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GENETICS

Genetic Studies with *Klebsiella pneumoniae*

GENETIC recombination following conjugation and phage transduction has been reported within and between several groups of enteric bacteria. It was decided, therefore, to look for evidence of genetic exchange in *Klebsiella pneumoniae*, and between this organism and others of the Enterobacteriaceae.

By means of the penicillin selection technique stable auxotrophic mutants were obtained in 17 *K. pneumoniae* strains. Strain *NCTC* 8821 readily yielded unstable auxotrophs with high rates of reversion to prototrophy, and may perhaps carry a mutation factor like that described by Jyssum[1] in strains of *Escherichia coli*. Strain 8821 did not, however, have a high rate of spontaneous mutation to streptomycin indifference.

Attempted transductions, employing 11 *Klebsiella* phages isolated from pooled sewage, gave only one suggestive result : a cysteine auxotroph of strain *NCTC* 8172 was apparently transduced to prototrophy at low frequency. The possibility that the rare prototrophs resulted, however, from transformations by deoxyribonucleic acid present in the crude phage lysate has not been excluded.

Mixed cultivation experiments were carried out according to the method of Miyake and Demerec[2]. These failed to yield any evidence of genetic recombination, occurring at the rate of at least 1 recombinant per 10^7 cells plated, between the different *K. pneumoniae* strains, between *K. pneumoniae* and *E. coli K-12*, HfrH, or *K. pneumoniae* and strain *NCTC 8706* of *Chromobacterium prodigiosum*.

An interesting syntrophic interaction was found between an unclassified auxotroph of *C. prodigiosum* 8706 and some *K. pneumoniae* auxotrophs. When well-washed cells of the *C. prodigiosum* mutant were spread on minimal *A* agar[3] plates, then incubated at 30°, few prototrophic revertant colonies appeared. Numerous reversions arose, however, if this auxotroph were incubated on minimal medium supplemented with a small amount of broth, or if washed mixtures of the *C. prodigiosum* and *K. pneumoniae* auxotrophs were incubated on minimal medium.

I wish to thank Prof. D. G. Catcheside for his advice, interest, and encouragement, and the Agricultural Research Council for the award of a research studentship.

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PSYCHOLOGY

Effects of Mental Activity on Vestibular Nystagmus and the Electroencephalogram

MOWRER[1] noted differences in head nystagmus elicited by rotation under conditions of 'excitement' and 'rest' in pigeons. Wendt[2] has indicated that "inward-directed revery states" reduce nystagmus and that the subject must "maintain an environment-directed orientation" to avoid replacement of nystagmus by wandering, autogenous eye movements. The present work was undertaken to ascertain how nystagmus, produced by a standard vestibular stimulus, is modified by different sets of instructions and, further, to determine the effects of these conditions on the electroencephalogram.

Subjects were seated in a light-proof room in the centre of a rotary apparatus, described in detail by Guedry and Kalter[3]. An adjustable biteboard held the subject's head in a standard position and a telegraph key, mounted on the right arm rest, was used as a signalling device. An Offner type *T* encephalogram, secured to the table, permitted the simultaneous recording of encephalograph (from a pair of parieto-occipital leads), horizontal nystagmus, subject key presses and a marker which denoted the periods of acceleration and deceleration. Subjects met three criteria : (*a*) no history of labyrinthine disorder ; (*b*) production of alpha activity when relaxed with eyes closed ; (*c*) quantifiable nystagmus. Of the seven candidates tested, only one was eliminated (due to a lack of alpha production). All subjects had previous turntable experience.

Three tasks were selected : (1) key pressing-signals made by the subject to indicate onset, cessation, and each experience of 90° of subjective rotation ; (2) mental arithmetic-subjects were instructed to divide 80 and each successive quotient by 5, ignoring the external environment, and proceeding as rapidly and accurately as possible (answers were recorded) ; (3) reverie-subjects were told to ignore the environment and simply to relax and daydream without directing their thoughts in any way. The tasks were given in the sequence above and repeated in the same order. The six trials were conducted successively on a single day.

All trials were conducted in the dark with eyes open, head upright, and rotation in a clockwise direction. Each trial began with a 30-second period of rotation at 1 r.p.m. followed by 14·9 sec. of $4 \cdot 5°/\text{sec.}^2$ acceleration to a constant velocity of 12·2 r.p.m. This speed was held for 2 min. before the subject was decelerated at $4 \cdot 5°/\text{sec.}^2$ to 1 r.p.m. After 1 min. of rotation at this velocity, the turntable was stopped and the trial ended. Prior to each trial, subjects alternately fixated markers for calibration purposes. They were located so as to provide horizontal eye sweeps of ±20° to either side of a central fixation point.

The slow-phase amplitude of the nystagmus was measured in centimetres, summed for the 14·9-sec. periods of acceleration and deceleration respectively, and converted to total degrees of slow-phase eye movement. The measurements made were the vertical distances from the peak of each nystagmic beat to the slow-phase base-line of that beat. Durations were calculated from the start of stimulation to the point where the primary nystagmus ended. The data from the encephalograph were tabulated by the 'seconds of alpha' technique, and the scoring covered only the periods of acceleration and deceleration.

Much less nystagmus was present during the daydreaming than during the key pressing or arithmetic trials (Fig. 1). The scored observations were subjected to separate analyses of variance and *t* tests. For acceleration, the conditions did not yield significantly different results. The deceleration results yielded the following significant relationships : (*a*) smaller total

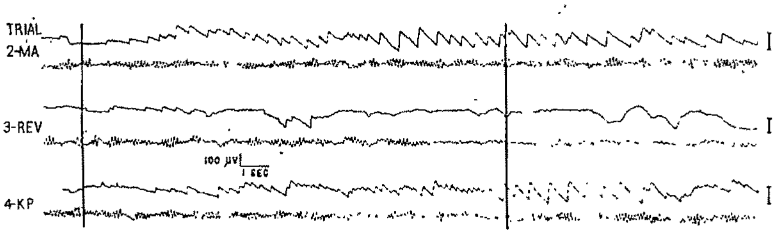

Fig. 1. Nystagmus and electroencephalograph tracings for subject 4 showing trials 2, 3 and 4, with the subject-tasks of mental arithmetic, reverie and key pressing, respectively (paper speed, 25 mm./sec.). The heavy vertical bars demarcate the period of 4·5°/sec.^{2} deceleration of 14·9 sec. duration. The calibrations to the right of each eye movement record indicate 20° eye excursions and the 100 μV. calibration refers to all electroencephalograph tracings. The amplifier time-constant for eye movements was 1·4 sec. Note that the contiguous task is a powerful variable in determining the qualitative as well as the quantitative aspects of nystagmus (during the period of deceleration, $MA = 363$, $REV = 151$ and $KP = 380$ degrees of slow-phase eye movement). The alpha rhythm as well as the nystagmic output are reduced during the reverie condition

degrees of nystagmic output during reverie than key pressing or mental arithmetic (0·03 and 0·001 levels respectively); (*b*) shorter durations of nystagmus for reverie conditions than for key pressing or mental arithmetic (0·01 levels); (*c*) more alpha during mental arithmetic than key pressing or reverie (0·02 levels).

The results demonstrate that nystagmus can vary with type of mental activity, thereby confirming Wendt's claim. However, it does not seem necessary that the subject be 'environment-directed' (as in the key pressing condition) in order to maintain nystagmus. Subjects frequently reported a lack of awareness of acceleration, and more often of deceleration, while doing arithmetic. Although this was also true for reverie states, the nystagmic output remained high during the mental work. Thus, general alertness would appear to be the important factor in maintaining nystagmus.

The failure to obtain significant differences from the acceleration results seems due to a combination of factors. One of these was an alerting 'end effect'. Two of the three subjects who knew the number of trials to be conducted produced more nystagmus during the last acceleration (reverie) than during any preceding trial; the third exceeded all but one previous trial. That such knowledge can 'alert' the subjects and increase the nystagmic output has been noted elsewhere[4]. The remaining factors were deliberately included in the design in order to determine the power of the mental set variable. They included: (*a*) the rapid change from mental activity to reverie required of the subjects; (*b*) the relatively brief period (30 sec.) between instructing the subjects to daydream and initiating the acceleration stimulus. Further work in progress demonstrates clearly that nystagmus declines significantly while subjects are daydreaming during both acceleration and deceleration when more favourable conditions are employed for the establishment of mental 'sets'[5].

It was anticipated that alpha activity would be more evident during reverie and would correlate negatively with nystagmic output. However, more alpha was present during the arithmetic task for the subjects studied here. Although this specific encephalograph finding may be peculiar to the particular group of subjects employed, or to the experimental conditions, the evidence from this investigation and others in progress indicates that: (*a*) nystagmus is markedly sensitive to instructions regarding mental activity while the pattern of the encephalograph is not; and (*b*) vestibular stimulation in combination with various attention-demanding tasks does not block alpha production which is going on[5]. It is frequently stated that various types of sensory input as well as mental activity will block alpha, but other reports note that such is not always the case[6-8].

That gross differences in nystagmic output depended on the mental 'set' of the subject (one subject gave no nystagmus at all during a day-dreaming session) and that this variable is so sensitive as to be manipulated within seconds by simple instructions should be of interest to clinicians seeking to evaluate vestibular dysfunction. It may also explain the lack of a 'constant reaction' and the 'temporary reduction in response' noted for some of the subjects recently studied by Suzuki and Totsuka[9].

Our findings suggest that: (*a*) alertness, whether 'environment-directed' or not, is a highly significant variable in the maintenance of a vigorous nystagmic response; (*b*) subjects should be given objective tasks as a control feature during vestibular experimentation; (*c*) under certain conditions, eye movements may give better indications of a subject's state of alertness than do encephalograph patterns.

We gratefully acknowledge the expert technical assistance of Stanton Warburton.

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The 'Psychological Refractory Phase' and Ageing

When, in psychological experiments, the subject is required to respond as rapidly as possible to a signal closely following another, an increase in response latency is observed[1]. It has become customary to refer to this aspect of high-speed sequential performance as the 'psychological refractory period' in analogy with changes in threshold of neurones, following the propagation of impulses, where the recovery is divisible into an 'absolute' and a 'relative' phase. Welford[1] has argued that the slowing of response to the second of two successive signals is due not to a temporary reduction of central sensitivity, but to the feature that "no two central organizing times can overlap". His view is derived from that of Hick[2], who postulated that in continuous performance the brain is occupied not only in dealing with a particular signal but also with the monitoring of a response to it.

The experiment briefly reported here differs from previous work on psychological refractoriness in three ways. First, the subject was required to deal simultaneously with two streams of signals, one regular, highly probable input (for which a response was made with the non-preferred hand), and the other irregular, with high temporal uncertainty (for which a response was executed with the preferred hand). Secondly, in contrast with most previous experiments, the 'second' signal, when pairs of signals were presented, always occurred after the response to the 'first' signal had been initiated. Intervals of 50, 150, 300 and 600 msec. between the beginning of a regular response and the onset of an irregular signal were employed. Thirdly, the regular signals were presented at two different rates; (*a*) at one every 2 sec.; (*b*) in another session, at one every 4½ sec. 34 subjects, ranging from 20 to 80 years of age, and divided into three age-groups, took part in the experiment. Approximately half the subjects in each group began with the faster rate.

The average response times to the irregular input for the three age-groups are shown in Table 1. (Although omitted to save space, the response latencies to regular signals also decreased with increased rate.)

Table 1. MEAN RESPONSE LAG (MSEC.) TO THE IRREGULAR SIGNAL

Age group	Interval (*R*1–*S*2)			
	50	150	300	600
	At 'fast' rate of the regular sub-task			
20–39 (11 subjects)	319	300	298	307
40–59 (12 subjects)	320	306	292	310
60–80 (11 subjects)	376	356	339	345
	At 'slow' rate of the regular sub-task			
20–39 (11 subjects)	329	295	299	317
40–59 (12 subjects)	358	302	299	323
60–80 (11 subjects)	419	374	364	374

Analysis of these results suggests that the period over which reaction times were lengthened is less than 150 msec. but more than 50 msec. for all subjects at both rates ($P < 0{\cdot}05$—$P < 0{\cdot}001$), and greater than 150 msec. but less than 300 msec. for the middle-aged and older subjects when the regular signals appeared at the faster rate ($P < 0{\cdot}02$). However, the magnitude of the delays is lower than would be predicted on Welford's theory, and it is doubtful if we can, without further qualifications, refer to the range of intervals over which response times increase as the 'refractory period', in the sense of 'dead time'. If the occupation of the central mechanisms by previous signals, or by feedback, prevents the commencement of the processes concerned with the subsequent signal, the response lag should equal the difference between the duration of the 'refractory period' and the time elapsing before the second signal is delivered, that is, it will give a curve with negative slope of 45°. The slopes obtained in the present experiment are considerably less than this in all cases, suggesting that the delays observed may be due not to the start of the processing of the irregular input being prevented, but to the decision mechanism operating less quickly for a short time after it had again become free to deal with signals. Such an interpretation, if correct, implies that the analogy with physiological refractoriness is more exact than hitherto assumed. Possibly it is important to distinguish between an 'absolute phase', in which signal analysis is held up until the central processes already begun have ended, and a 'relative phase' during which the speed of decision is lowered. The duration of both phases is likely to be a function of signal probability, and it is in suggesting this relationship that our results differ from previous ones. Certainly, in our investigation the distinction is of interest, because although for the older subjects an increase in the frequency of regular signals leads to a lengthening of the 'relative refractory period', what can be inferred about the duration of the 'absolute phase' from the slope points to a decrease (mean difference between the two rates × age: at 50 msec. $P < 0{\cdot}02$, at 150 msec. $P < 0{\cdot}05$, at 300 msec. $P > 0{\cdot}05$, at 600 msec. $P < 0{\cdot}02$; mean difference in slopes 50–150 msec. intervals at the two rates: $P > 0{\cdot}05$ for young subjects, $P < 0{\cdot}001$ for both the middle-aged and old groups).

These observations suggest the possibility that amplification of a routine activity may serve, in the case of the older adults, to improve selective responsiveness of central mechanisms to a high-information signal. If such an amplification causes the level of 'vigilance'[3] or 'arousal'[4] to be increased, perhaps via the reticular formation, it is conceivable that decision processes are thereby enhanced. There are, of course, many factors determining the level of activity in the reticular system[5]; but there is direct neurophysiological evidence that one of them may be the rate of sensory inflow[6]. In addition to our observations, the more recent results of comparative experiments[7] would seem to support such an interpretation.

In an attempt to explore these theoretical possibilities, we are now engaged in examining older adults, employing different sensory channels for each stream of signals. Preliminary results suggest that response latencies and refractory delays to visual signals tend to decrease still further with an auditory sub-task. As a further step we are attempting to develop a suitable technique for observing refractoriness in continuous performance, when the size of the ensemble of possible signals varies.

The work is supported by a grant from the Nuffield Foundation. Our thanks are also due to A. C. Staniland for help with the design and construction of apparatus, and to D. J. Stone for statistical advice.

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FORTHCOMING EVENTS

Monday, April 10

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m. —Discussion on "Applications of Electrical Phenomena at Liquid Helium Temperatures" opened by Dr. R. E. Hayes.

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. A. E. Canham: 'The Use of Electric Light and Heat in Horticulture".

Royal Aeronautical Society, Historical Group (at 4 Hamilton Place, London, W.1), at 7 p.m.—Discussion Evening—"A Quiz on Little Known Early Aircraft".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. H. W. Tilman: "Voyage to Iles Crozet and Kerguelen".

Monday, April 10—Saturday, April 15

First International Congress on "Metallic Corrosion" (at Imperial College, Prince Consort Road, London, S.W.7).

Tuesday, April 11

British Occupational Hygiene Society (at Birkbeck College, Malet Street, London, W.C.1)—Conference on "Training in Occupational Hygiene".

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

Institution of Electrical Engineers, Measurement and Electronics Sections (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. Woods: "Precision Instruments for Co-axial Line Measurements up to 4 Gc./s.". (Supported by a paper by Mr. D. Woods entitled "The Concept of Equivalent Source E.M.F. and Equivalent Available Power in Signal-Generator Calibration".)

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 7 p.m.—Prof. M. W. Thring: "Direct Electrical Power Generation by Thermal and Chemical Means".

Tuesday, April 11—Thursday, April 13

Royal Photographic Society, Scientific and Technical Group; Royal Geographical Society; and the British Association for the Advancement of Science (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7)—Symposium on "Photography on Expeditions".

Wednesday, April 12

Institute of Physics and Physical Society, Low Temperature Group (at 47 Belgrave Square, London, S.W.1), at 11 a.m.—Meeting on "Techniques for Generating Very High D.C. Magnetic Fields".

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. L. R. Raymond: "The Petrology of the Permian Rocks of North-east Yorkshire and South-east Durham—The Lower Magnesian Limestone"; Dr. D. K. Bailey: "Intrusive Limestones in the Keshya and Mkwisi Valleys, Northern Rhodesia".

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Dr. R. N. Curnow: "Optimal Programmes for Varietal Selection".

British Institution of Radio Engineers, Electro-Acoustics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. D. E. Mullinger: "Vibration Analysis and Testing".

Institution of Electrical Engineers, Education Discussion Circle (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Teaching Physics to Engineers", opened by Dr. J. Topping.

Society for Analytical Chemistry (joint meeting with the Oil and Colour Chemists' Association, at the Wellcome Building, Euston Road, London, N.W.1), at 7 p.m.—Scientific Papers.

Women's Engineering Society (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Miss Sabey: "Road Research".

Royal Aeronautical Society, Graduates' and Students' Section (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Mr. B. S. Shenstone: "Man-Powered Flight—Progress to Date".

Thursday, April 13

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Miss Mary F. Hay, Mr. H. R. Lindner and Dr. T. Mann, F.R.S.: "Morphology of Bull Testes and Seminal Vesicles in Relation to Testicular Androgens"; Mr. T. W. Glenister: "Organ Culture as a New Method for Studying the Implantation of Mammalian Blastocysts".

Institution of Mechanical Engineers; Institution of Civil Engineers; and the Institution of Electrical Engineers (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—H.R.H. The Prince Philip, Duke of Edinburgh, K.G.: "Resources, Human and Material, of the Commonwealth" (Graham Clark Lecture, 1961).

Friday, April 14

Association of Applied Biologists (in the Metallurgy Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 10.15 a.m.—Annual General Meeting.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Grade B Assistant to teach Biology to G.C.E. Advanced level and to Pre-nursing classes with some General Science; and a Grade B Assistant to teach Physics to G.C.E. Advanced level and to O.N.C. classes—The Principal, Peterborough Technical College, Easterfield Road, Peterborough (April 15).

Senior Research Scientist (of high academic standing in biochemistry, and preferably with a medical qualification), to take charge of a new laboratory unit and undertake research into the biochemical aspects of cancer—The Secretary, Marie Curie Memorial Foundation, 124 Sloane Street, London, S.W.1 (April 15).

Senior Lecturer in Mathematics—The Principal, Nottingham Training College, Clifton, Nottingham (April 17).

Chair of Zoology tenable in the Durham Division of the University—The Registrar, University Office, 46 North Bailey, Durham (April 22).

Lecturer (preferably with qualifications in historical geography with special reference to Scotland) in the Department of Geography—The Secretary, The University, Aberdeen (April 22).

Research Fellow in Mathematics—The Secretary, Royal College of Science and Technology, Glasgow (April 22).

Senior Lecturer (prepared to initiate research in some branch of pure or applied mathematics) in Mathematics—The Secretary, Royal College of Science and Technology, Glasgow (April 22).

Statistician, Scientific Officer (with a good honours degree in mathematics or science and a knowledge of statistical techniques as applied to agriculture or biology) at the Agricultural Research Council's Unit of Statistics (University of Aberdeen)—The Secretary, Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1 (April 22).

Lecturer in the Department of Physics—The Registrar, University College of North Wales, Bangor, North Wales (April 24).

Lecturer in Cytology in the Departments of Botany and Zoology in the Faculty of Science—The Registrar, The University, Manchester 13 (April 25).

Assistant Lecturer (Grade B) in Geography—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (April 29).

Assistant Mycologist (preferably male, with M.Sc. or B.Sc. degree in botany, forestry or agriculture) at the Forest Research Institute, Rotorua, New Zealand, for the investigation of fungi associated with forest trees and/or fungal taxonomy—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B.13/8/1 (April 29).

Assistant Lecturer or Lecturer in Dental Biochemistry—The Registrar, The University, Liverpool, quoting Ref. CV/N (April 30).

Assistant Director (with a recognized degree or equivalent qualification, and a wide experience in agricultural botany or in crop husbandry)—The Director, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (May 1).

Research Fellow (with a minimum of three years postgraduate research experience) in the Department of Chemistry, University College, Ibadan, Nigeria, to join a group working on the chemistry of the West African Flora, in particular to investigate the bitter principles of the West African Cucurbitaceae—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 1).

Research Assistant in Dental Biochemistry, to pursue investigations into the metabolism of bone and teeth—The Registrar, The University, Liverpool, quoting Ref. CV/N (June 30).

Assistant Lecturer, Grade B (with a degree or equivalent qualification, teaching experience, and able to teach to Higher National Certificate standard—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex.

Assistant Lecturer (preferably with a Ph.D. or equivalent postgraduate research experience, and a knowledge of electronics or radiochemistry) in Physical Chemistry—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

Assistant Professor (Psychologist) in the University Department of Psychiatry—The Dean of Medicine, University of Alberta, Edmonton, Alberta, Canada.

Biology Technician, to work at the Royal Veterinary College assisting a research fellow of the Zoological Society of London in physiological and animal breeding work—The Assistant Secretary, Royal Veterinary College, Royal College Street, London, N.W.1.

Demonstrators (2) in the Department of Horticulture, to assist with practical classes on vegetable crops and ornamental plants respectively—Prof. O. V. S. Heath, F.R.S., University of Reading Horticultural Research Laboratories, Shinfield Grange, Shinfield, Berks.

Experimental Officer, for work on biochemical problems associated with the diseases of farm animals and the fundamental study of protein and nucleic acid biosynthesis—The Secretary, Agricultural Research Council, Field Station, Compton, near Newbury, Berks.

Graduate Chemist or Physical Chemist (age 23 to 30 years). for work on techniques employed in the fractionation of proteins in human plasma. The work includes the development of laboratory procedures for use on a small production scale—The Secretary, Lister Institute of Preventive Medicine, Elstree, Herts.

Lecturer (with a good honours degree and preferably a higher degree) in Mathematics—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

Lecturer (with a special knowledge and/or experience in spectrophotometry and colorimetry) in Physical Chemistry—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

Lecturers in Geography, Pharmacy, Biochemistry, Inorganic Chemistry, Botany, Mathematics, Physics, Statistics, and Zoology (Comparative Anatomy)—The President, University College, P.O.B. 399, Addis Ababa, Ethiopia.

Master to teach Chemistry to "A" and "S" level G.C.E.—The Headmaster, The Perse School, Cambridge.

Master to teach Mathematics, very largely in the Sixth Form—The Headmaster, Bolton School, Bolton, Lancs.

MINERALOGIST (preferably with Ph.D. degree)—Department of Geology, University of New Brunswick, Fredericton, New Brunswick, Canada.

RESEARCH ASSISTANT (Senior Technician grade, with laboratory experience in chemistry or biochemistry, industrial or hospital), for interesting research in amino-acid metabolism in health and disease—Dr. Woolf, Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford.

RESEARCH ASSISTANT (with an honours degree in physiology, physics or a biological subject), for experimental research in human lung function and to assist in teaching—The Finance Officer, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1.

RESEARCH ENGINEER IN THE DEPARTMENT OF MATERIALS, to assist in setting up and developing a high-temperature research project—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

RESEARCH FELLOW (with a first degree in physics or metallurgy) IN THE DEPARTMENT OF MATERIALS, to undertake research in the fundamental deformation and annealing mechanisms in graphite—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

SENIOR BIOCHEMIST (with an appropriate science degree or the A.R.I.C.) within the Nottingham No. 2 Group of Hospitals, to work under the direction of the Chemical Pathologist at the City Hospital—The Group Secretary, Nottingham No. 2 Hospital Management Committee, Sherwood Hospital, Nottingham.

SENIOR TECHNICIAN or TECHNICIAN (with instrument or physics workshop experience) IN THE RADIOTHERAPY DEPARTMENT, Oldchurch Hospital, for duties which include Physics Workshop, Isotope Laboratory, Radium and Mould Room work—The Group Secretary, Romford H.M.C., Oldchurch Road, Romford, Essex.

SENIOR TECHNICIAN (with experience of normal histological techniques), for work with an electron microscope—The Secretary, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1.

TECHNICAL ASSISTANT (female) IN THE PHYSICS DEPARTMENT, for duties concerned with the medical applications of radioactive isotopes (training will be given in the specialized techniques involved)—The Secretary, Leicester Royal Infirmary, Leicester.

TECHNICIAN (preferably with previous experience in histological techniques) IN THE RESEARCH DEPARTMENT—The Secretary, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Association of British Chemical Manufacturers. A Report of Meetings held in London and Manchester on 10th and 15th June, 1960, concerning Origin Rules, Certification, and Segregation of Stocks, by the Association of British Chemical Manufacturers. Pp. iii+42. (London: Association of British Chemical Manufacturers, 1960.) 3*s.* 6*d.* [111

Report of the Seventh Commonwealth Entomological Conference, 6th–15th July, 1960. Pp. iii+399. (London: Commonwealth Institute of Entomology, 1960.) 50*s.* [111

National Union of Teachers. University Entrance: The Basic Facts. Pp. 64. (London: National Union of Teachers, 1961.) 4*s.* [111

Royal Institute of Chemistry. Lectures, Monographs and Reports, 1960. No. 3: Energy Exchange in Interactions Between Ions and Molecules. By V. N. Kondratiev. Pp. ii+16. 4*s.* net. No. 4: Chemistry in Everyday Life—The Contribution of the D.S.I.R. By Sir Harry Melville. Pp. 16. 4*s.* net. No. 5: Sugar and Its Industrial Applications. By Dr. L. F. Wiggins. Pp. ii+44. 5*s.* 6*d.* net. (London: Royal Institute of Chemistry, 1960.) [111

British Association for Commercial and Industrial Education. Policy in Perspective: Report of the BACIE Annual Conference held at Cambridge, 22–24 September, 1960. Pp. 126. (London: British Association for Commercial and Industrial Education, 1960.) 10*s.* [111

The Incandescent Heat Company, Ltd. Bulletin No. V. 10C: A Complete Service in Industrial Heat Engineering. Pp. 29. (Smethwick, Birmingham: The Incandescent Heat Company, Ltd., 1961.) [111

Kodak Limited. About Kodak. Pp. 24. (London: Kodak Limited, 1961). [111

Association of Universities of the British Commonwealth. Report of the Executive Council, together with the Accounts of the Association for the year 1st August, 1959, to 31st July, 1960. Pp. 30. (London: Association of Universities of the British Commonwealth, 1960.) [111

Coal Tar Research Association. Review of Coal Tar Technology. January–June, 1960. (Vol. 12, Part 1.) Pp. ii+63. (Gomersal, Leeds: Coal Tar Research Association, 1960.) [191

Ministry of Power. Dust Prevention and Suppression. Instructional Pamphlet No. 3: The Application of Water to Machine Undercutting and to Deep Hole Extra High Pressure Infusion of Coal Seams. Pp. iii+8+2 plates. (London: H.M. Stationery Office, 1960.) 1*s.* 6*d.* net. [191

Commonwealth Mycological Institute. Mycological Papers, No. 79: Dematiaceous Hyphomycetes, II. By M. B. Ellis. Pp. 23. (Kew: Commonwealth Mycological Institute, 1961.) 7*s.* net. [191

Department of Scientific and Industrial Research. D.S.I.R. Studentships and Fellowships 1961. Pp. 28. 1*s.* 9*d.* net. D.S.I.R. Research Grants, 1961. Pp. iv+8. 1*s.* net. (London: H.M. Stationery Office, 1961.) [191

Department of Scientific and Industrial Research. Memoirs of the Geological Survey, Scotland. The Geology of Ben Nevis and Glen Coe and the Surrounding Country. By E. B. Bailey and H. B. Maufe. Second (revised) edition by Sir E. B. Bailey, with Economic Chapter by T. R. M. Lawrie. Pp. x+307+13 plates. (London: H.M. Stationery Office, 1960.) 35*s.* net. [191

Society for Analytical Chemistry. Report of the Analytical Methods Committee, 1959. Pp. vi+27. (London: Society for Analytical Chemistry, 1960.) [191

Other Countries

United States Department of Commerce: Coast and Geodetic Survey. Technical Bulletin No. 13: A Singular Geodetic Survey. By L. G. Simmons. Pp. ii+14. (Washington, D.C.: Government Printing Office, 1960.) 15 cents. [111

University of California Publications in Geological Sciences. Vol. 34, No. 6: Displaced Miocene Molluscan Provinces Along the San Andreas Fault, California. By Clarence A. Hall, Jr. Pp. 281–308. 1.25 dollars. Vol. 38, No. 2: Mississippian and Devonian Paleontology and Stratigraphy, Quartz Spring Area, Inyo County, California. By R. L. Langenheim, Jr., and Herbert Tischler. Pp. 89–152. 1.25 dollars. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1960.) [111

This is Du Pont International. Pp. 16. (Geneva: Du Pont de Nemours International S.A., 1960.) [111

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturais. Dundo–Lunda–Angola. Museu do Dundo. Subsidios para o Estudo da Biologica na Lunda. Publicacoes Culturais No. 53: Nova Contribuicao ao Estudos dos Tabanideos (Diptera: Tabanidae) de Angola. Por J. A. Travassos Santos Dias. Pp. 125. (Lisboa: Companhia de Diamantes de Angola, 1960.) [111

Proceedings of the United States National Museum, Smithsonian Institution, Washington. No. 3430 (Vol. 111): Cydnidae of the Western Hemisphere. By Richard C. Froeschner. Pp. 337–380+13 plates. No. 3443 (Vol. 112): Cultural Sequences in Hokkaido, Japan. By Lt.-Col. Howard A. MacCord. Pp. 481–503+14 plates. No. 3445 (Vol. 112): *Lithoglyptes spinatus*, a Burrowing Barnacle from Jamaica. By Jack T. Tomlinson and William A. Newman. Pp. 517–526. No. 3446 (Vol. 112): Notes on Mysidacean Crustaceans of the Genus *Lophogaster* in the U.S. National Museum. By Dr. O. S. Tattersall. Pp. 527–547. No. 3447 (Vol. 112): The Fairy Shrimp *Branchinecta campestris* from Northwestern United States (Crustacea: Phyllopoda). By James E. Lynch. Pp. 549–561. (Washington, D.C.: Government Printing Office, 1960.) [111

Chicago Natural History Museum. Fieldiana: Zoology. Vol. 35, No. 7: Birds of the Philippine Islands: Siquijor, Mount Malindang, Bohol, and Samar. By Austin L. Rand and Dioscoro S. Rabor. Pp. 221–441. 4 dollars. Vol. 39, No. 40: Bermudian Cephalopods. By Gilbert L. Foss. Pp. 419–446. 60 cents. Vol. 39, No. 41: Three New Histerid Beetles from the Pacific Northwest, with Records and Synonymies of Additional Species (Coleoptera: Histeridae). By Rupert L. Wenzel. Pp. 447–463. 50 cents. (Chicago: Chicago Natural History Museum, 1960.) [111

United States Department of Commerce: Weather Bureau. Technical Paper No. 15: Maximum Precipitation for 1, 2, 3, 6, 12, and 24 Hours. Part XXV: Arkansas. Pp. ii+86. (Washington, D.C.: Government Printing Office, 1960.) 50 cents. [111

United States Department of the Interior: Geological Survey. Water-Supply Paper 1480: Evaporation Control Research, 1955–58. By Robert R. Cruse and G. Earl Harbeck, Jr. Pp. iv+45. 50 cents. Water-Supply Paper 1621: Surface Water Supply of the United States, 1959. Part 1–A: North Atlantic Slope Basins, Maine to Connecticut. Prepared under the direction of J. V. B. Wells. Pp. viii+276. 1 dollar. Water-Supply Paper 1629: Surface Water Supply of the United States, 1959. Part 6–A: Missouri River Basin above Sioux City, Iowa. Prepared under the direction of J. V. B. Wells. Pp. x+415. Water-Supply Paper 1633: Surface Water Supply of the United States, 1959. Part 9: Colorado River Basin. Prepared under the direction of J. V. B. Wells. Pp. xi+506. 1.75 dollars. (Washington, D.C.: Government Printing Office, 1960.) [111

United States Department of the Interior: Geological Survey. Water-Supply Paper 1302: Compilation of Records of Surface Waters of the United States through September 1950. Part 1–B: North Atlantic Slope Basins, New York to York River. Prepared under the direction of J. V. B. Wells. Pp. v+679+plate 1. Water-Supply Paper 1304: Compilation of Records of Surface Waters of the United States through September, 1950. Part 2–B: South Atlantic Slope and Eastern Gulf of Mexico Basins, Ogeechee River to Pearl River. Prepared under the direction of J. V. B. Wells. Pp. v+399+plate 1. Water Supply Paper 1315–B: Compilation of Records of Surface Waters of the United States through September, 1950. Part 11-A: Pacific Slope Basins in California except Central Valley. Prepared under the direction of J. V. B. Wells. Pp. v+461–874+plate 2. Water-Supply Paper 1420: Floods of August-October, 1955, New England to North Carolina. By Dean B. Bogart. Pp. ix+854. (Washington, D.C.: Government Printing Office, 1960.) [111

United States Department of the Interior: Geological Survey. Water-Supply Paper 1634: Surface Water Supply of the United States, 1959. Part 10: The Great Basin. Prepared under the direction of J. V. B. Wells. Pp. viii+247. Water-Supply Paper 1636: Surface Water Supply of the United States, 1959. Part 12: Pacific Slope Basins in Washington and Upper Columbia River Basin. Prepared under the direction of J. V. B. Wells. Pp. x+402. 1.50 dollars. (Washington, D.C.: Government Printing Office, 1960.) [191

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PHYSICS

RESEARCH AND DEVELOPMENT IN INDUSTRY

ALTHOUGH in the debate in the House of Commons on the nuclear power programme on February 3 the location of nuclear power plants in Britain was discussed, and some anxiety was expressed over the preservation of amenity, the serious criticism voiced in the latest report of the Nature Conservancy was not mentioned, and the debate dealt with many of the same issues as that on the economic situation which followed on February 6 and 7. These issues are also discussed in the PEP report, *Growth in the British Economy**, to which frequent reference was made in the latter debate. The debate on February 3, centred on a motion of Mr. J. Eden, which was accepted for the Government by Mr. J. C. George, Parliamentary Secretary to the Ministry of Power. The motion urged the Government, while giving due weight to considerations of public safety, the preservation of landscape, and the need to make power available from the cheapest and most efficient sources, to maintain the nuclear power programme at a level that would encourage the development of manufacturing experience and capacity, and provide the best possible prospects for exporting British designs, products and skill. Mr. Eden questioned the wisdom of development proceeding at the rate of about one new nuclear power station a year, and thought that serious waste of man-power and resources was involved in the maintenance of the industrial consortia and in competitive tendering, even by the three consortia now remaining out of five. He was also concerned that some of the stations now under construction might be obsolete fairly soon, and superseded by stations which could be built at lower capital cost and produce power more efficiently. He referred particularly to duplication of research and also asked for more positive encouragement in connexion with exports.

Some of these points were also emphasized by Mr. N. Ridley and by Mr. A. Neave. Mr. Neave referred to the bearing of the load factor, and said that with a load factor of 75 per cent, the cost of nuclear generation might soon approximate to 0·5*d*. per unit. He also urged that the Atomic Energy Authority should make full use of the industrial consortia in the design and development of reactor systems and suggested that, in particular, the Authority should be encouraged to look into new systems, and the advanced conversion of existing reactor systems. He stressed the need for the industry and its technologists to be able to plan ahead with some confidence that the programme would not again be curtailed, but was obviously doubtful whether it is economic to maintain the three industrial consortia, employing altogether some 1,000 scientists and engineers, to produce one station for every three years per consortium. Mr. R. Gunter was also concerned about the effective and economic use of the limited trained man-power in Britain; but Mr. D. Price challenged the view that there is duplication of research and development and thought we should keep at least two of the consortia. It is not easy, he pointed out, to build up again a team that has once been disbanded, and he strongly supported the view that the consortia should be encouraged to participate in development. He thought the area of advance in research on reactor design should be broadened and that, besides associating the consortia more closely with the Atomic Energy Authority, we should make more use of the European Nuclear Energy Agency. Mr. T. H. H. Skeet was concerned more particularly with the design of reactors for export, pointing out that the nuclear power plant of 300–500 MW. produced for the United Kingdom may be too large for some overseas markets.

In replying on the debate, Mr. J. C. George stated that the commissioning of the experimental fast reactor at Dounreay is proceeding satisfactorily; but in regard to controlled thermonuclear reactors, the Authority has broadened its programme of research with a number of experiments aimed at developing the advanced techniques required. He claimed that the present programme, with six nuclear power stations under construction, two more to be started in the next twelve months, and permission sought for another, is sufficient to sustain an industry capable of competing with success abroad and expanding to meet our own needs. He quoted figures for falling costs, from £160 per kW. at Berkeley to £123 at Trawsfynydd and £110 at Dungeness, and said that, besides two stations already sold abroad, there are possibilities of two in India, one in Brazil and one in Japan, apart from the export of research reactors. As regards small reactors, the first might well be on merchant ships, but it does not seem possible, he said, to construct a small nuclear station to provide power at anything like an economic price.

This debate showed that the House of Commons is fully alive to the importance of research and development as a basis in economic growth, and to the need for care to avoid waste of trained man-power. These factors also received some emphasis, though to a lesser extent, when the economic situation generally was debated. Mr. R. E. Prentice, who directed attention first to the wastage of skills, was primarily concerned with this in respect of the re-training of skilled men who had lost their existing jobs, and with apprenticeship; but although he and other speakers referred to the lack of support for the Government's scheme of training young people, no reference was made to the waste caused through the inappropriateness of apprenticeship schemes and the maintenance of periods of apprenticeship which are quite unnecessary under modern conditions. On this point generally, Mr. C. Fletcher-Cooke was forthright, condemning the hoarding of skilled labour, and urging that the skilled labour force should be expanded by putting through group apprenticeship and other means of industrial training with real urgency. He also advocated an intensive study of the whole question of the psychology of the mobility of labour.

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* *Growth in the British Economy: a Study of Economic Problems and Policies in Contemporary Britain.* Pp. xii+256. (London: Political and Economic Planning; and George Allen and Unwin, Ltd., 1960.) 30*s*. net.

So far as research and development are concerned, the comments made in the debate related rather to the insufficiency of research in two particular industries, namely, the shipbuilding and machine tool industries, though Mr. S. R. McMaster stressed the need for adequate expenditure on research and development generally as a factor in encouraging higher productivity, and referred to the need to afford adequate support for university research. Both these industries have been the subject of recent inquiry. The machine tool industry was sharply criticized by the Mitchell Committee for its lack of expenditure on research and development, which appears to be a primary reason why imports of machine tools into Britain remain at a high level, and Britain's share of the trade of major importing countries, excluding eastern Europe and China, is less than half Western Germany's and no greater than that of Switzerland. Further, only 1·3 per cent of the employees in the machine tool industry are qualified scientists, compared with figures of 2·2 per cent in electrical engineering, 3·7 per cent in chemical industry and 2·7 per cent in the aircraft industry, and only 0·29 per cent of its technical men are employed on research.

In this comparison, the shipbuilding industry shows up even worse. Only 588 qualified scientists and engineers were employed in the shipbuilding and ship repairing industry at the beginning of 1959—about 0·6 per cent of the number of employees—and Mr. Austen Albu, in a recent broadcast talk on the price of tradition in industry, questioned whether more than 70 or 80 graduates are employed in the shipbuilding and marine engineering industry, and of these more than half are probably employed on Admiralty research. The report from the Department of Scientific and Industrial Research entitled *Research and Development Requirements of the Shipbuilding and Marine Engineering Industries**, which was freely mentioned in the debate, was highly critical of the industry; but the published material has been the subject of some criticism as representing only a part of the report. It was published however, with the concurrence of the Shipbuilding Conference, as representing the shipbuilding and marine engineering industries, and its recommendations have been agreed between that Conference, the Ministry of Transport and the Department of Scientific and Industrial Research. That should assure the implementation of its recommendations; but it is disturbing to find that objections have been raised by the boiler-makers to the comparisons of efficiency in the United Kingdom and elsewhere, and particularly the analysis of labour costs.

Neither here nor elsewhere are closed minds likely to encourage growth in the British economy, and the conclusions of the report are disturbing enough in their brevity. The world shipbuilding industry is itself facing a major and probably prolonged recession, and there is no indication that the United Kingdom industry has on balance any marked technical or economic advantage over its major foreign competitors, apart from its large home market. The total effort at present devoted to research and development in the field of shipbuilding and marine propulsion is insufficient in relation to the serious problems now facing the industry; in particular, practically no organized research has yet been applied to production and management problems with the object of increasing the productivity of labour and capital and reducing costs. While adequate effort is probably being devoted to problems of hull resistance and propeller design, the development of propulsion installations is handicapped by the organizational structure of the industries producing propulsion units and auxiliaries and, in consequence, by the insufficient use of available research facilities.

The figures quoted in this report for the research effort do not quite agree with those given by Mr. Albu. Of the total professionally qualified staff of 1,240, 247 are estimated to be employed on research and development, and the total expenditure on research and development in the industry is estimated at about £1·5 million, or 1·0 per cent of net output. When the contributions made to co-operative research in the British Shipbuilding Research Association, the Parsons and Marine Engineering Turbine Research and Development Association and the British Welding Research Association and to the Ship Division at the National Physical Laboratory are added with the Government contributions, total annual expenditure on research in and for the industry is probably about £2·5 million, or 1·7 per cent of net output; in marine engineering alone, however, it is about £1·9 million, or about 4·4 per cent of net output, a figure which compares favourably with most other manufacturing industries. Nevertheless, there is some evidence tending to confirm that British shipbuilding costs have recently been high by comparison with those of major competitors, and there is no evidence to suggest that over the industry as a whole labour productivity has significantly increased, in spite of increased investment in production plant and machinery. Average construction times in 1957–59 were at least double those in Germany, Sweden and Japan, and it seems certain that the fullest use of shipyard space and capital equipment is handicapped by labour conditions and by the impracticability of shift working.

The report recommends that the Department of Scientific and Industrial Research and the Shipbuilding Conference, in collaboration with the Ministry of Transport, should take further steps to examine the research and development needs of the industry as a whole, and to review the facilities for carrying out research. Arrangements should be made for a concurrent economic and technical study of these needs in marine engineering to provide background for consideration by the Council for Scientific and Industrial Research of the reports it is to receive from the two research associations on their organization and programmes, and this study should be carried out by the Department of Scientific and Industrial Research with the assistance of the Ministry of Transport. The Department and Ministry should also consider with the industry what assistance can most appropriately be given to the industry's own efforts to promote research into production techniques and methods aimed at increasing productivity and reducing costs. As an immediate stimulus to development, the Department of Scientific and Industrial Research should give priority consideration to proposals for development under contract in these industries and with the Ministry of Transport, and the shipping industry should consider what further steps should be taken to study the industry's future technical requirements.

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* Department of Scientific and Industrial Research. *Research and Development Requirements of the Shipbuilding and Marine Engineering Industries*. Pp. vi+18. (London: H.M. Stationery Office, 1960). 1s. 6d. net.

The President of the Board of Trade, Mr. R. Maudling, in concluding the debate on the economic situation, agreed that research and development generally need expansion. He also expressed the hope that the Mitchell Report on the machine-tool industry would point the way to a considerable expansion of research and development facilities in that industry—the report recommended that the industry's expenditure on research should be trebled. Expansion there and in the shipbuilding industry, however, as well as in nuclear power, needs to be considered in relation to the country's research and development effort generally and to the available resources in scientific and technical man-power. It is clear that the broad question is one to be considered further by the Minister for Science and his Advisory Council for Scientific Policy, for it is in this context that proposals for space research are properly considered, and also the steps to be taken to meet other shortcomings in the research effort of Britain to which the survey carried out for the Advisory Council and outlined in its last annual report pointed.

COLOUR VISION

Mechanisms of Colour Discrimination

Proceedings of an International Symposium on the Fundamental Mechanisms of the Chromatic Discrimination in Animals and Man, held in Paris, 25–29 July, 1958. Edited by Yves Galifret. (Sponsored by the International Council of Scientific Unions.) Pp. viii+296. (London and New York: Pergamon Press, 1960.) 63*s*. net.

THIS book is a report of a very interesting conference at which ten invited papers were given. These introduced a series of valuable discussions which are fully reported. About seventy per cent of the report is in English, twenty per cent in French and ten per cent in German. The topics discussed fall under the following headings: (1) zoological inventory (including a paper on colour vision in insects by Von Frisch); (2) neurophysiological problems (including papers by Rushton on retinal processes and by Galifret on the optic nerve pathways); (3) visual pigments of colour vision: (4) psychophysical problems.

Perhaps the most interesting single issue was the relation between the trichromatic theory (Young. Helmholtz) and the opponent-colour theory (Hering). The exposition of the trichromatic theory (and of its limitations) by Stiles formed an admirably clear statement. Hurvitch gave an interesting exposition of the opponent-colour theory. It was unfortunate that his paper was entitled "The Tetrachromatic Scheme", thereby suggesting that the opponent-colour theory is an alternative to the trichromatic theory. The same information may be analysed (i) in terms of three primaries, or (ii) in terms of (*a*) total luminance (which may be called the white-black difference), (*b*) the red-green difference and (*c*) the yellow-blue difference. The psychophysical data and the electrophysiological observations of De Valois show that both these analyses are 'represented' in the visual cortex. Many other experiments, including the recent work of Svaetchin and MacNicol and of Wagner and Wohlbarst. indicate that signals relevant to both analyses are present at lower levels. It is very probable that eventually the trichromatic and opponent-colour concepts will be seen as aspects of one theory, as has happened with the wave-particle 'conflict' in physics.

Dartnall, who gave a very interesting paper on the biochemistry of cone pigments, opened with the statement that "no-one can say *with certainty* that he has ever had a cone pigment in a test-tube", and although this statement may be a little too cautious, the biochemical side of colour vision is certainly very difficult. Rushton, using biophysical methods, has been able to obtain the absorption spectra of two cone pigments and to show that one is absent in protanopes, but this does not identify the molecular structures.

This is not a book for the general reader. but I recommend it most strongly to all who are working in any part of this field. I would like to record my gratitude to M. Pieron who, as chairman, led the discussions and drew out so many ideas of value. and to M. Galifret both for his own paper and for editing this volume.

R. W. DITCHBURN

RAINFALL IN BRITAIN, 1957

British Rainfall, 1957

Report on the Distribution of Rain in Space and Time over Great Britain and Northern Ireland during the Year 1957 as recorded by about 5,000 Observers. (Ninety-seventh Annual Volume). Air Ministry: Meteorological Office. Pp. iv+245. (M.O. 663.) (London: H.M. Stationery Office, 1960.) 32*s*. 6*d*. net.

THE volume of *British Rainfall* under review is the ninety-seventh of the series and covers the year 1957. It contains the usual tables, charts. and descriptions of the annual, seasonal and monthly amounts of rainfall and numbers of days with rain over Great Britain and Northern Ireland based on observations at 5,022 stations. There are also the usual tables of heavy rainfalls with descriptions of the weather situations in which they occurred.

The general pattern was a decidedly wet first quarter, a very dry second quarter, a wet third quarter and a dry last quarter. The year as a whole had an average rainfall over England but a few per cent more than average over Scotland, Northern Ireland and Wales. Evaporation, too, was about average on the whole with a large value for June, the sunniest since 1909. The largest falls of rain in one day were 6–8 in. over the Camelford area of Cornwall in thunderstorms on June 8.

A new feature in this volume is a snow survey which is for the winter 1956–57 and gives a description of snowfalls and a table of the number of days of snow lying in the mountainous areas.

G. Stanhill, of the National Vegetable Research Station, contributes an article on the variance and co-variance of evaporation and rainfall for seven well-distributed stations, which brings out the importance of taking the variance of evaporation into account in computing the variance of the water content of the soil.

An important change in the main table of stations is the omission of the particulars of the type of gauge to make room for the National Grid reference locating the position of the gauge to the nearest 100 metres. This is a reflexion on one hand of the necessity for accurate knowledge of position in connexion with the more detailed studies of areal distribution of rainfall now required, and on the other hand of the great progress towards standardization of equipment and exposure produced by the much-intensified programme of inspection of gauges by the Meteorological Office.

The preface shows that consideration is being given to the need for continuing the use of such terms as 'rain-day', 'drought', etc., specified arbitrarily in terms of the rainfall amount of one day or a period in view of the possibility of readily obtaining frequency distributions of amounts or spells by the new machine methods of data-processing now coming into use. Comments on this point are particularly invited by the Meteorological Office.

No review of *British Rainfall 1957* would be complete without a mention of the fact that 1957 was the last year of official service of Dr. J. Glasspoole, who joined the British Rainfall Organization in 1916 and was associated with its work for more than forty years.

EXPLORING THE OCEANS

Frontiers of the Sea

The Story of Oceanographic Exploration. By Robert C. Cowen. Pp. 307+21 plates. (London: Victor Gollancz, Ltd., 1960.) 25*s*. net.

IN an expanding branch of science there is a clear need for the good 'popular' book, which can present achievements and aims not only to the general reader but also to scientists in other fields, and may even enable the specialist to see his own work in a truer perspective. Oceanography has been well served by such books in recent years, and that of Robert Cowen is a worthy addition to their number. The author sets out to cover the whole field of marine science, putting present knowledge in its historical setting and indicating the probable lines of future advance. He has achieved success in this comprehensive aim by a balanced selection of topics, which he has treated clearly and vividly.

After a historical introduction, the author discusses present ideas on the origin of the Earth and the oceans. He then describes some of the remarkable new features of bottom relief discovered by recent expeditions. An account is given of the use of oxygen isotope and radiocarbon techniques in the study of sediments and of geophysical methods for probing deeper into the ocean floor. In a chapter on ocean currents, the Gulf Stream is selected as an illustration of the complex structure revealed by modern techniques, and the sub-surface Cromwell Current as one of the major discoveries of recent years. The study of life in the oceans is represented by a chapter on plankton and a brief review of the larger forms of life, giving special attention to deep-living animals.

Having surveyed the present scene, the author looks to the future and to the possibility of exploiting the resources of the oceans, as a source of food and of minerals. In a final chapter on "The New Oceanography", he describes the bathyscaphe, the mesoscaphe and the abyssal submarine, the *Aluminaut*, now being developed. The 'Mohole' project and the problems of the disposal of radioactive wastes at sea are also discussed.

In a book with so many excellent features one is reluctant to look for faults. Some of the line drawings, however, are far from precise, and it would be almost impossible for anyone unfamiliar with the instruments concerned to understand the working of, for example, the water bottles in Fig. 4 or the Kullenberg corer in Fig. 12. There are several factual errors, a serious one being the statement, on p. 201, implying that the nutrient substances account for 6 per cent of the salinity of the sea, an estimate which is too great by several orders of magnitude.

K. F. BOWDEN

HISTORICAL GEOLOGY

Géologie Stratigraphique

Par Prof. Maurice Gignoux. Cinquième édition complétée d'un addendum par Léon Moret. Pp. iv+760. (Paris: Masson et Cie., 1960.) 71 NF

THE data of stratigraphy are in themselves as dry as the dust of the Permian deserts. Undergraduate students of geology often look on stratigraphy as mere meaningless lists of names of formations and of fossils which occur in them. And of course the details of stratigraphy are meaningless unless they are related to specific problems. That is why *Principles of Stratigraphy* by C. O. Dunbar and J. Rodgers has such appeal; it concerns itself with problems of facies interpretation, and the relevance of all the detail is obvious to the reader. Similarly, L. J. Wills's *Physiographical Evolution of Britain* was such a success because the detail was related to problems of diastrophism. Much of the data of stratigraphy is, however, only relevant to problems of the subdivision and correlation of successions, which are problems not in themselves interesting though they may be important in the working out of tectonic structures. It is obviously pointless that the student should burden his memory with excessive detail of this kind. Nevertheless, a certain modicum of general knowledge of successions every student must have; and he does need a text-book to which he can turn for such information when he has to orientate himself in the local setting of a particular problem. Ever since 1926, M. Gignoux's *Géologie Stratigraphique* has been available for this purpose.

It is in many ways a first-class text-book which covers the general stratigraphy of Western Europe very fully and provides also useful information about broad features of the stratigraphy of the rest of the world. Its outstanding characteristic is clarity and simplicity. It can and should be read in French by every student of geology with the least knowledge of the language. It is true that a translation of the fourth edition (1950) is now available. (This fifth edition, published since the death of the author, differs only in having an appendix giving references to a great many recent papers.) But reading this book in French leads the undergraduate student so naturally and painlessly to a knowledge of the French technical terms he will need in reading original papers that it is well worth his trouble.

In its successive editions the book has been considerably extended and continuously brought up to date. Numerous references to original papers cited in footnotes have been added in each edition. In many respects, however, the book does have an old-fashioned look—names like *Glyphioceras*, *Anthraconauta* and *Posidonomya* have not been emended, the Palæocene is not recognized, and the Calabrian (Villafranchian) remains in the Pliocene.

The great feature of this book is its treatment of the Mesozoic and Cainozoic of France and North Africa. There is no better introduction to the complicated stratigraphy of the Alps than can be had from the splendidly lucid sections of successive chapters devoted to this subject. The new appendix to this edition by Prof. L. Moret contains a special section with numerous references to recent literature on the French Alps, with his own critical comments. This is a particularly valuable addition to that part of the book which is of most interest to the non-French reader.

S. SIMPSON

THE JESSE P. GREENSTEIN MEMORIAL SYMPOSIUM

Amino Acids, Proteins and Cancer Biochemistry

Papers presented at the Jesse P. Greenstein Memorial Symposium, Division of Biological Chemistry, American Chemical Society, September 16, 1959. Edited by John T. Edsall. Pp. ix+244. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 7 dollars.

JESSE GREENSTEIN died in 1959 and his scientific publications cover the three decades, 1930–60. All his work carried a sense of building, stone on stone—a painstaking insistence on the surest foundations so that the final result should be as sound as man could make it. He was a pioneer in amino-acid, peptide and protein chemistry all his life and, for many years, was also actively pursuing a vast programme of work on the enzymology of cancer tissues. This dual thread of endeavour running through his life's work was reflected in the contributions to the symposium held in his memory, lending them a coherence which makes their record in this volume a most gratifying one to read.

The first part contains a study by Winitz and other colleagues at the National Cancer Institute extending the series of nutritional experiments using water-soluble, chemically defined diets, which they began with Greenstein in 1957. There are papers on the structure of ribonuclease (Scheraga *et al.*) and of carboxypeptidase *A* (Neurath *et al.*); on the chromatographic evaluation of protein mixtures (Sober and Peterson), and a stimulating contribution by Alton Meister on the activation of amino-acids and the biosynthesis of peptide bonds.

The second part of the symposium stems from Greenstein's interest in cancer research. Weinhouse enlarges on Greenstein's concept of the biochemical uniformity of tumours: that different tumour tissues resemble one another in their biochemical characteristics more closely than they do their tissues of origin. He relates this generalization to a critical consideration of the 'deletion' hypothesis of the cause of cancer and comments on Greenstein's evidence for multiple enzyme deletion in neoplasms, concluding, pertinently, that since so many enzymes appear to suffer deletion after a cell becomes malignant, evidence of far greater cogency will be required before cancer initiation can be ascribed to any specific change of this type.

Roberts and Simonsen, and Saul Kit contribute chapters respectively on the free amino-acids and on the nucleic acids of normal tissues and tumours, and the symposium ends with a fine flurry of fact and speculation over which Racker and Britton Chance preside with easy control. Much of their argument is concerned with the search for a rate-limiting factor in the carbohydrate metabolism of cells, with special reference to the ascites tumour cell. The significance of adenosine diphosphate and of inorganic phosphate in this connexion is expounded and stress laid on the 'compartmentation' of adenosine diphosphate—and of reduced diphosphopyridine nucleotide—into cytoplasmic and mitochondrial forms. A comparable distinction of inorganic phosphate into endogenously formed P_i and slowly diffusible extracellular P_i, underlines the importance of knowing 'readily available' substrate and coenzyme concentrations when considering rate-limiting conditions in the cell. Explanations of both the Pasteur effect and of the well-known phenomenon of lactate production in the respiration of tumours are tentatively given with the aid of the hypotheses outlined. Britton Chance, it must be added, pursues the subject to the point of digital computer representation.

Certain interesting differences come to light in these two chapters, including a basic one on the estimation of hexokinase activities, and another on the effect of glucose on respiring ascites tumour cells (pp. 178 and 199). They may be taken as typifying the general liveliness of the text throughout the book.

"No-one can measure the full impact of the contributions of an outstanding scientist", writes Dr. Edsall in his preface; but those colleagues of Dr. Greenstein who have written in this symposium have finely acknowledged their debt and friendship.

F. H. MALPRESS

Beyond the Planet Earth

By Konstantin Tsiolkovsky. Translated from the Russian by Kenneth Syers. Pp. vi+190. (London and New York: Pergamon Press, 1960.) 15s. net.

WRITTEN in 1920, this mildly prophetic tale is described as a 'scientific fantasy'. It is well above the ordinary run of science fiction, in that the characters behave as normal human beings for most of the time, the laws of physics are also undistorted, and the author was clearly a competent scientist. As to prophecy, the longest shot occasionally finds its mark, and any consistency between fantasy and later fact need not affect its attractiveness as a fable. The preface has a lot to say about Tsiolkovsky's work on rockets, which seems to have been done unobtrusively and unsubsidized, but led to his being named as the "father of the *Sputniks*"; and some other aeronautical priorities seem to have slipped in under this umbrella. The book itself was intended to be entertaining, and is a good story.

The characters are a lively bunch; Franklin, Galileo, Helmholtz, Laplace, Newton, and the inventive Ivanov. They start operations in lush headquarters remote from modern life, which has by 2017, when they start up, become depressing. The base is a castle in the Himalayas (it will be remembered that Jules Verne had already annexed the Carpathians). They work things out, get into orbit, live graciously without gravity, find gold and precious stones on the Moon, and, after some years of resourceful exploration, land happily in the Indian Ocean to start the 'space era' going. The chief re-entry problems are the dreadful colds and influenza to which they succumb on returning to the rigours of ordinary life. A simple and almost unsophisticated story, it has been very readably translated; Mr. Syers has the Arthur Ransome touch.

G. R. NOAKES

Technology, Engineering and Safety

Vol. 2. Edited by R. Hurst, R. N. Lyon and C. M. Nicholls. (Progress in Nuclear Energy, Series IV.) Pp. xvi+799+xii. (London and New York: Pergamon Press, 1960.) 105s. net; 15 dollars.

THE present volume is the fourth in a series of eleven, and consists of a selection of thirty-four papers presented at the 1958 Geneva Conference on the peaceful uses of atomic energy, together with a short introduction by C. M. Nicholls. The papers are presented under the following chapter headings: Reactor Chemistry; Reactor Materials; Engineering Experience and Practice; Engineering Studies; and Engineering Aspects of Reactor Safety. The editors have recognized the difficulty of making a representative selection from the large number of relevant papers, and acknowledge that in some cases the choice has been somewhat arbitrary. The book covers a very wide field and includes a number of papers on various aspects of advanced reactors, such as the liquid metal fuelled reactor and molten fluoride systems which do not, however, figure prominently in present-day reactor development programmes. Nevertheless, the information presented forms a useful and interesting record of the considerable research and development work undertaken in support of these ambitious concepts. The eight papers on various aspects of reactor safety form an interesting group, and those dealing with the containment problem constitute a useful introduction to a difficult subject about which there is still a considerable diversity of opinion.

The volume represents a convenient—if somewhat expensive—summary of various aspects of reactor technology and safety, especially for those who do not have ready access to all the Geneva Conference papers. As such, it forms a useful addition to the present series.

D. ENGLISH

High-Energy Electron Scattering Tables

By Robert Herman and Prof. Robert Hofstadter. Pp. ix+278. (Stanford, Calif.: Stanford University Press; London: Oxford University Press, 1960.) 68s. net.

THIS volume of tables is designed to serve as a handbook for those concerned with high-energy electron scattering. It contains numerical values of nuclear charge density distributions, form factors of nucleons and light nuclei and cross-sections for electron-electron scattering. There is also a number of excellent figures showing point proton cross-sections and other data. The tables are preceded by a review of the theoretical basis of the calculations which may well be of wider interest, as it includes a discussion of Born approximation calculations and their accuracy, the choice of models for nuclear density distributions, and also a more detailed treatment of electron scattering by nucleons and deuterons.

A large part of the work on these topics has been carried out at Stanford University by Prof. Hofstadter and his associates. This book gives further indication of the power of the electron scattering techniques they have developed for the investigation of nuclear structure and sizes.

D. F. JACKSON

The Year Book of the Royal Society of London, 1961

(No. 65.) Pp. 302. (London: The Royal Society, 1961.) 21s.

THE *Record of the Royal Society of London* for 1897 outlines in its preface the purpose of the then newly inaugurated *Year Book* as "containing information which is liable to change". The *Year Book* of the Royal Society is a most valuable compendium for anyone who wishes to keep track of many leading scientists. Much information can be gleaned from its 302 pages, concerning Fellows and Foreign Members, membership of the many committees and boards, standing orders, Council procedure, medal awards, lectures, financial trusts and funds, special memberships and appointments, and a report of Council for the year ended September 30, 1960—a year in which the Society celebrated the tercentenary of its foundation. Appended to the report of Council are twenty-five reports on research work carried out by individuals holding Royal Society research fellowships. Without doubt, the lists of Fellows and Foreign Members is one of the most useful sections of the *Year Book*. However, anyone who is constantly having to refer to this part of the *Year Book* will be well aware that the information available is only valid to January 1, and that continual reference is necessary to the supplementary 'fly-Sheet', when it becomes available. As has been advocated in these pages in previous years, it is to be regretted that publication of the *Year Book* is not withheld until May, by which time it should be possible to include the new Fellows elected in the current year.

INTERSTELLAR AND INTERPLANETARY COMMUNICATION BY OPTICAL MASERS

By Dr. R. N. SCHWARTZ and Prof. C. H. TOWNES*

Institute for Defense Analyses, Washington, D.C.

LONG-RANGE communication by radio-waves is already well known, and the possibility of interstellar communication by radio-waves in the microwave region has been suggested in several interesting proposals[1-3] to search for signals from intelligent beings on planets associated with nearby stars. The supposition is that curiosity such as our own would motivate advanced civilizations associated with stars other than our Sun to make determined efforts to communicate with whatever other intelligent life might exist on neighbouring planetary systems. Radio-waves have, because of our present state of technological development, dominated the field of very long-distance communication, and perhaps for this reason these proposals gave particular attention only to the radio region. It appears, however, that we are now not very far from the development of maser oscillators and other appropriate apparatus in or near the optical region which will also allow detectable light signals to be beamed between planets of two stars separated by a number of light years.

Our own maser techniques in the optical and nearby spectral regions are still in a rudimentary stage; no such operating device was known a year ago[4,5]. Another ten years should bring very marked development. Further, only historical accident seems to have prevented discovery of optical masers thirty or more years ago, in which case they would probably already have been in an advanced stage of development. This implies that a separate civilization might have inverted our own history and become very sophisticated in the use of optical or infra-red masers rather than in the techniques of short radio-waves.

We propose to examine the possibility of broadcasting an optical beam from a planet associated with a star some few or some tens of light-years away at sufficient power-levels to establish communications with the Earth. There is some chance that such broadcasts from another society approximately as advanced as we are could be adequately detected by present telescopes and spectrographs, and appropriate techniques now available for detection will be discussed. Communication between planets within our own stellar system by beams from optical masers appears *a fortiori* quite practical.

Optical Maser Characteristics

Present ruby masers have produced pulsed optical beams of 10-kW. power[6], or still shorter pulses of perhaps 100-kW. peak power. This radiation is concentrated in a band-width of about 0·02 cm.$^{-1}$. Another type of maser, operating continuously at about 0·02 W., emits a wave which has been shown to be in phase over the entire maser reflector surface (about ½ in. in diameter) and to be concentrated in a frequency-interval of about 10 kilocycles per sec.[7]. The latter case is much closer to theoretical expectations[4] for an ideal maser in so far as coherence is concerned. There seems to be no general reason, other than the necessary dissipation of power, why solid-state optical masers cannot operate continuously at high power and with a short-term monochromaticity close to theoretical expectations, or hence with frequency-widths very much less than 1 megacycle/sec.

Now consider directivity of the beam from an optical maser. If a maser produces a wave of wave-length λ with constant phase over a surface of diameter d, the angular width of the radiating beam is approximately λ/d. However, it is obvious that its angular width can be still further reduced if it is operated in conjunction with an auxiliary optical system. Imagine that an ideal lens is put in the beam of focal length d and diameter d. Then, at the focal point the entire beam has a diameter approximately λ. If this focal point is made to coincide with that for a much larger ideal lens or reflecting mirror of diameter D and focal length D or larger, the beam emerges from the latter with an angular diffraction width determined by its aperture, or λ/D. To obtain this result, it is not necessary actually to focus the beam to a small spot; this was assumed only for heuristic purposes. The limiting factors in achieving an intense, directed beam probably reside, not in the source, but in the more familiar technical problems of producing large mirrors of the required accuracy and controlling the optical distortions due to heating by the beam.

For the sake of discussion, two optical maser systems will be assumed with the following characteristics:

System (*a*). Power-level, 10 kW. continuous; frequency, ~ 5000 Å.; band-width, ~ 1 megacycle/sec. or 3×10^{-5} cm.$^{-1}$; diameter of reflector (D), 200 in. (maximum size of present telescopes); beam-width (λ/D), 10^{-7} radian.

System (*b*). A group of 25 masers, each of the same maser characteristics as system (*a*), but with an effective system aperture, D, of 4 in. (and therefore a beam width of 1 sec. of arc, or 5×10^{-6} radian). The entire group is to be pointed in the same direction within the accuracy of the beam-width.

The beam-width of system (*a*) could be attained in a system operating from a platform above (or in the absence of) a planetary atmosphere and of such accurate dimensions that diffraction is the limiting factor. (See following discussion.) When operating from within an Earth-like atmosphere, the atmospheric turbulence restricts the effective band-width to that which would be achievable in a 4-in. telescope system, suggesting system (*b*) in this case as a more reasonable choice.

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Detectability of Maser Signals

Two primary criteria for detection of a maser beam emanating from near a star are: (1) it must produce enough photons per unit area at the receiver to be detectable with a lens of practical size and in a reasonable time; (2) it must be distinguishable from the background of stellar light.

The maser system operated from above an atmosphere (system (*a*)) would produce a beam of intensity, I, where: $I = \text{flux}/\lambda(D)^2 = 10^{18}$ W./sterad. at 5000 Å. or, per wave-number; $I_\nu = I/\Delta\nu = 3 \times 10^{22}$ W./sterad. cm.$^{-1}$ in a band-width $\Delta\nu = 3 \times 10^{-5}$ cm.$^{-1}$.

The intensity of radiation from system (*b*) would be less by a factor of 100. Under the most favourable conditions, a star of magnitude 8·3 can be seen by the naked eye; hence, the system (*a*) beam could be seen by eye at 0·1 light-year. With ordinary binoculars against the normal sky, the range would be about 0·4 light-year. Corresponding distances would be less by a factor of 10 for system (*b*).

From a distance of 10 light-years, the system (*a*) could be seen visually through the Hale 200-in. telescope or could be photographed with ordinary techniques with an exposure of about 1 min. This time of detection gives about 10^6 quanta of signal, and presumably could be much shortened by special effort. The distance of 10 light-years is significant because, as noted by G. Cocconi and P. Morrison[1], there are seven stars of nearly the same luminosity and spectral characteristics as our Sun within this distance. System (*b*) would require correspondingly about a 1½ hr. exposure in the Hale telescope for detection at 10 light-years. Thus criterion (1) is well satisfied.

The beam intensities indicated here are quite sufficient to communicate between planets of our solar system. They must, of course, compete with a background of light reflected from the planetary surface. The intensity of sunlight scattered by the Earth's surface is less than $1/\pi \times$ cross-sectional area $\times$ solar constant $= 6 \times 10^{16}$ W./sterad., so that the maser beams could be easily seen against the background of the Earth as viewed visually through an optical filter a few hundred angstroms wide, or as a bright spot superimposed on the image of the planet in a telescope of moderate size. This affords an alternate communications technique with some advantages for specific cases over radio communications. For example, because of directivity, light could be beamed from Mars or the Moon to a particular part only of the Earth. Furthermore, equipment for receiving simple signals need not be much more than the human eye.

Consider now the question of detection of such a signal from a distant planet against the background of light from its star. There is some, but little, hope of resolving the two spatially. The Earth and Sun subtend an angle of about ½ sec. of arc at a distance of 10 light-years. Hence light of equal intensity from the two might be resolved at this distance by a telescope of very high quality. However, one can perhaps more easily resort to high spectral resolution in order to discriminate between maser and stellar radiation.

We may take the spectrum of our own Sun as representative of the light to be discriminated against The intensity per unit wave-number of the continuum has a maximum near 5000 Å. of about:

$$I\nu \text{ (5000 Å.)} = 1{\cdot}2 \times 10^{21} \text{ W./sterad. cm.}^{-1}$$

with a total intensity:

$$I = 3 \times 10^{25} \text{ W./sterad.}$$

Here it is seen that the maximum stellar intensity per wave-number is 1/25 that of maser system (*a*) or about four times that of maser system (*b*). The band-width of 1 mc./sec. chosen for the maser might possibly be reduced to increase the relative intensity of maser to star, but possible difficulty in obtaining high-power masers with this stability and some other considerations which will be mentioned later make use of such narrow band-widths unattractive.

For the Sun, I_ν is lower than the value of 5000 Å. by more than an order of magnitude below 2500 Å. and above 15,000 Å., two orders of magnitude below 2000 Å. and above 4μ. Superimposed on this continuum is a discrete structure, the Fraunhofer absorption lines, and emission spectra in the far ultra-violet. The most pronounced absorption lines of Ca II, Sr II and Na provide windows several angstroms wide in which the intensity is less by an order of magnitude than that of the continuum. Of course, it is to be remembered that the Earth's atmosphere limits terrestrial observation to wave-lengths between 2900 Å. and some few microns.

A logical selection in transmission frequency would be either to use a frequency as far in the violet as atmospheric absorption permits, or to pick a prominent Fraunhofer line (as the Ca II *H* or *K* line). In either case, a gain by a factor of about 10–20 in the ratio is to be had over transmission at 5000 Å., the choice probably being dictated by the availability of a suitable maser material to produce the desired frequency. Were it only a question of the solar intensity, another two or three orders of magnitude could be gained by recording above the Earth's atmosphere in the 1400–1800 Å. region. However, the loss of reflecting power in mirror systems and the anticipated difficulties in maser operation at these wave-lengths make the region unattractive—even if recording above the atmosphere were not already too awkward. The advantages of transmission in the infra-red over that at 5000 Å. would be offset by the widening of the beam were diffraction the limiting factor as in system (*a*).

Assuming a maser system (*a*) operating, say, within the Ca II *H* or *K* absorption lines, the ratio of maser to Sun intensity is about 300 in the narrow band-width of 3×10^{-5} cm.$^{-1}$. A spectrometer with resolution comparable to 0·01 cm.$^{-1}$ (that is, somewhat better than a resolution of 10^6 at this frequency) would give a maser signal just equal to the stellar background. Present grating spectrographs of high speed and resolving power give a resolution close to this figure (for example, the echelle spectrograph of G. Harrison[8]). The use of interferometric techniques can provide a resolution of a few orders better than this. An instrument comparable to the coudé spectrographs developed for use in conjunction with the Hale 200-in. telescope and with the required resolution appears capable of producing an acceptable photographic image with an exposure of a little more than an hour. Much less resolution than 0·01 cm.$^{-1}$ would still yield signals detectable against background, although the maser signal would then not be so obviously narrower than some spectral emission lines.

Maser system (*b*) would give an intensity just three times that of stellar background, again in the band-width 3×10^{-5} cm.$^{-1}$. While convenient detectors operating within such a narrow band-width might be developed, none is yet available. A spectrometer of resolution 0·01 cm.$^{-1}$ would afford a maser signal 1 per cent as large as background, which might allow marginal discrimination.

One must consider the possibility that changes in Doppler shifts during the period of exposure will be larger than the spectral resolution used and thus weaken the image. The Earth's orbital or rotational motion, the orbital motion of a satellite around the Earth if such is used as an instrument platform, and corresponding motions of the transmitting source may all produce Doppler shifts which can change the frequency and smear out the maser signal. The maximum rate of variation of the fractional change of frequency due to a Doppler shift $\Delta\nu_D$ from a signal source following a circular path of radius r with angular speed ω is given by:

$$\frac{1}{\nu}\left|\frac{d(\Delta\nu)_D}{dt}\right|_{\max.} = \frac{\omega^2 r}{c}$$

where ν is the frequency and c the velocity of light. Taking the motions within our own solar system as illustrative, one obtains maximum values of 2×10^{-10} per sec. for an Earth-based transmitter mainly due to rotation of the Earth and 10^{-11} per sec. for a Moon-based transmitter. For these Doppler changes on both the receiving and sending end of the transmission to be contained within the required limits of resolution of 0·01 cm.$^{-1}$, the times of photographic exposure in the visible spectrum, then, would be restricted to about an hour. This is, as already noted, the approximate time needed for a good spectrum if the transmitter were at a distance of 10 light-years. Alternatively, both sender and receiver might, of course, compensate for his own cyclical Doppler shifts, which would be well known to him.

The question of exposure, or integration time, has a different connotation depending on whether the observer is engaged in a search of the spectrum or has found a likely line. In the first situation, hundreds of angstroms of the spectrum can be examined in a single photograph and, consequently, the spectral search of a given star is not unduly hampered by fairly long times of exposure. The spectral line sought can be expected to be exceptionally narrow, at an abnormal frequency for the type of star in question, and varying in intensity. Observation of any of these characteristics should lead to closer examination. Once a likely line has been located, a photo-detection system of higher sensitivity could be used to reduce the required integration times to minutes for the purpose of recording modulations in the signal. Actually, with a 200-in. telescope and an efficient dispersing system, the modulation could be visible to the eye.

Discussion

Thus, it appears that if one postulates the existence of an optical maser system beamed towards us of the characteristics of system (*a*) at a distance of the order of 10 light-years, it is within the state-of-the-art for us to detect it. System (*b*) is marginally detectable, or detectable with good signal to background, if very narrow-band optical receivers are developed. One should hence consider the feasibility and expense of such systems as judged by our own experience, and of course in the light of any fundamental difficulties.

Advantages accruing from the coherence of radiation over a very large aperture, or from the theoretical possibility of obtaining coherence among several maser sources are nullified if the system is required to operate from within an Earth-like atmosphere. In this case, system (*b*) above, perhaps augmented in either power-level or numbers of system elements, appears most advantageous. An appreciable increase in the number of elements would not necessarily involve prohibitive expense. Accepting a criterion of minimum detectability as a signal of only 1 per cent of the background, system (*b*) as defined would be sufficient for the job. An added difficulty in working within an atmosphere should be noted. Local air turbulence caused by hot lens and mirror surfaces in contact with the atmosphere would widen the beam even more unless well controlled. The power density reflected from the mirror surface of system (*a*) is close to that of direct sunlight. Such problems, connected with handling a large amount of power in a carefully controlled optical system would probably be some of the most troublesome ones.

The unique advantages of maser sources can be better utilized in operation from a very high-altitude balloon, a space platform, or natural Moon—a possibility that may not have been seriously considered a few years ago but should be more acceptable to-day. The capability of the ideal system (*a*) could possibly be met by using a single large mirror, although the very large mirrors with which we have had experience have not attained the highest angular resolution possible (the Hale 200-in. has an angular resolution of about 1/3 sec. of arc (ref. 9)). The task of obtaining the limit imposed by diffraction may be a difficult one. However, the beam intensities of system (*a*) might also be met by a bank of smaller mirrors of higher accuracy, perhaps operated in phase (the beam intensity varies in such case as the square of the number). In this connexion, it should be noted that the 36-in. mirror to be used in the current *Stratoscope II* high-altitude balloon programme[10] is a casting of fused quartz which is expected to achieve its theoretical angular resolution of 1/10 sec. of arc.

It may be both an encouraging enlargement of possibilities, and at the same time an unwelcomed complication, to find that the frequency of the hydrogen line in the micro-wave region is not the only reasonable place at which to search for possible interstellar communications, and that the optical region also seems a logical one. What other methods are we overlooking which might appear natural to some other civilization? Far ultra-violet and infra-red are absorbed by most imaginable atmospheres friendly to life, and hence would be avoided unless reception above the atmosphere is to be expected. Beams of charged particles would be subject to inconvenient bending in interstellar fields. We see no way of producing neutron beams or electromagnetic waves much shorter than the ultra-violet[4] with adequate intensity. On the other hand, the rapid progress of science implies that another civilization, more advanced than ourselves by only a few thousand years, might possess capabilities we now rule out—they may have already been able to send us an exploratory instrumented probe. Since none has yet been seen, perhaps it would be appropriate to examine high-resolution stellar spectra for lines which are unusually narrow, at peculiar frequencies, or varying in intensity.

Note added in proof. Dr. W. S. Boyle and Dr. R. J. Collins have independently arrived at similar conclusions (private communication).

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A PROPOSED DUAL NEUROHUMORAL ROLE OF ACETYLCHOLINE: ITS FUNCTIONS AT THE PRE- AND POST-SYNAPTIC SITES

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ACCORDING to the generally accepted concept of neurohumoral transmission, the arrival of a conducted nerve impulse at the presynaptic terminals of a cholinergic fibre causes them to liberate acetylcholine, which then diffuses across the exceedingly narrow (50–200 Å.) synaptic gap and combines with specific receptors at the postsynaptic site; the combination results in the production of a localized postsynaptic potential, which, if excitatory, triggers off a propagated nerve action potential[1]. In non-cholinergic fibres, other transmitter agents are considered to take equivalent roles; although several naturally occurring substances have been proposed as such, the only other neurohumoral agent which has been demonstrated reasonably convincingly is norepinephrine as the transmitter of the adrenergic postganglionic sympathetic fibres.

From the foundations laid by Loewi, Dale, Feldberg and their collaborators, subsequent work has largely confirmed the foregoing hypothesis. It has, moreover, provided the basis for explaining the mechanisms of action of several classes of drugs, particularly those acting on the peripheral divisions of the autonomic nervous system, its effector organs, and the motor end-plate of skeletal muscle. However, several observations in various laboratories, including my own, during the past few years, while not entirely inconsistent with the hypothesis, cannot be fully explained by it as presented above. The working hypothesis proposed here to explain these phenomena is that the acetylcholine mobilized by a conducted presynaptic impulse may act at two sites: postsynaptically, as outlined above, but prior to that at the presynaptic terminal itself, to bring about the release from the terminal of sufficient additional acetylcholine or of some other neurohumoral agent to initiate the characteristic postsynaptic effect. The proposed action of acetylcholine at its site of liberation is equivalent to its long-postulated role as a local hormone in smooth muscle and other tissues.

There is little claim to originality in the present proposal: it has been invoked in part before, directly or indirectly, to explain isolated phenomena. However, it is believed that the evidence which follows is now sufficient to present it as a general concept.

Histochemical Distribution of Acetylcholinesterase in Nervous Tissue

Determinations conducted on homogenates of various neural tissues have shown that, with certain exceptions, there is fairly good correlation between the concentrations of choline acetylase, acetylcholine and acetylcholinesterase, and that all three entities are present in relatively large amounts in cholinergic neurones (see ref. 2 for references). With the development of a microscopic histochemical technique for cholinesterases[3], and its subsequent modification to permit selective localization of acetylcholinesterase[4,5], it was possible to demonstrate that, in the cat, known cholinergic neurones (for example, anterior horn cells, preganglionic autonomic neurones) contain high concentrations of acetylcholinesterase throughout their entire lengths, whereas adrenergic neurones (for example, the great majority of those in the superior cervical and stellate ganglia) contain scarcely detectable concentrations. All the neurones of the parasympathetic ciliary ganglia are heavily stained for acetylcholinesterase; consequently, the small number of heavily stained neurones of the sympathetic ganglia were considered to be those giving rise to the cholinergic fibres to the sweat glands and the cholinergic vasodilator fibres. On the other hand, a limited number of cat sympathetic ganglion cells show intermediate staining, an observation which has been confirmed quantitatively by Sjöqvist and Holmstedt[6]. Likewise, the sensory neurones of the dorsal root ganglia, which are generally considered non-cholinergic, show intermediate grades of staining[5]. In the rabbit and monkey, in contrast to the cat, the differences between the autonomic cholinergic and adrenergic neurones are not nearly so pronounced; all the latter appear to contain relatively low but appreciable concentrations of acetylcholinesterase; again, concentrations in the sensory neurones in these species are intermediate[5]. The same situation was found in the brain of the rat[7]: all neurones giving rise to somatic motor fibres (for example, the hypoglossal nucleus) and parasympathetic preganglionic fibres (for example, the Edinger–Westphal nucleus) and those of certain correlation centres (for example, the amygdaloid and caudate nuclei) were heavily stained; others (for example, the magnocellular division of the red nucleus) were practically unstained, whereas many (for example, the supraoptic nucleus and lateral thalamic nuclei) showed intermediate grades of staining. As a possible explanation for these observations, it was proposed a few years ago that the terms 'cholinergic' and 'adrenergic' might define the predominant, but not necessarily the exclusive, transmitting agent of a given neurone, and that those containing intermediate concentrations of acetylcholinesterase might liberate both acetylcholine and another transmitter[5].

Superior Cervical Ganglion

From the comparative effects of lipid-soluble and insoluble anticholinesterase agents, it has been shown that the neuronal acetylcholinesterase is separated by the cell membrane into external and internal fractions[8,9] and that transmission in the cat superior cervical ganglion is affected by inhibition of only the former[10]. At that site, the external, or functional, acetylcholinesterase is entirely presynaptic[11], in contrast to the neuromuscular junction, where most of the enzyme is postsynaptic[12]. From this it was inferred that acetylcholinesterase serves different primary functions at the two sites. In the course of an investigation of the physiological function of acetylcholinesterase in the ganglion, my colleague, Volle[13], determined the mean threshold doses of intra-arterially injected carbamylcholine and acetylcholine for producing postganglionic activation in series of normal and preganglionically denervated cats, both before and after inactivation of the ganglionic acetylcholinesterase by di*iso*propylfluorophosphate. To our surprise, the thresholds to both agents were markedly elevated by denervation; with carbamylcholine, a nearly thirty-fold elevation was evident prior to administration of di*iso*propylfluorophosphate, whereas a five-fold elevation in the acetylcholine-threshold was unmasked only after inactivation of acetylcholinesterase. To explain these findings, we proposed that the threshold dose of acetylcholine or carbamylcholine in normal cats activated only the presynaptic terminals, causing them in turn to liberate acetylcholine immediately adjacent to the postsynaptic receptor sites, which in turn activated the ganglion cells. Following denervation, the higher thresholds were presumed to reflect those required for direct excitation of the ganglion cells. Consequently, it would appear that the primary function of the presynaptic ganglionic acetylcholinesterase is to limit the effect on the presynaptic terminals themselves of acetylcholine liberated there during transmission or the resting stage. Other investigators have published evidence of the susceptibility of the preganglionic terminals to activation by acetylcholine in pseudorabies-infected rats[14,15], and in normal cats[16], although the interpretation of the latter findings has been questioned[17].

Hypothalamico-Neurohypophyseal Tract

The work of Scharrer and Scharrer[18] and others has provided strong evidence that the hormones of the neurohypophysis are formed in the supraoptic and paraventricular nuclei of the hypothalamus, transported within their axons through the infundibulum, and stored until liberated in their terminations, the Herring bodies, within the neurohypophysis. From the work of Pickford and her associates[19,20], it appears likely that the hypothalamic control of neurohypophyseal secretion is regulated by cholinergic fibres. In an attempt to localize the cholinergic pathways involved, Abrahams[21], while visiting my laboratory, conducted a histochemical survey of the distribution of acetylcholinesterase in the hypothalamus of the dog. Unexpectedly, the enzyme was found in moderate concentrations only in the neurones of the supraoptic and paraventricular nuclei and their axons, and not in any of the presynaptic fibres adjacent to them. From the evidence cited earlier, this indicated that the only cholinergic neurones in the area of the hypothalamico-hypophyseal tract are the same ones which are assumed to elaborate the neurohypophyseal secretions. Among the suggestions offered at that time was that the same neurones might, indeed, liberate at their terminals both acetylcholine and the endocrine secretions, the latter under the influence of the former. More direct evidence of this possibility, and independent arrival at the same proposal, has recently been published from the laboratory of De Robertis. From an electron microscopic study of the neurohypophysis of the toad, these investigators discovered the presence in the neuronal terminals of two distinct populations of vesicles. Those of one group were dense to electrons, had an average diameter of 1150 Å., and could be traced back to the hypothalamus; they were identified as the neurosecretory product. Those of the other group were considerably less dense, averaged 420 Å. in diameter, and appeared only at the terminals; they were considered to be identical with the synaptic vesicles that have been described at various presynaptic sites[23], and may represent the storage form of acetylcholine and other transmitters[24]. Following this report, we have examined the neurohypophysis of the cat histochemically, and have found that the nerve fibres and associated Herring bodies stain consistently for acetylcholinesterase (Koelle, G. B., and Geesey, C., unpublished work). While indirect, the evidence is thus strong that the hypothalamico-neurohypophyseal fibres liberate both acetylcholine and endocrine secretions, and that the latter are under cholinergic control. In this situation, there is no participation of an immediate postsynaptic site.

Afferent Vagus

The primary sensory fibres are the only component of the peripheral nervous system for which a transmitter has not been identified. On the basis of their low contents of acetylcholine and choline acetylase, and the inconclusive effects of anti-cholinesterase agents on transmission[25], they are generally considered non-cholinergic. Matsumura, in my laboratory (unpublished work), has recently repeated the procedure developed by De Castro[26] for the establishment of functional re-innervation of the preganglionically denervated superior cervical ganglion by the afferent neurones of the peripheral vagus located in the nodose ganglion. Histochemically, the regenerated vagal fibres which synapsed with the superior cervical ganglion cells showed definite acetylcholinesterase activity, but were stained much less intensively than the normal preganglionic fibres. Ganglionic transmission was potentiated by eserine. It was blocked by hexamethonium and tetraethylammonium, in the same dosage-range as produced blockading of intra-arterially injected acetylcholine; however, much higher doses were required to produce equivalent blockade in normal ganglia. While alternative explanations are equally likely, these findings are compatible with the proposal that acetylcholine normally takes an intermediate or subsidiary role in liberating another primary transmitter of the afferent vagal fibres.

Postganglionic Adrenergic Fibres

During the past few years, Burn and Rand[27-29] have developed an interesting new concept of the mechanism of action of tyramine, ephedrine and related non-catechol sympathomimetic amines. Stated in brief, they have obtained evidence that the major component of the action of such amines consists in the liberation of endogenous catecholamines from storage sites in the vicinity of the peripheral terminations of the postganglionic adrenergic fibres. Furthermore, they have shown that a cholinergic mechanism probably is involved in the liberation of catecholamines by the adrenergic fibres following their stimulation. Evidence for the latter phenomenon is based on two types of findings: following atropinization, the injection of acetylcholine or nicotine produces sympathomimetic effects at peripheral sites; after depletion of the peripheral stores of catecholamines by pretreatment with reserpine, stimulation of sympathetic nerves to many organs produces cholinomimetic effects. The location of the peripheral stores of catecholamines is still conjectural. Burn and Rand have considered both the terminations of the adrenergic fibres themselves and adjacent chromaffin cells as possibilities. Without excluding the likelihood of the latter, the former is consistent with the present proposal; it is strengthened by the finding of Burn and Rand that the stores are depleted of catecholamines following sectioning and degeneration of the sympathetic fibres. Again recognizing the possibility of alternative interpretations, this might then represent another example of the release of acetylcholine from axonal terminals in response to the nerve impulse, and its triggering in turn the subsequent release of the actual neuroeffector transmitter, norepinephrine, from the same terminals. It is interesting to speculate that this process may occur in the sympathetic neurones containing moderate concentrations of acetylcholinesterase, whereas the release of norepinephrine from those practically devoid of the enzyme may occur normally without the mediation of a cholinergic mechanism. Most of these points have been discussed by Burn and Rand[29].

Peripheral Terminals of Motor and Sensory Fibres

The terminations of somatic motor nerves have been shown to respond to injections of various cholinomimetic drugs by the discharge of antidromically conducted impulses, simultaneously with postsynaptic activation of the motor end-plate[30,31]. This might be construed as reflecting a physiological situation similar to that proposed for the superior cervical ganglion; that is, that normally the acetylcholine first liberated by the terminals promotes the liberation of additional acetylcholine from the same source to act as the transmitter of the neuroeffector.

More provocative is the question of the possible role of acetylcholine or a similar agent at the peripheral terminals of sensory fibres. It is well established that a great variety of sensory terminals or end-organs (for example, carotid chemo-receptors and stretch receptors, Pacinian corpuscles, taste buds) are activated by injected acetylcholine, and that this action is blocked by ganglionic blocking agents such as hexamethonium, tetraethylammonium, and nicotine[32]. However, it is a moot point whether this reflects the participation of acetylcholine in their physiological activation by normal stimuli[33], or whether it represents a purely pharmacological phenomenon[34].

Yet there is a remarkable parallelism between the events which occur at the dendritic terminals of crustacean stretch receptors[35], or at the Pacinian corpuscle[36], and those recorded at the motor end-plate in response to stimulation of the motor nerve fibre or local application of acetylcholine[37]. The initial response to pressure at the tip of the afferent fibre within the bulb of the Pacinian corpuscle is the development of a transducer or generator potential; this, like the end-plate potential at the neuromuscular junction, is relatively slow, graded, and is not propagated. At the region of the first node of Ranvier, the transducer potential triggers the propagated nerve action potential, just as the end-plate potential initiates the propagated muscle action potential. Hence, the transducer potential of the Pacinian corpuscle has the characteristics of a localized, chemically induced response. In their electron microscopic study of the corpuscle, Pease and Quilliam[38] noted that the enclosed nerve terminal contains inclusions similar to the synaptic vesicles found elsewhere at presynaptic sites. The sum of these observations leads to the suggestion that the normal mechanism for the initiation of the transducer potential, in response to pressure, may be the liberation of free acetylcholine or a similar agent by the same terminal on which it then acts. The nerve filament and central core of the Pacinian corpuscle of the cat have been shown histochemically to contain both acetylcholinesterase and non-specific or pseudocholinesterase[39], although the latter undoubtedly predominates[40,41]. A similar ratio for the two enzymes has been noted in the carotid body of the cat, in which the chemoreceptor cells contain considerable pseudocholinesterase and the nerve fibres a low concentration of acetylcholinesterase[4]. As in the terminals of the hypothalamico-neurohypophyseal tract, the Pacinian corpuscle represents a possible site of acetylcholine function where apparently no actual postsynaptic site is involved.

Conclusions

In the foregoing discussion, evidence from several lines of investigation has been presented in support of the proposal that acetylcholine, released at the presynaptic terminals by a nerve impulse, may have a dual role in synaptic and neuro-effector transmission: an action at the same site effecting the release of another transmitter, or possibly of additional quanta of acetylcholine, and a transmitter action at the postsynaptic site. This does not necessarily imply that acetylcholine plays any direct part in the propagation of the conducted nerve

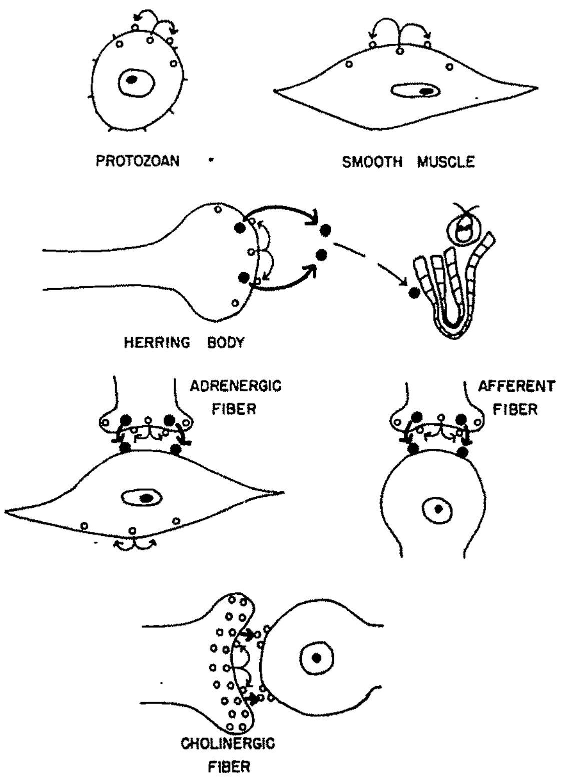

Fig. 1. Diagrammatic representation of the (1) direct influences of acetylcholine localized to its sites of liberation (protozoans, smooth muscle), (2) indirect influences through liberation of hormones acting at remote sites (from Herring bodies) and of neurohumoral agents acting at adjacent sites (from adrenergic and primary afferent fibres), and (3) direct influences at adjacent sites as a neurohumoral transmitter (from cholinergic fibres)

impulse, as proposed by Nachmansohn[42], nor does it preclude consideration of that possibility. The two actions considered here are presumably localized.

The postsynaptic transmitter role of acetylcholine seems now too well documented to require further discussion. There is also considerable evidence for its action at sites of release where no process of intercellular transmission is involved. The most direct demonstration of this phenomenon is in the ciliated protozoon, *Tetrahymena gelii*, which contains acetylcholinesterase, and in which acetylcholine appears to exert some control over ciliary activity[43]. Higher in the evolutionary scale, acetylcholine is involved in the beat of the cilia in the gills of the clam, *Mytilus edulis*; here, it is apparently formed by, and acts on, the ciliary epithelium without the intervention of any neural mechanism[44]. Likewise, in mammalian smooth[45] and cardiac[46,47] muscle, acetylcholine probably has a role as a 'local hormone', in that it appears to be liberated by the same muscle fibres on which it acts in the regulation of tone and rhythmicity, perhaps through modification of membrane permeability; superimposed on this inherent mechanism is the influence of acetylcholine liberated from the postganglionic parasympathetic fibres.

The proposed physiological activity of acetylcholine may be summarized in Fig. 1. At some sites, it has only a localized action on the cells in which it is formed, as in the last-quoted examples. Among such localized effects are included the liberation of secretory substances acting at a distance, as in the neurohypophysis, and, in the nervous system, of neurohumoral agents such as norepinephrine in adrenergic fibres, and perhaps an unidentified transmitter in the primary afferent fibres. In the so-called cholinergic fibres, sufficient acetylcholine is liberated, quite possibly in two steps, to act both locally, as above, and as the transmitting agent at the postsynaptic site. A universal role of acetylcholine at all synaptic and neuroeffector sites is not necessarily implied. In some cases, another agent or agents may have similar functions at the presynaptic site, just as other neurohumoral transmitters act post-synaptically where so-called non-cholinergic fibres are involved. Furthermore, the possibility is not excluded that a given fibre might liberate more than one transmitter acting post-synaptically, as previously proposed[5]. Finally, in isolated instances, synaptic transmission may occur without the mediation of a neurohumoral agent[48].

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THE GASSIOT COMMITTEE OF THE ROYAL SOCIETY AND METEOROLOGICAL RESEARCH

By DR. D. C. MARTIN, C.B.E.

Assistant Secretary of the Royal Society

IN 1871, Mr. John Peter Gassiot conveyed to the Royal Society £10,000 of Italian Irrigation Bonds for the purpose of assisting magnetical and meteorological observations with self-recording instruments, primarily in the Kew Observatory. The Society set up its Gassiot Committee in the same year, and, apart from the Society's Sectional Committees, its Finance, Library and Scientific Relief Committee, it is one of the oldest of the Society's committees. The income of the Gassiot Fund has since 1871 been applied to the purposes set out in the Gassiot Trust Deed. Until 1910 the proceeds were passed first to the Kew Committee, then to the National Physical Laboratory Account of the Royal Society when the National Physical Laboratory had responsibilities in respect of the Kew Observatory. In 1910 the management of Kew Observatory was transferred to the Director of the Meteorological Office, with provision for scientific advice from the Gassiot Committee. Since 1920, when the Meteorological Office was incorporated into the Air Ministry, the income of the Gassiot Fund of the Royal Society has been paid annually to the Air Ministry on the recommendation of the Gassiot Committee in accordance with John Peter Gassiot's wishes.

In 1942, after consultation with the Air Ministry, it was agreed that the Royal Society should cooperate with the Meteorological Research Committee to assist the Air Council in the development of meteorological research on a wider basis and the Gassiot Committee was given terms of reference which included the following: (1) to advise the Royal Society on matters relating to pure research in meteorology and to supervise the disposal of such funds as may be made available by Council for this purpose; (2) to recommend as to the work of the meteorological and magnetic observatories with which the Society is connected; (3) to administer the Gassiot and other trust funds applicable to their maintenance. The Council of the Society asked the Committee to formulate plans for work on atmospheric composition, photochemistry, ultra-violet spectroscopy, synoptic meteorology and infra-red spectrometry. The Committee set up three sub-committees to assist it in encouraging this new programme of work, the sub-committees being entitled: (*a*) Atmospheric Composition and Motion; (*b*) Photochemistry of the Atmosphere; (*c*) Atmospheric Radiation and Temperature. With the aid of a grant, which was initially £5,000 per annum, and was later increased to £7,000 per annum, a worthwhile programme of work has been undertaken at a number of university centres. Among these were pioneering ozone studies by Prof. G. M. B. Dobson, at the University of Oxford, which have led to the creation of the permanent ozone survey service of the International Ozone Commission of the World Meteorological Organization; ultra-violet spectroscopic studies by Dr. T. W. Wormwell at Cambridge; infra-red work by Prof. P. A. Sheppard at the Imperial College of Science and Technology; research on molecular spectra by Dr. R. W. B. Pearse at the Imperial College; excitation of spectra by Sir Harrie Massey at University College, London; measurement of absorption cross-section by Prof. R. W. Ditchburn at the University of Reading; studies of airglow and auroral phenomena by Prof. D. R. Bates at the Queen's University of Belfast, and by Mr. J. Paton at the University of Edinburgh; and study of atmospheric winds by Mr. J. A. Ratcliffe at the Cavendish Laboratory, Cambridge. These studies have been carried out in close co-operation with various programmes of the Meteorological Office and Prof. T. G. Cowling of the University of Leeds, as well as Prof. D. R. Bates and others, contribute to the theoretical aspects of the work. Annually, the Gassiot Committee presents a report on its work to the Royal Society Council, and this report is forwarded to the Secretary of State for Air. From time to time, on the recommendation of the Gassiot Committee, special Royal Society discussion meetings have been held on subjects in the Gassiot programme. Prof. G. M. B. Dobson was the Committee's chairman during 1942–47, when he was succeeded by Sir David Brunt. Since 1952, Sir Harrie Massey has been chairman.

As early as 1953 the Gassiot Committee set up a small *ad hoc* sub-committee to consider whether atmospheric research, using rockets, might be initiated in Great Britain, and it organized a conference on the subject which was held in Oxford in that year. In 1955 a grant of £50,000 was received by the Royal Society from the Air Ministry for a programme of investigation of the upper atmosphere using high-altitude rockets. The Gassiot Committee created a new sub-committee to co-ordinate work at a number of university centres and to co-operate with the Ministry of Supply, particularly the Royal Aircraft Establishment. This programme, using *Skylark* rockets, has been very successful and has now been incorporated into the larger United Kingdom Space Research Programme which owes its beginning to the initiative taken by the Gassiot Committee in 1953.

During the past few years the organization of research programmes under the ægis of the Meteorological Research Committee has been re-organized, and some of the work hitherto conducted by the Gassiot Committee is considered to be more appropriately carried out directly by the Meteorological Research Committee itself through extra-mural contracts with university scientists. Some of those who have been supported in the past through the Royal Society will continue on the basis of such contracts and others wishing to carry out meteorological research should make application to the Director-General of the Meteorological Office, who is willing to support such research at university centres.

The Meteorological Office is, however, also anxious to continue its long and intimate connexion with the Gassiot Committee, and the Office recognizes that it provides an excellent medium for encouraging a wider interest in the problems of the atmosphere in universities, not least in subjects which border on meteorology. Arrangements have therefore been made for continued co-operation with the Meteorological Research Committee, it being agreed that the Gassiot Committee will concentrate its efforts on those problems of the physics of the atmosphere and allied subjects that are not at present dealt with by the Meteorological Research Committee. Initially, the Gassiot Committee, supported by an Air Ministry grant, will encourage further research in the photochemistry of the atmosphere and will promote, through discussion and other means, interest in other aspects. Close co-operation will be maintained with the Meteorological Research Committee by arranging joint meetings to discuss the Gassiot research programme. It is hoped that university physicists and others interested in its programme and not already in contact with the Gassiot Committee will approach the Royal Society.

The time-honoured association between the Meteorological Office and the Gassiot Fund, instituted in 1871, is to take a new form, which is warmly welcomed by the Royal Society. The Secretary of State for Air has approved the recommendation of the Meteorological Committee that two wholetime fellowships for research in the fields of seismology and geomagnetism respectively be established.

The income of the Gassiot Fund, now paid annually to the Air Ministry as mentioned above, will be regarded as a contribution to one of the fellowships, which will be tenable at Kew Observatory, and both fellowships are to be known as Gassiot fellowships, thereby preserving the historic connexion with John Peter Gassiot. The Royal Society Council accepted the invitation to nominate two representatives to the selection committee for these appointments, and Sir Edward Bullard and Sir Basil Schonland agreed to serve.

This story, which began ninety years ago, is an example of how a relatively small gift placed in the hands of a responsible body by a benefactor of science to encourage scientific endeavour has led to the development of a significant programme of co-operative research on a scale beyond his wildest dreams.

CARL LUDWIG GIESECKE (1761–1833)

By JOAN M. EYLES

SIR CHARLES GIESECKE, best known as a mineralogist, was born in Augsberg on April 6, 1761. His real name was Johann Georg Metzler, and his reasons for changing his name have never been satisfactorily established. He studied at Göttingen under J. F. Blumenbach, from whom he received his grounding in mineralogy. In his early years he was devoted to music and the stage, associating with Mozart, and taking some part in writing "The Magic Flute". This period of his life, perhaps some thirteen years, was mainly spent in Vienna. In 1794 he resumed his mineralogical studies, and travelled extensively to collect minerals, first in Austria and Germany, later in the Scandinavian countries.

In 1805, Giesecke was in Copenhagen, and was then commissioned by King Christian of Denmark to journey to Greenland to examine its minerals and prepare maps. He arrived in Greenland in 1806 and spent nearly seven years there. His private collection of minerals, which he had left in Copenhagen, was destroyed when the English navy bombarded the city in 1807. While in Greenland he discovered the famous cryolite occurrence at Ivigtut, on the Arsuk Fiord. Specimens of this rare mineral, a double fluoride of sodium and aluminium, had been sent to Denmark in the eighteenth century by missionaries, but the place from which they had been obtained was not known until Giesecke located it. This deposit later became of great importance to the aluminium industry. On his return to Denmark in 1813, Giesecke's ship called at Leith, and he spent some time in Edinburgh with Scottish mineralogists. Here he found some of the minerals that he had collected in Greenland. The ship in which they had been dispatched to Denmark had been captured by a French privateer, which, in turn, had been captured by an English frigate.

While in Edinburgh, Giesecke learnt that the Royal Dublin Society wished to appoint a professor of mineralogy. He applied for the post, and was elected by a large majority over several other candidates, although at the time he did not know enough English to lecture in the language—an indication of the esteem in which his achievements as a mineralogist were held. Giesecke held this post in Dublin until his death. In 1816, he was made a knight of the Danish Order of the Dannebrog, and became known as Sir Charles Giesecke. Thenceforward, apart from a brief return to Denmark and his native Augsberg between 1817 and 1819, he made Dublin his home. Here he lectured on mineralogy, arranged the valuable collections of the Royal Dublin Society, and also made mineralogical journeys into the west of Ireland. His published work relates mainly to Greenland and Ireland. He died suddenly in 1833, and the museum of the Society was closed for a fortnight as a mark of respect for his memory. His portrait, by Raeburn, was presented to the Society in 1817.

OBITUARIES

Dr. C. V. Drysdale, C.B., O.B.E.

Charles Vickery Drysdale, well known for his work on the design of electrical measuring instruments, died at Bexhill on February 7, at the age of eighty-six.

He was born in 1874, his parents being Dr. Charles R. Drysdale and Alice Vickery Drysdale. Like his father, his mother also was medically trained and was well known as Dr. Alice Drysdale Vickery (retaining her maiden name Vickery for professional purposes). She was born at Barnstaple, Devon, in 1846 and was fully trained in medicine and surgery for six years in London and Paris, but even in 1880 the British examining bodies refused to accept women. She went to Dublin, however, and obtained diplomas at King and Queen's College of Physicians certifying her as physician and licentiate in medicine and midwifery.

Dr. Drysdale was educated privately, and at Finsbury Technical College, and the Central Technical College, South Kensington. He was associate head of the Electrical Engineering and Applied Physics Department of the Northampton Institute (London) during 1896–1910.

He became a fellow of the Physical Society in 1898, and was a member of Council 1936–39 and Duddell medallist in 1935. He was also a member of the old Optical Society, of which he became president for 1903–4. A founder fellow of the Institute of Physics in 1920, he served on the Board during 1924–25 and was a vice-president during 1932–36 and editor of the *Journal of Scientific Instruments* during 1927–28. He was a Fellow of the Royal Society of Edinburgh and a member of the Board of Managers of the Royal Institution (1934–36). In 1920 he delivered the Kelvin Lecture on "Modern Marine Problems" at the Institution of Electrical Engineers, of which he was a member. He was honorary secretary of the Students' Section of the Institution of Electrical Engineers in 1894. He was also a Fellow of the Royal Statistical Society.

In addition to his scientific activities, Dr. Drysdale was a staunch member of the Malthusian League, of which he had been secretary, president and editor of the League's *Journal* during 1907–16. In 1921 and 1925 he had acted as president of Neo-Malthusian International Conferences in London and New York. He was a member of the Athenæum Club.

He was well known for his work on optical instruments, and for many years (1902–29) was examiner to the Spectacle Makers' Company. Such a wide range of interests is seldom found in one individual.

During 1910–16 Dr. Drysdale was closely associated with Mr. H. Tinsley, during part of this period in partnership, as makers of electrical measuring instruments. There he supervised the manufacture of many of his inventions like the a.c. potentiometer and the polyphase wattmeter, which are still made to-day as high-precision instruments. One of his great achievements was the phase-shifting transformer, the basis of servo-mechanisms in use throughout the world.

I first met Dr. Drysdale in January 1918 when he joined the staff of the Admiralty Research Laboratory at Parkeston Quay, Harwich. There he applied his wide knowledge of alternating current theory and electrical instrument design to Service problems. He designed sensitive mechanically tuned a.c. relays of sub-audio frequency, which could be actuated at a considerable range by the electromagnetic field surrounding a cable, lying on the sea bed, carrying alternating current of the same frequency as that of the relay. Afterwards (1919–21), at the Admiralty Experimental Station at Shandon (Gareloch), he made use of this system in the development of a 'leader cable' with visual indication on the bridge, to show the navigating officer the position of his ship, port or starboard, relative to the cable. Certain anomalies in this otherwise simple and useful system were observed, however, and this led Dr. Drysdale to make a very thorough full-scale investigation of the field distribution below and above the sea-surface around a submarine cable carrying alternating current. This work was done in Gareloch (off the Clyde) near Shandon and Rhu. The results of this important investigation, combined with those of theoretical and model-scale experimental work by S. Butterworth at the Admiralty Research Laboratory, Teddington, were published in the *Philosophical Transactions of the Royal Society* in 1924. This provided an explanation of the ambiguous indications of Dr. Drysdale's experimental leader cable. During the course of this research be became the first superintendent of the Admiralty Research Laboratory, Teddington, in 1921, a post which he held until 1929. In that year he was appointed director of scientific research, Admiralty, in succession to Sir Frank E. Smith. While serving in this post, in which he was in charge of all Admiralty research establishments, he was made a C.B. in 1932. He retired from Admiralty service in 1934.

He was a prolific writer, having published many original scientific and electrical engineering papers in *The Journal of the Institution of Electrical Engineers, The Electrician, The Proceedings of the Physical Society, The Journal of Scientific Instruments*, etc., as well as many contributions on Malthusian and Neo-Malthusian principles. One of his most important scientific papers is the one to which I have referred (*Trans. Roy. Soc.*) above. His book on *The Foundations of Alternating Current Theory* (published in 1910) was based on a series of lectures, extending over five years, which he gave at the Northampton Institute. This was one of the earliest efforts of which I am aware to emphasize the close analogy which exists in the theory of electrical and mechanical oscillations. At the end of the First World War he contributed to a collected work on *Mechanical Properties of Fluids*, in which he dealt with submarine signalling and the transmission of sound through water. His best-known book, *Electrical Measuring Instruments* (two vols.), in collaboration with Mr. A. C. Jolley, contains the work of many years study, theoretical and experimental, of the principles of design and operation of all kinds of electrical measuring instruments.

Dr. Drysdale lived a very full and energetic life, having a wide range of interests. He always took a great delight in seeing his theoretical deductions verified by an experiment. I remember him, however, working on an experiment at the Royal Institution, after his retirement from the Admiralty, to demonstrate that the basis of Einstein's theory of relativity was wrong. At the time of his death he was busy writing another book.

In 1898 he married Bessie Ingram Edwards, who died some years ago. They had one daughter, Eva, who died young, and an adopted son, Eric, who is married and lives in South America.

A. B. Wood

Prof. A. H. Cox

The death of Prof. A. Hubert Cox on February 14, 1961, will be sadly mourned by his many students dispersed all over the world and by geologists in Britain and in Canada. After graduating at the University of Birmingham as a chemist, he studied in Strasbourg where he carried out research work on benzenoid compounds and a great variety of other chemical problems.

During the First World War he became engaged on chemical work of great national importance which ultimately caused him to turn from chemistry to mineralogy and finally to stratigraphic and structural geology. Even prior to becoming professor of geology in the University College of South Wales and Monmouthshire, he had carried out important research work on the Ordovician rocks of Wales. His enthusiasm and energy in tackling the difficult and unknown structure and petrology of Cader Idris became symbolic of his future interest in the geology of the Principality. His name will always be associated with the pioneering work that was done in the early part of this century on the older rocks of Wales and, in particular, with the establishment of type-sections in Pembrokeshire. Conscious of the contribution which geology and geologists should make to industry, he devoted himself to the problems connected with the development of the coals of South Wales. He was among the first to apply geophysical methods to the determination of geological structures, and his use of resistivity measurements in the Coal Measures became the basis for a great deal of the modern work in this field of geophysics. He was among the first to illustrate how resistivity methods could be used to locate water-saturated strata in the Coal Measures and how those could be used in the development of the mines. His earlier training as a chemist made him singularly able to conduct investigations into the incidence of silicosis, as it was then called, among the miners of the South Wales coalfield. So tireless were his efforts to secure knowledge of the environmental factors concerning this disease among miners and so conscious was he of his duty as a scientist to the community, that his work became responsible for the complete revision of the pensions schemes for miners afflicted with pneumoconiosis.

Likewise, his contribution to the welfare and the development of the University College, Cardiff, will always be remembered and revered, and very specially by the many students entrusted to his care. Through many of these he was able to make profound contributions to the understanding of the structure of the Canadian Rockies and more especially to that of the Selkirk Mountains. His arduous work helped to establish the location of several important reserves of gold and silver and he can be said to have been among the pioneers of the scientific development of the mineral resources of Western Canada.

W. D. Evans

NEWS and VIEWS

Director of the National Lending Library for Science and Technology: Dr. D. J. Urquhart

The Department of Scientific and Industrial Research recently announced that Dr. D. J. Urquhart has been appointed the first director of the National Lending Library for Science and Technology at Boston Spa, Yorks. Dr. Urquhart was educated at Barnard Castle School and the University of Sheffield where he graduated in 1931 with an honours degree in physics. Afterwards he obtained a Ph.D. in the Metallurgical Faculty at Sheffield, and then he worked in the research department of a steel works. In 1938, Dr. Urquhart was appointed to the staff of the Science Museum Library. During the War he was at the Ministry of Supply, engaged mainly in the technical administration of armaments inspection. In 1948, he was transferred to the Information and Intelligence Division of the Department of Scientific and Industrial Research, where he was responsible for the reorganization of the Headquarters Library and Records and carried out a number of surveys of the use made of technical literature in different industrial centres. In November 1956 he became responsible for planning the National Lending Library. Since then he has been wholly concerned with the planning and creation of the new library, which has included the introduction of Britain's Russian translating programme in co-operation with the National Science Foundation's programme in the United States. Dr. Urquhart writes frequently on library and technical information topics. In recent years he has made a number of notable contributions in this field, starting with a paper he read to the University and Research Section of the Library Association in January 1957 on the new National Lending Library. It was in this paper that Dr. Urquhart estimated the size and requirements of the National Lending Library, and forecast the huge growth of Chinese scientific literature in the future. He dealt with this subject again in a paper to the Library Association last year. In 1958, at the International Conference on Scientific Information in Washington, he reported the results of an interesting analysis of the pattern of borrowing from the Science Museum Library which has served to guide the acquisition policy of the National Lending Library.

Civil Engineering at Swansea: Prof. O. C. Zienkiewicz

Dr. O. C. Zienkiewicz, who has been appointed to succeed Prof. B. G. Neal (see *Nature*, 188, 896; 1960) in the chair of civil engineering at the University College of Swansea, will bring to his new appointment experience of both British and American universities.

After graduating from the Imperial College of Science and Technology in 1943, Dr. Zienkiewicz conducted research into some problems of gravity dam design under Profs. A. J. S. Pippard and R. V. Southwell at the same institution. In 1945 he joined Sir William Halcrow and Partners, and for four years he gained experience in the design and construction of dams and hydro-electric projects. He was then appointed a lecturer in civil engineering at the University of Edinburgh where he remained until 1957, when he joined the staff at Northwestern University, Evanston, as an associate professor, becoming a full professor in 1960. Dr. Zienkiewicz's research interests have ranged widely, embracing both fluid mechanics and stress analysis. Apart from his work on gravity dams, several of his early papers were concerned with the behaviour of surge waves and with problems of hydrodynamic lubrication. His main interest at present is in the field of thermal stress analysis in elastic and viscous materials, and he is investigating applications to the design of concrete dams, reactor shields and other shell structures. Several lines of approach are being considered, notably structural models and analogue methods as well as analytical and numerical procedures. The building of new accommodation for all the Departments of Applied Science is to commence at Swansea in 1962. Dr. Zienkiewicz will therefore have the opportunity to plan further developments in civil engineering, specially suited to his own research interests, and he must also establish arrangements for teaching the much greater number of undergraduates to be admitted in the near future. His colleagues in this task will be Prof. R. H. Macmillan (Mechanical Engineering) and Prof. W. Fishwick (Electrical Engineering), now chairman of the Department.

Zoology at Ghana : Prof. E. E. Edwards

Prof. E. E. Edwards, who retired this year from the chair of zoology at the University College of Ghana, has become director of a new Entomological and Parasitological Research Unit under the National Research Council established by the Ghana Government in 1958. He will therefore be able to continue his interests in tropical entomology and parasitology which were a feature of the University Zoology Department under his professorship. He was appointed to Achimota, then the University College of the Gold Coast, in 1952, and during his occupancy of the chair was responsible for the planning of the zoological laboratories at the new Legon site of the College.

Prof. R. D. Purchon

Prof. R. D. Purchon, who has now been appointed to succeed Prof. Edwards, goes to Ghana from the chair of zoology at the University of Malaya in Singapore, which he has held for eleven years. During that time he established a very active school for the study of the animal ecology of Malaya, with particular emphasis on marine biology. Facilities for this type of work are already well founded in Ghana, where Accra has served as a centre for much of the previous work of the University College in this field.

British Participation in Space Research

In asking leave of the House of Commons on March 21 to introduce a Bill to provide for the establishment of a Minister of Space Research and Development, and for connected purposes, Mr. Woodrow Wyatt argued that no real progress in this important new field would be made until Britain had a Minister in the Cabinet responsible for co-ordinating everything to do with space. The Americans were spending £3 10*s.* a year per head of their population on space development, and the Russians an equivalent sum because they knew that the resultant advances in technology would be injected into other branches of industry. Britain, however, was spending not more than 1*d.* per head, and to fall 3 or 4 years behind in any department gravely handicapped technologists and made their task of keeping Britain in the forefront more difficult. Mr. Wyatt asserted that too many Ministries had a fringe interest and none an overriding concern in space matters, and that the Minister for Science was not sufficiently interested. He referred to the profit to be made out of communication satellite systems, but alleged that effort was being duplicated and that the Postmaster-General was indifferent to the work being done in this field by firms like the English Electric Co., Hawker-Siddeley, and Pye, and maintained that the future of Britain's export trade and her standard of living depended on not being left out of the space age. Leave to introduce the Bill was granted and the Second Reading ordered for May 5.

British Funds for Oceanographic and Hydrographic Research

At the Committee stage of the Navy Estimates 1961–62 in the House of Commons on March 14, several questions were raised on the vote of £21 million for scientific services, an increase of £1·7 million. This represents about 5 per cent of the Navy Vote of £400 million, but Mr. C. Ian Orr-Ewing, Civil Lord of the Admiralty, admitted that the true expenditure on research and development was considerably more, including expenditure incurred through the Ministry of Aviation. The 98-in. Isaac Newton telescope now being erected at Herstmonceux would cost about £780,000, of which half would come from Navy Votes and half from the Treasury, and it would not only be used by the Royal Greenwich Observatory, but would also be available for general use by other astronomers in the United Kingdom. Replying to specific questions about hydrographic work, Mr. Orr-Ewing said that R.R.S. *Discovery II* would cost some £570,000 this year, of which £285,000 would come from the Navy Vote and £285,000 from the Development Fund. Its maximum range would be 15,000 miles and its cruising speed would be 9–10 knots. It would be 260 ft. long with a loaded draught of 18 ft., and it was planned to have a scientific plotting room at deck-level; electronic hydrographic, biological, chemical and general laboratories, a low-temperature laboratory and an underwater instrument room with a trunk leading through the bottom of the ship. About the same amount as last year would be spent on research and development of nuclear propulsion, but there was a considerable increase in salaries, wages and allowances, resulting from adjustments to keep those of the scientific staff with salaries of scientists in industry and elsewhere. The vote included a grant-in-aid to the National Institute of Oceanography and a subscription to the International Hydrographic Bureau.

Water Research in Britain

In a written answer to a question in the House of Commons on March 20 regarding Department of Scientific and Industrial Research grants for research on the utilization and conservation of water supplies, the Parliamentary Secretary for Science, Mr. D. Freeth, said that direct expenditure in such research could not be separated precisely from that which had an indirect bearing on the subject. The best estimates of current expenditure (1960–61) by the Department and co-operative research associations were: Geological Survey and Museum, £32,350; Hydraulics Research Station, £93,100; Water Pollution Research Laboratory, £148,650; British Coke Research Association, £18,750; British Leather Manufacturers Research Association, £6,800; British Paper and Board Industries Research Association, £9,500; Water Research Association, £35,000; current grants to universities for special research work, £11,000.

Students Awards in Scotland

In a written answer in the House of Commons on March 28, the Secretary of State for Scotland, Mr. J. S. Maclay, said that, with the agreement of the local authority associations, from next October awards would be made by the Scottish Education Department to full-time university students, students training as teachers and students taking advanced full-time or sandwich courses at other establishments. Although the education authorities would no longer make the awards, their local officers would assist the Department by interviewing students and making local inquiries when questions of special difficulty arose, and they would be responsible for advising young people in their area as to facilities available for further education. They would continue to award bursaries to other further education students and to school pupils. There will be standard maintenance allowances for students to whom the Scottish Education Department made awards at the rate fixed for the university or college which the student actually attended. These rates have been fixed on an interim basis as announced on December 19, and it is intended to undertake a comprehensive review of the rates during the coming year.

The Match-making Industry in Britain

To mark their hundredth anniversary, the well-known match-making firm of Bryant and May has issued an attractive booklet which describes not only the history of the company but also changes in the way matches have been made over the years. Matches were invented in 1827 by an Englishman named John Walker, whose substances were: potassium chlorate, sulphide of antimony and gum arabic. A piece of fine sandpaper was included in each box. Herbert Spencer called this invention the greatest boon and blessing to come to mankind in the nineteenth century. Match-making materials have varied little since the time of the first British patent granted to Francis May in 1855. The head of the 'strike-anywhere' match contains chlorate of potash (to provide the oxygen) and sesquisulphide of phosphorus, while a safety-head match consists mainly of chlorate of potash and sulphur, providing flame when rubbed against the amorphous phosphorus on the side of the box. The materials in both heads are bound together by special Scottish glue. The sticks are treated with paraffin to help them burn and ammonium phosphate to prevent smouldering. A feature of the match industry is the curious way certain districts in Britain will buy certain types of matches—only the box is different—and no other.

U.S. Federal Expenditure on Science

In its ninth issue of *Federal Funds for Science*, the National Science Foundation analyses Federal expenditure on scientific research and development in the fiscal years 1959–61, during which period the total increased from 6,200 million dollars to 7,700 million dollars in 1960 and an estimated 8,200 million dollars in 1961; the corresponding obligations are 7,400, 8,600 and 8,500 million dollars, respectively, and of the last, about 10 per cent is for basic research (Surveys of Science Resources Series. Pp. iii+89. Washington, D.C.: Government Printing Office, 1960. 50 cents). As in 1960, some 90 per cent of the total is expected to be administered by the Department of Defense, the Atomic Energy Commission and the National Aeronautics and Space Administration. 44 per cent of Federal obligations for basic research in 1960 were allocated to educational institutions, 30 per cent to Federal agencies and 14 per cent to profit organizations. Obligations to the National Science Foundation increased by one-third in 1960 to 72 million dollars, and an increase to 84 million dollars is anticipated in 1961. Of obligations totalling about 1,900 million dollars for basic and applied research in 1960, the physical sciences, including mathematics and engineering, accounted for 56 per cent, the biological sciences for 27 per cent and the remaining 17 per cent went to the psychological, social and other sciences. Of this 'other sciences', about 85 per cent is accounted for by research into the space sciences administered by the National Aeronautics and Space Administration. Trends in expenditure and obligations over the period 1940–61 are also analysed in the report.

The Australian Scientist

It is a fair wind that finds among the farrago of scientific journals one that is devoted to science in its broadest fields. The tendency in the past has been for most countries to produce at some time or other a scientific showpiece, and it was logical that in time Australian scientists should wish to make their contribution. *The Australian Scientist*, a new journal (1, No. 1 (February 1961). Pp. 70. Subscription rates (1 year): Australia, Fiji, New Guinea, New Zealand £A4 4*s*. 0*d*.; Commonwealth countries (excluding the above) £Sterling £3 12*s*. 6*d*.; Non-Commonwealth countries £Sterling £4 5*s*. 0*d*. Liechhardt, Sydney: Tasman Productions, 9–23 Upward Street, 1961), does much more than just act as a showroom for Australian science, and represents a major contribution to the communication of science in general. As would be expected from the strong editorial board, consisting of Prof. A. A. Abbie, Prof. S. T. Butler, Dr. A. S. Fraser, Prof. R. J. W. Le Fevre, Prof. H. Messel, Sir Mark Oliphant, Prof. R. N. Robertson, Prof. R. H. Thorp and Sir Samuel Wadham, the first issue sets a very high standard of material and presentation. The pattern of the journal follows the traditional form of many of the world's established journals, but rather than just represent science it also acts as a spokesman; and it is planned that a section of letters to the editor will commence as soon as practicable.

PHYSICS DEPARTMENTAL LIBRARY

The first issue contains, besides the foreword by Dr. D. A. Cameron, Minister for Health and Minister in Charge, Commonwealth Scientific and Industrial Research Organization, a forthright article entitled "New Deal in Science Education", by Prof. H. Messel, and articles on mast cells, automatic computation, seashore life in Australia, genetics, civil engineering, cosmic radiation, plasma physics, the cyclotron, drugs and the heart, all by Australian authorities, and aimed at the greatest possible circle of readers. The outward appearance of the journal is most striking, and all articles are generously illustrated, and some even include a portrait of the author. While certain illustrations are much enhanced by colour, it does not seem that some warrant it (Figs. 4 and 5 on pp. 64–65), and their reduction to black and white would have, at least, done something towards lowering the cost of production and hence the price of the journal.

Birds killed by Poisonous Seed-Dressings

A REPORT, issued jointly by the British Trust for Ornithology and the Royal Society for the Protection of Birds, is presented in an *Intelligence Unit Bulletin* (No. 13, March 1961) issued by the Council for Nature, and provides firm evidence that many, if not most, of the large numbers of birds found dead in various parts of Britain in the spring of 1960 had been poisoned by consuming dressed corn either directly or by eating some other creature that had itself eaten the corn. The report lists 67 localities from Aberdeenshire to Kent in which dead birds were found; in 59 of these cases seed dressings are believed to have been the cause of death. Sufficient corpses were analysed to point to dieldrin as the seed dressing principally concerned. The most numerous birds reported dead were wood pigeons; there were almost as many pheasants, and smaller numbers of rooks, crows, chaffinches, partridges and blackbirds. Among insectivorous and predatory birds killed were two each of the tawny owl, buzzard and wren, and single specimens of the barn owl, lapwing and kestrel. A few mammals were also reported killed in the same way, among them three dogs, three cats, two hares and singletons of the fox, weasel and hedgehog. Fear is expressed that another great slaughter of birds is likely this spring, due to the heavy sowing programme which, as a result of the wet autumn and winter, farmers will have to undertake in the next few weeks. An appeal is made therefore to all naturalists to send information of any dead birds they may find to Peter Conder, Royal Society for the Protection of Birds, 25 Eccleston Square, London, S.W.1. The basic facts required are the date on which kills began, the numbers and species of birds and mammals involved, and the name of the seed dressing used nearby. One or two of the corpses of birds larger than pigeons, from each kill, would also be most useful for chemical analysis to determine the cause of death.

Nature Conservancy: New Appointments

LORD HURCOMB has been appointed chairman of the Nature Conservancy in succession to Mr. Arthur Bryce Duncan. Previously, Lord Hurcomb was chairman of the Committee for England, and is succeeded by Prof. A. R. Clapham. Lord Howick of Glendale has been appointed as a member of the Nature Conservancy. Sir Charles Connell, Prof. J. H. Burnett and Major S. F. Macdonald Lockhart have joined the Conservancy's Scottish Committee; the vacancies having arisen through the retirement of Prof. J. R. Matthews, Major D. C. Bowser and Mr. Michael Noble. Dr. A. M. Massee and Prof. D. L. Linton have replaced on the Committee for England Sir William Taylor and Dr. A. S. Watt, who have retired. On the Committee for Wales, Prof. P. F. Wareing and Mr. W. Vaughan Lewis fill the vacancies arising from the retirement of Prof. F. W. Rogers Brambell and Mrs. I. M. Vaughan.

The Textile Institute Scholarships

THREE scholarships are being offered by the Textile Institute: *The Textile Institute Scholarship*, which is a three years scholarship, total value £1,050, for the study of textile technology, and is open to young people who are, or have been, or intend to be, engaged in the cotton spinning or cotton weaving section of the textile industry in the United Kingdom; *The Peter Coats Scholarship*, which is for two or three years to the value of not more than £100 per annum, tenable at the textile department of any technical college in the British Isles, and is open to students who wish to take a course leading to a college diploma or other qualification in subjects connected with the cotton industry; *The Rowland Spencer Scholarship* is for training or research, to the value of £100 per annum, for persons already engaged in the textile industry and undertaking a part-time course at a recognized technical college. The closing date for applications for all scholarships is May 1, and application should be made to the General Secretary, Textile Institute, 10 Blackfriars Street, Manchester 3.

Gulbenkian Foundation Grants

THE trustees of the Calouste Gulbenkian Foundation of Lisbon have announced the grants, totalling about £550,000, made during the second half of 1960 (making a total of more than £1 million for the year). Of these, grants in the United Kingdom and British Commonwealth total about £70,000. Among the grants in aid of science are: £2,000 per annum for five years to the University of London Institute of Archæology (total £10,000) for the academic part of a postgraduate course in the care and restoration of ancient buildings; £2,000 for a gas liquefier for the Gulbenkian Radio-carbon Dating Laboratory, to the University College of Rhodesia and Nyasaland; £29,949 for cytological work at the Institute of Histology and Embryology, University of Lisbon; £3,750 to the Fondation Européenne pour les Echanges Internationaux to enable postgraduate science students to study in Portugal.

University News: Belfast

ADRIAN P. ROBERTS has been appointed senior lecturer in engineering mathematics. Mr. Roberts has been a lecturer in electrical engineering at the Imperial College of Science and Technology.

The College of Aeronautics, Cranfield

THE following appointments have been announced: *Lectureships*, E. R. Norster and J. H. Dunmore (aircraft propulsion); H. B. Gatland (electrical engineering); D. Hodgetts and G. H. Tidbury (automobile engineering). *Senior research fellowships*, Dr. G. T. Csanady (Department of Aerodynamics); Dr.

W. D. G. Bennett (Department of Materials). *Research fellowships*, D. Hyde (Department of Flight); A. Kaye (Department of Materials).

Leeds

THE following appointments to lectureships have been announced: Dr. J. F. Bowers (mathematics); Dr. A. Myers (physics); Mr. M. Wells (electronic computing).

London

DR. D. J. BOHM, Research Fellow in the University of Bristol, has been appointed to the University chair of theoretical physics tenable at Birkbeck College. The following titles have been conferred: that of reader in spectroscopy in the University of London, on Dr. Edna M. F. Roe in respect of her post at the Institute of Cancer Research: Royal Cancer Hospital, and that of reader in chemical pathology in the University of London, on Dr. I. D. P. Wootton, in respect of his post at the Postgraduate Medical School of London.

Mr. Desmond Curran, senior psychiatrist at St. George's Hospital, has been appointed to the University chair of psychiatry tenable at St. George's Hospital Medical School. Mr. D. J. Wiseman, assistant keeper at the British Museum, has been appointed to the University chair of Assyriology tenable at the School of Oriental and African Studies. Dr. C. Long, head of the Biochemistry Division at the Institute of Basic Medical Sciences, has been appointed to the University readership in biochemistry tenable at that Institute.

Announcements

ANY material that has to do with the life of Alan Gregg, late of the Rockefeller Foundation, will be welcomed by his biographer. This should be sent to Dr. Wilder Penfield, Montreal Neurological Institute, Montreal, Canada.

THE annual conference of the Museums Association will be held in Plymouth during June 19–23, under the presidency of Dr. D. B. Harden. Further information can be obtained from the Secretary, Museums Association, 33 Fitzroy Street, Fitzroy Square, London, W.1.

A CONFERENCE on "The Control of Noise" is to be held at the National Physical Laboratory during June 26–28. Admission is by personal invitation and anyone interested should write to the Director, National Physical Laboratory, Teddington, Middlesex, from whom further information can be obtained.

THE Italian Society of Chemotherapy is organizing a symposium at the beginning of June. Among the subjects to be considered are: new penicillins; antitumoural antibiotics; activity and use of nitrofurane derivatives; radioprotective drugs. Further information can be obtained from the Secretariat of the Italian Society of Chemotherapy, Via Andrea del Sarto 21, Milan.

THE Northern Branch of Aslib is organizing a residential conference at St. Mary's College, University of Durham during April 20–22. To be included in the programme are a number of papers: "The Battle of the Books"; "O. and M. in the Library"; "The Function of the Library in Research Projects"; "The New University Library in Newcastle upon Tyne"; "The National Lending Library at Thorp Arch". Further information can be obtained from Miss E. Boddy, c/o Edgar Allen and Co., Ltd., Imperial Steelworks, Sheffield, 9.

"AUTOMATION—Men and Money" is the title of the first British conference on the social and economic effects of automation to be organized by the British Conference on Automation and Computation. The conference is to be held at Harrogate during June 27–30. Further information can be obtained from Miss Elizabeth Ellett, British Institute of Management, 80 Fetter Lane, London, E.C.4.

THE four hundred and fifth meeting of the Biochemical Society will be held in the University of Birmingham during April 28–29, when there will be a colloquium on "Metabolic Pathways of Foreign Compounds". Further information can be obtained from Dr. W. J. Whelan, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1.

THE eleventh meeting of holders of Nobel Prizes will be held in Lindau during June 26–30. Among those present who are to speak are: Prof. A. I. Virtanen (Helsinki); Prof. L. Ruzicka (Zurich); Sir Robert Robinson (London); Prof. P. Debye (Ithaca). Further information can be obtained from the Secretariat der Tagungen der Nobelpreisträger, Lindau, Bodensee, Postfach 11.

A MEETING of the Welsh Soils Discussion Group, to be held on May 3, will take the form of a full-day field meeting in the Aberystwyth hinterland. The theme of "Glaciation as Related to Soil Profile Development" will be continued by a field examination of (*a*) soil profiles revealed in forest roads in Dovey Forest and (*b*) cliff sections along the coast south of Aberystwyth towards Aberaeron. Particulars can be obtained from Mr. James A. Taylor, Department of Geography, Alexandra Road, Aberystwyth.

THE sixth Nuclear Congress, organized by the Comitato Nazionale per l'Energia Nucleare, is being held at the Palazzo dei Congressi, Rome, during June 13–18. Three symposia are being included in the programme: "The Thorium–Uranium Cycle"; "Nuclear Applications in Agriculture"; "Autoradiographic Techniques in Medical Science". A study day on "Conventional Engineering Problems in Nuclear Plants" is also being arranged. Further information can be obtained from Ufficio Stampa e Relazioni Pubbliche, CNEN, via Belisario 15, Rome.

AN informal discussion on the effects of ionizing radiations on organic and biological solids will be held at the Laboratoire de Chimie Physique, 11 Rue Pierre Curie, Paris V$^{\text{eme}}$, during July 3–4, under the joint auspices of the British Biophysical Society and the Société de Chimie Physique. Information and application forms can be obtained from one of the organizers, Prof. J. A. V. Butler, Chester Beatty Research Institute, Fulham Road, London, S.W.3: Dr. L. H. Gray, Mount Vernon Hospital, Northwood, Middlesex, England; or M. Magat, 11 Rue Pierre Curie, Paris V$^{\text{eme}}$, France.

THE name of T. Kobayashi (Japan) as one of the vice-presidents of the International Union of Geological Sciences has been omitted from a note appearing in a recent issue of *Nature* (April 8, p. 132).

PLANT VIRUS DISEASES

A GENERAL paper-reading meeting of the Association of Applied Biologists was held at the British Museum (Natural History), Cromwell Road, London, on February 10. Dr. C. Potter was in the chair. Although not advertised as a symposium it might well have been, for it had a coherent content of new and unpublished work on diseases caused by plant viruses in crops.

Several themes, apart from the obvious ones implicit in the titles, linked the different contributions. The first was the ubiquity of soil-transmitted viruses, some known and some suspected to be transmitted by nematodes. Another was seed-transmission which recurred in several contexts, and a third, carried or 'pick-a-back' viruses. These are viruses that cannot be transmitted alone by arthropods, but may be so in the presence of another virus that is transmissible by a vector.

All these aspects of transmission were mentioned in the first paper on "Some Celery Viruses", by Mr. M. Hollings (Glasshouse Research Institute). Among viruses isolated from celery crops was celery yellow vein, transmissible by grafting and by manual inoculation of infected sap, as well as through naturally infected soil. It reacts strongly to antisera prepared against a strain of tomato black ring virus, which is transmitted by seed, soil and probably nematodes. Cucumber mosaic and lucerne mosaic viruses were isolated from the field. A previously unrecorded virus, provisionally called celery yellow spot, could be transmitted from celery to celery only when cucumber mosaic was present ; cucumber mosaic alone did not cause the symptoms, and yellow spot alone was not transmissible. Another previously undescribed virus, celery yellow net, was transmitted by grafting or manual inoculation only during the winter months; no aphid or other vector was found.

Mrs. M. A. Watson (Rothamsted Experimental Station) described carrot motley dwarf virus which causes a disease of carrots known to growers as purple top. In an experiment, infection in July caused a loss of about one ton per acre of carrots for every additional 15 per cent of plants infected. The willow-carrot aphid, *Cavariella aegopodiae*, retained the virus for many days when adequately fed on infected plants. Motley dwarf may be a complex of two viruses, motley dwarf proper and red leaf. Motley dwarf proper was transmissible by aphids and by manual inoculation to *Nicotiana* spp. but not by aphids thereafter. Red leaf has not been transmitted by manual inoculation, and does not seem able to infect *Nicotiana* spp. Possibly its presence enables motley dwarf to be transmitted by aphids, and it may be transmitted by seed in an attenuated form. Motley dwarf virus was infective in saps extracted with phenol, but infectivity was completely destroyed by addition of ribonuclease to a final dilution of 0·01 mgm./l., 3 min. before inoculation, suggesting that infective material in phenol extracts is nucleic acid.

Dr. J. A. Tomlinson (National Vegetable Research Station) showed that lettuce crops can be kept reasonably free from damage by lettuce mosaic virus in the summer, by sowing seed containing less than 0·1 per cent of seed-infection. In winter, cucumber mosaic caused a disease resembling that caused by lettuce mosaic. Cucumber mosaic is not transmitted by seed so control measures based on seed hygiene were less successful in winter than in summer. An unidentified virus caused symptoms similar to those of arabis mosaic when inoculated to *Chenopodium* spp., but was symptomless in lettuce.

Dr. R. Markham (Agricultural Research Council Virus Research Unit) presented a joint paper with Dr. R. MacLeod on "A Persistent Aphid-transmitted Virus Disease of Tobacco". This virus, provisionally called tobacco yellow net, was not manually inoculable, but was transmitted by *Myzus persicae*, which could retain it for several days after a single adequate feeding on infected leaves. It resembled the 'vein-distorting' component of K. M. Smith's tobacco rosette complex of viruses, and, like 'vein-distorting' virus, was able to act as a carrier for the other component of the complex, mottle virus, which was otherwise not transmissible by aphids. Yellow net might be easier to handle biochemically than other aphid-transmitted persistent viruses which usually have less amenable hosts than tobacco. However, vein-distorting virus, which it resembles, has proved disappointing in this respect.

At the afternoon session emphasis was on soil-transmitted viruses. Some of these, for example arabis mosaic, have previously been known to be transmitted through infected soil, but nematodes have only recently been shown to be the vectors.

Mr. R. M. Lister (Scottish Horticultural Research Institute), in a joint paper with Dr. A. F. Murant, described seed-transmission of arabis mosaic, raspberry ringspot and tomato black ring viruses in a wide variety of hosts, including many weeds. These viruses are disseminated by distribution of vegetative material from nematode-infested sites, but dissemination of infected seed may spread them even more widely. However, such spread is limited by the susceptibility of the wild plants to virus and vectors, and the amount of seed that becomes infected. Infected weeds may have poor ability to survive in competition with healthy ones and in cultivation. Even if they survive, it may be in sites free from suitable nematodes. These factors probably reduce the chances of virus being spread by seed transmission.

Speakers in the discussion also thought that the three viruses are mainly spread by infected propagating material, but the importance of the discovery that they are transmitted by seed was stressed. Mr. Lister mentioned that the viruses were pollen-transmitted, and distribution by pollen may be more effective than by seed.

Dr. B. D. Harrison (Rothamsted Experimental Station) gave a joint paper with Dr. R. D. Winslow on arabis mosaic virus. It included one of the first accounts of the behaviour of a nematode-transmitted virus in relation to its vectors. The virus has previously been associated with important diseases of raspberry, strawberry and cherry, very recently with white clover, marrow and celery. The patchy distribution of diseased plants in crops coincides with that in the soil of *Xiphinema diversicaudatum*, the vector of arabis mosaic virus. Adult nematodes usually transmit better than larvæ, but both are infective after being kept without plants for several weeks. The virus remains viable a much shorter time *in vitro*, and must be in some way stabilized in the nematodes; possibly it multiplies in them. Galls that develop on roots of plants infested with *X. diversicaudatum* are caused by nematode feeding and not by virus infection. Evidence is accumulating that transmission of different nematode-transmitted viruses is specific to particular species of nematodes. Ecological studies showed that in some districts hedgerow plants are important hosts of arabis mosaic and of *X. diversicaudatum*, but in others the vectors, and probably the virus, are more widely distributed in the fields. An interesting discussion centred around the possibility that cultivation, and not preference of the nematodes for a particular environment, determined this distribution. A grass field that had not been cultivated for thirty years had nematodes only near its hedgerows, but Dr. F. Raw did not think thirty years to be a particularly long time for soil animals not to have returned after cultivation.

An important paper by Mr. R. Cropley (East Malling Research Station) described the identification and symptoms of viruses isolated from cherry trees with rasp leaf and leaf roll diseases which are widespread and damaging in sweet cherry orchards. Rasp leaf was found associated with raspberry ringspot virus, but the symptoms, of reduction in leaf size and rough enations on the backs of the leaves, can also be caused by arabis mosaic virus. Cherry leaf roll virus is readily transmissible by manual inoculation to many herbaceous hosts. Symptoms, thermal inactivation and particle size and shape are characteristic of soil, or nematode-borne 'ringspot' viruses, but leaf roll seems to be distinct from those already known. It is manually inoculable from cherry to *Prunus pennsylvanica*, and from tobacco to cherry and strawberry. Virus can be detected in young leaf tissues in the spring, but not in summer and autumn.

In the discussion Dr. Posnette emphasized the importance of cherry leaf roll ; he thought that degeneration of orchards often attributed to age or poor husbandry was really caused by the virus. It was suggested that Tomlinson's lettuce latent virus (see paper No. 2) might be the same as cherry leaf roll virus.

An interesting symposium can be made to sound dull by the use of the dread word 'practical'. However, this series of papers gave information of practical importance to farmers and growers, as is only proper for applied biologists, but also contributed to general understanding of plant virus problems and reported advances of a more fundamental kind in virus research. There was clear indication of trends that could become important in the future.

MARION A. WATSON

PHYSICAL CONDITIONS OF SOIL

On March 1 about eighty members of the National Vegetable Research Station Association attended talks and demonstrations on "Soil Physical Conditions affecting Plant Growth", given by the staff of the Chemistry and Irrigation Sections at Wellesbourne.

It is well known that the overall physical condition of a soil, often referred to as soil structure, and, in the surface layers, soil tilth, has considerable effect on the growth of plants. Furthermore, applications of bulky organic materials, such as farmyard manure and compost, have given yields of vegetables much higher than those produced by inorganic fertilizers alone. The general reason advanced for this difference is that the bulky organic manures improve the physical condition of the soil as well as providing plant nutrients. While many opinions have been expressed, in text-books and elsewhere, as to which of the soil physical properties are important, for example, water-holding capacity and aeration, there is still a dearth of experimental evidence on the relations between measurable specific physical properties of the soil and plant growth.

Experimental work in progress at Wellesbourne on the following subjects was described and demonstrated: soil aeration; soil moisture characteristics; the uptake of nutrients by plants, especially in the early seedling stage; the distribution and activity of the roots of crop plants; and the effect of soil physical conditions on the responses of plants to applications of irrigation water.

At present, there is no satisfactory method for measuring soil aeration, and hence there is no means of assessing the importance of this factor in crop production. The distribution of oxygen in a soil may affect plant growth by influencing the ability of plant roots to take up nutrients, and by altering the activities of the micro-organisms and thus the amount of nutrients available to the plant roots. Work, so far, has been confined to the latter topic. With the view of using the activities of the soil micro-organisms as indicators of availability of oxygen, the effect of oxygen concentration on them is being studied. Actively metabolizing micro-organisms in soil are covered with a thin layer of water which is exposed to the soil atmosphere. Since large differences exist between the oxygen concentrations at different points in the water layer, techniques have been devised which enabled the activities of the soil micro-organisms to be correlated with the oxygen concentration in the water at their surfaces. It has been shown that the respiration of the soil organisms and nitrification were not inhibited, and denitrification and anaerobic decomposition of soil organic matter were not induced, unless the concentration of oxygen at the microbial surfaces was less than about 10^{-6} M. Using microbial activities as indicators of oxygen concentration, physical theories of the distribution of oxygen in columns of moist soil crumbs are being tested, and efforts are being made to find out if it is possible to obtain the parameters necessary for the theories from simple measurements on field soils.

In field experiments at Wellesbourne during 1954–61, applications of farmyard manure produced large increases in the yield of a wide range of vegetables. Measurements of the moisture characteristics of the manured and of the unmanured soils during the growth of a crop of leeks showed that at planting time in July the available water capacity of the two soils was the same, but by October the available water capacity of the manured soil was 24 per cent greater than that of the unmanured soil. Further, the extra water was held at tensions less than 0·33 atmosphere, and, therefore, would be expected to be readily available to plants. These results could explain the large yield response of leeks to the application of farmyard manure which is especially marked in dry seasons.

The results of growth analyses of carrot crops grown with and without farmyard manure showed that, during the very early stages of growth, the rate of increase of dry weight was much greater on plots where farmyard manure had been applied, and that this initial difference could affect the final yield of the crops. Chemical analyses of the seedlings revealed differences in composition which were larger than might have been expected from the results of laboratory assessments of the available nutrients in the treated and untreated soils. Experiments on the uptake of nutrients by seedlings in the first five days after emergence have shown that the amount of potassium taken up during this period affects the rate of growth of the seedlings for about 35 days, irrespective of the potassium nutrient treatment given after the first 5-day period. While it appears that potassium was involved in the stimulation of growth of the seedlings on the farmyard manure-treated plots, the reason for the much greater uptake of potassium by seedlings on these plots is not known.

The effect of soil conditions on the distribution and activity of plant roots is important to the nutrient and water economy of the plant, but, unfortunately, there is little fundamental information on this subject. Some work has been done at Wellesbourne on the distribution of the root systems of various vegetable crops, and attention is now focused on devising suitable techniques for the investigation of the factors affecting root activities in defined soil systems.

In the midland, eastern and south-eastern counties of England, crop production is limited in four years out of five by insufficient rainfall. Irrigation is now widely practised in these areas and, in general, produces increased yields of most crops. However, the response to applications of irrigation water is affected by the physical condition of the soil, and on certain soils, notably silts and fine sands, yields can be decreased if irrigation water is applied in such a way that the surface of the soil is beaten down to form a 'cap'. Methods of irrigation to overcome this disadvantage are now being studied, together with the causes of the detrimental effect of the 'cap' on plant growth.

F. HAWORTH

SCIENCE AND EDUCATION IN COMMUNIST CHINA

IN a paper presented to the American Association for the Advancement of Science at its New York meeting on December 27, 1960, Dr. T. H. E. Chen, describing the position of science and scientists in Communist China, explains that to the Chinese Communist science and politics were inseparable, and every scientist had to pledge himself unreservedly and unquestioningly to the Communist party: the study of politics and ideology was as important as the study of science. Ever since 1949, increasing pressure had been exerted on China's scientists to conform politically and ideologically, and scientists were required to follow the direction of the Communist party in their scientific work. Political interference was found in many areas and the idea of objective science was rejected. As regards the present position, Dr. Chen said there was a new interest in science and technology, and technical institutes at secondary and higher levels were producing large numbers of engineers, technicians and technologists. Better facilities for scientific study had been provided since 1956–57, living conditions and salaries for scientists had been improved, and laboratory and library facilities expanded. The predominant emphasis remained on applied science and technology and also violent opposition to science for its own sake. Moreover, scientists were so hemmed in by political restrictions and burdened with political requirements that there was little room for initiative and creativity. There had, however, been a mushroom growth of new ideas in technical innovations and improvements, but despite such spectacular results, much was lacking from the point of view of a long-range and balanced programme of scientific development. There was reason to believe, however, that beneath the outward conformity a good deal of resistance to Communist pressure still existed among scientists.

In a paper on the training, growth and utilization of scientific and technical man-power in Communist China, presented at the same meeting, L. A. Orleans, of the Library of Congress, reviewed briefly the educational system in Communist China, and especially its capacity to produce scientists and technicians, and then considered the professional man-power of China, its competence and the effectiveness with which it is used. Mr. Orleans stressed the importance of using with discretion data on Communist China. Despite some revolutionary educational reforms, the framework of the educational system is approximately that which existed under the Nationalist regime, but it is supplemented by a great variety of educational facilities which do, in fact, provide training in various subjects and skills with different degrees of complexity and intensity. Between 1949 and 1957 enrolment in primary schools increased by 40 million, in secondary schools by about 6 million and in institutions of higher education by 300,000; during 1958 enrolment in primary schools increased from 64·3 million to 86 million; in secondary schools from 7 million to 10 million; and in universities and institutes from 440,000 to 660,000; but inevitably the already inadequate education received by most pupils deteriorated further. The most glaring weakness in higher education is probably over-specialization: there is virtually no possibility of interchange among specialists, and in most institutions physics, for example, is no longer taught as a distinct and integrated subject. Despite the limited facilities, teachers and funds, the system provides a large number of people with an appreciation for education, and a relatively small number with the knowledge necessary to lead the drive towards industrialization and world status.

Mr. Orleans accepts the figure of 625,000 as most closely reflecting the number of persons with higher education at present living in Communist China, more than half of whom are under thirty years of age and almost 80 per cent are men. During the past few years the number of professional workers has been growing at about 10 per cent per annum, and under the Communists the greatest increase has been in engineering, followed by education and agriculture. Of 170,000 with engineering degrees, 90 per cent have received them since 1949, while of 38,000 graduates in the natural sciences, two-thirds are Communist trained. Great inefficiency appears to exist in the estimates of future man-power requirements and in placing graduates. Most of the serious scientific work is being done in the Chinese Academy of Sciences, which in 1958 had almost 6,000 research workers (almost 75 per cent being assistants, trainees or technicians), and its affiliated institutes; even here scientific research was integrated with production. In 1958, the 650 research institutes in science and technology employed a staff of 82,100, of whom 18,100 were research and technical workers, 14,700 in industry and communications, 1,200 in agriculture and related fields and 2,200 in medical science and public health, but it cannot be assumed that all have completed professional training. Many engineering graduates are, in fact, little more than skilled technicians and many science graduates advanced laboratory technicians. China undoubedly possesses a limited number of first-rate scientists, but, at least in the immediate future, her achievements in science, as in overall economic growth, will depend on how much help is obtained from the U.S.S.R. More and more persons will graduate from the advanced institutions now established to train scientists and technologists, but their function must be problematic while China continues the policy of adapting present knowledge to immediate needs rather than sponsoring creative research.

TREATMENT OF RINGWORM BY GRISEOFULVIN

TINEA CAPITIS (ringworm of the scalp) is a dermatomycosis mainly affecting groups of children of school age. Although not serious, the disease is conspicuous, and affects the morale of children, keeping them from school and from participation in normal childhood activities. The bacterial complications to which it may give rise are sometimes serious.

The disease is carried home from school or from a group of playmates, and a reservoir of infection is then established among adults. In some communities in Israel, the proportion of adults suffering from ringworm is higher among women than among men. On the other hand, in Moslem communities it appears to be the men who maintain the disease and run the risk of serious infection, because of their custom of shaving their heads, which is frequently done without due attention to hygiene. Moslem women are protected by the care they give their hair.

Ringworm is particularly widespread in communities where life is organized around the family or the tribe or where people live together in crowded conditions. Some persons contract ringworm more easily than others exposed to the same risk, and the disease is more frequent among children suffering from protein deficiency. In the (then) Belgian Congo, Vanbreusghem examined thousands of children and found that its prevalence varied between 5 and 40 per cent. It was higher in areas where serious protein deficiency was known to occur and kwashiorkor was present. The same applied to cases treated in hospital: in Bukavu, 89 per cent of the children suffering from kwashiorkor were infected with ringworm. At present it is impossible to say whether this arises from a lack of vitamin A accompanying protein deficiency, a change in the sebaceous secretion of the scalp, or some abnormality of the skin or hair related to kwashiorkor and favouring the development of fungi.

In North Africa, in some parts of Central Africa, in the Eastern Mediterranean basin, in certain parts of the United States where there is a high percentage of Negroes, and in various parts of eastern Europe, ringworm may affect 30–40 per cent of the children. The health authorities of several countries have decided to attack this problem and the World Health Organization is giving assistance to the Province of Syria, the United Arab Republic and to Tunisia. In Yugoslavia, where a systematic campaign against ringworm is under way, the Organization has assisted a demonstration project of mass griseofulvin treatment, directed by Prof. E. I. Grin. The campaign being carried out and a general survey on ringworm and its treatment are outlined in a recent issue of the *WHO Chronicle**.

The dermatophytic fungi (*Trichophyton, Microsporum*) live and multiply at the expense of the keratin they digest; as a result, the growth of the hair is impaired and it falls out, leaving bald patches.

The treatment used until quite recently consisted in desquamation of the parts affected so as to remove the diseased keratin, and the disinfection of the patches in order to prevent contamination of newly formed keratin. For this purpose, fungicidal ointments and pomades, and even X-ray therapy, which causes depilation, were employed. None of these methods gave reliable and permanent results. It was necessary to find a new treatment, acting internally instead of externally, that would enable keratin-producing cells to resist the fungus, and continue to resist it after they had become keratinized.

The discovery of griseofulvin provided an antibiotic satisfying these requirements. Isolated in 1939 from *Penicillium griseofulvum*, then from several other species of *Penicillium*, the antibiotic was identified chemically and used in horticulture against certain fungal plant pathogens. Its powerful fungistatic action attracted the attention of dermatologists, and, starting in 1958, investigations were carried out on its use in the treatment of certain human and animal dermatomycoses. The antibiotic gave better results than any form of treatment previously applied. It was more active when administered orally than parenterally, was not found in the serum of treated subjects, but, on the other hand, could be detected in their hair. In therapeutic doses it did not have any harmful or irreversible side-effects. As a result of these outstanding properties it was decided to use it for mass treatment.

Following mass immigrations from highly endemic areas after 1948, ringworm, which had been almost unknown in Israel, again developed into a public health problem. The number of new cases reached 4,000–6,000 per annum. The good results obtained elsewhere with griseofulvin encouraged Israeli health authorities to adopt it for a mass campaign, after confirming its effectiveness and harmlessness in a preliminary trial with a group of women and children in hospital at Tel-Hashomer.

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* *WHO Chronicle*, 15, No. 2; February 1961.

Those most seriously affected are children aged 5–14 years. Although in most cases the condition clears up spontaneously at puberty, some victims bear the marks of the disease throughout their lives.

Trichophyton violaceum is the most widespread parasite ; favus caused by *T. schoenleini* is also encountered. In the Arab village where the campaign was carried out there were 441 *T. violaceum* and 5 *T. schoenleini* cases. Griseofulvin was administered in single doses of 25/30 mgm./kgm. of body weight a day. At the end of three weeks there was a marked improvement in most cases. After eight weeks, 99 per cent of the *T. violaceum* infections and 60 per cent of the *T. schoenleini* infections were cured. All cases were cured after treatment for ten weeks. Twenty-five cases which were followed up after 2–6 months did not suffer any relapse.

DYNAMICS AND ENERGETICS OF SWIMMING IN WATER-BEETLES

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KINEMATIC and dynamic measurements[1] were carried out on *Acilius sulcatus* L. (Dytiscidae). The resistance coefficient c_w of the *Acilius* torso, as a criterion of its flow adaptation, amounts to 0·23 for a Reynolds' number, R_e of 5×10^3 to 8×10^3. Consequently, *Acilius* produces only 5·75 times as much resistance for a given velocity as a drop-shaped body of best adaptation, having a comparable frontal area, but only half as much as a corresponding ball. This very favourable value results from the dorsoventral flattening of the body, the greatest height of which lies in the front third and the greatest width in the rear third of the body, where the epipleura are pulled out so that the borders are sharp. There is no essential difference in velocity between dorsal and ventral flow, and there is no enlargement of the resistance coefficient by a smaller R_e as a result of the preformed places of vortex separations (Fig. 1). The body ends in a half-round edge ; this hinders the vorticity of the plane separating dorsal and ventral flow. Measurements prove the hydrodynamic efficiency of this body-form ; the resistance rises to about 120 per cent if the flow comes from behind, and to about 122 per cent if the edge is cut off. This body-form also possesses excellent swimming stability.

The single propulsion factor is the part of rudder-stroke force consumed by metapodial swinging that lies in the direction of movement, the forward drive. With each rudder-stroke, the forward drive impulse, I_R, that is, the time integral of the propulsive force during the rudder stroking, is reduced by the counter-drive impulse, I_V, which results from the forward pulling of the metapodials. The fraction of the total impulse available for propulsion, $\eta = I_R/(I_R + I_V)$, is the efficiency of underwater swinging as a propulsive factor. It can equal 1·0 in an ideal situation and it can be determined graphically in a complicated way for the stationary (η_1) and freely swimming (η_2) animal as follows : slow-swimming movement, $\eta_1 = 0{\cdot}84$, $\eta_2 = 0{\cdot}72$; medium-swimming movement, $\eta_1 = 0{\cdot}87$, $\eta_2 = 0{\cdot}78$; fastest-swimming, $\eta_1 = 0{\cdot}91$, $\eta_2 = 0{\cdot}81$. The η values for freely swimming animals are somewhat lower than for stationary ones, since the pulling forward of the metapodials happens in water which is moving in relation to the swimming animal. The faster the animal swims, the better is the efficiency ; the greater the applied power, the better it is utilized. The levels of efficiency are surprisingly high with an average of 80 per cent. This is explained by the morphological and kinematic peculiarities of the metapodials.

In the rudder-stroke the surface of the leg, F, and the stroking speed, v, are large. Therefore, the induced resistance W, proportional to Fv^2, is very high. The leg stands with broad sides and with swimming hairs spread perpendicular to the direction of stroking ; v is usefully maximal, when the leg is perpendicular to the median. During the 'partial thrust time' the expended force is changed in propulsion from 98 per cent to 100 per cent, and further to 70 per cent. The resulting harmful side-force amounts to a maximum of only 23 per cent of the forward drive.

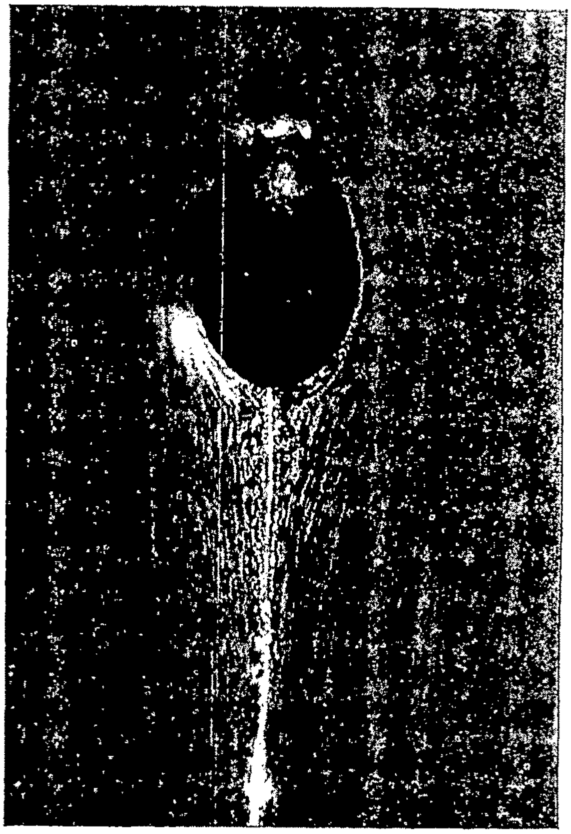

Fig. 1. *Acilius sulcatus*, L. Flow around the frontal area ; taken with an associated reference system at $v = 35$ cm./sec. The laminar flow changes at the widest position of the torso into turbulence. The eddying (and therefore the resistance) is moderate ($R_e = 6{,}000$)

When the metapodials are pulled forward, v is somewhat smaller (an average of 90 per cent of the rudder-stroking speed); F becomes extensively reduced through the collapse of the swimming hairs and the forward-pulling of the leg close to the body, which first is turned around in the highly resistant rudder-position, when the body-speed has reached its minimum. Therefore, according to the above, the resistance generated by the forward-pulling of the leg is small; compared with the outstretched rudder-leg (100 per cent) it amounts to only 6·2 per cent by drawing after, 14·4 per cent by pulling forward close to the body and 31 per cent by turning into the new rudder-position.

The tarsus, also possessing swimming hairs, performs the chief propulsion, namely, 81·8 per cent of the total forward drive compared with 16·9 per cent of the tibia and 1·3 per cent of the femur. According to careful measurements, the part of total forward drive produced by the swimming hairs alone amounts to 67·7 per cent. Furthermore, they raise the forward drive of the solid leg to 230 per cent, the counter drive only to 45 per cent and consequently the effective forward drive to 300 per cent. Therefore, as 'split surfaces' they constitute an excellent rudder principle (Fig. 2). Besides, they move automatically and therefore they consume no muscle force. Moreover, through their uneven distribution, they turn the tarsus at the start of rudder-stroking automatically into working position. They occupy 79·8 per cent of the leg surface, and, with a 67·7 per cent total forward drive component, they attain 54 per cent of the forward drive of an equally large uninterrupted surface. They attain the basic advantage of their mobility and automatic movement as a result of their lattice arrangement with a loss of only 46 per cent of the forward drive against the theoretical maximum. The leg-thrust amounts to 480 per cent of the body resistance. So, for the *Acilius* torso, the rudder legs are very efficient locomotive organs.

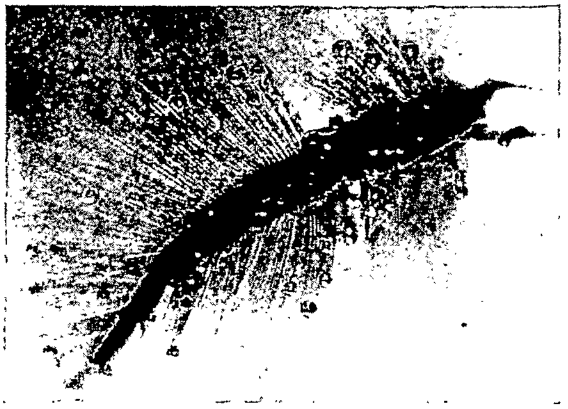

Fig. 2. Left metapodium of *Acilius sulcatus* L. at rudder stroking. The manner of the distribution of the swimming hairs and the spread, as also the enormous enlargement of the stroking area, are completely defined (taken in a flow apparatus)

The smaller the rudder-energy E_R, the greater is the energy available for other body-processes E_x, since the sum of these is equivalent to the total available energy, E. Only a fraction of the rudder energy E_R is used for the production of work, which is useful for locomotion, as a result of vorticity (η_1), through lateral drive (η_2) and counter-drive (η_3). It has been determined that $\eta_1 = 0·50$, $\eta_2 = 0·77$ and $\eta_3 = 0·80$; consequently, the total level of efficiency, $\eta_{\text{total}} \approx 0·30$. 70 per cent of the mechanical energy produced is lost in the leg movement itself. For a muscle efficiency, $\eta_4 = 0·30$, becomes $\eta_{\text{chem.}} = 0·10$. That is, the available chemical energy is transformed into work for locomotion to the extent of 10 per cent. Comparing the minimal chemical energy which is calculated for swimming at speed v with the total available energy which can be determined from respiration measurements at resting turn-over, it follows that *Acilius* expends 100 per cent when $v = 25–30$ cm./sec. (flight), 42 per cent when $v = 20$ cm./sec. (fast continual swimming), 2 per cent when $v = 7$ cm./sec. (slow continual swimming), only 5 per cent by normal distribution of movement and with a warm atmospheric condition; with a cool atmospheric condition it expends only 1 per cent of its available energy corresponding to the resting turn-over. This means that in daily life no notable energy losses result through locomotion ($E \gg E_R$; $E_x \approx E$), but, if necessary, extremely fast swimming is possible under optimal use of this great energy (flight, seizing of plunder; $E \approx E_R$). With *Paramecium*[2], the corresponding values amount to ½–12 per cent, with jumping grasshoppers[3] 100 per cent, and with running suckers[4] 60–100 per cent. With running insects they have not been investigated, but certainly they are less favourable. The quoted dynamic and energetic sizes present for the first time a quantitative idea of the excellent adaptation of water insects to the flowing medium.

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COSMIC RAY AGES OF THE TREYSA AND SIKHOTE-ALIN METEORITES

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IT has been proposed[1,2] that the cosmogenic production-rate, dependent on depth, of stable nuclides in iron meteorites can be estimated from the measured rare gas ratios. One of the criticisms that has been directed at the scheme[3] is that (while it incorporated experimental data on the Sikhote-Alin and Treysa meteorites) different exposure ages were assigned to these meteorites; whereas, it is claimed, direct measurements[4,5] lead to approximately equal ages.

On re-examination of the data, an error was found in our calculation of the helium-3 production-rate in

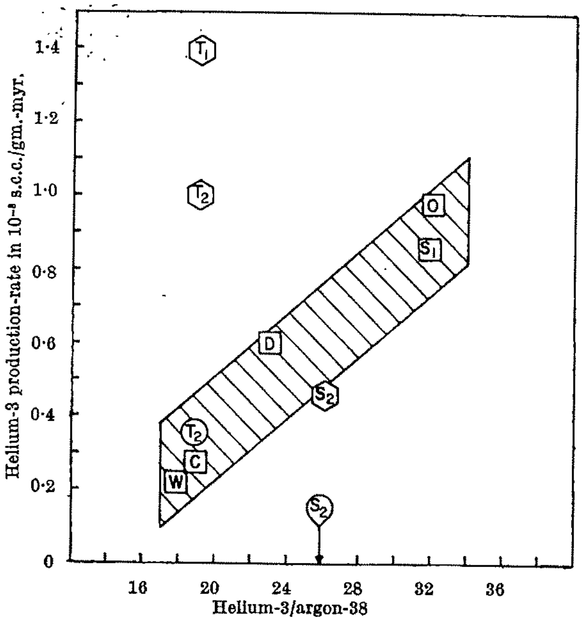

Fig. 1. Production-rate of helium-3 plotted against ratio of helium-3/argon-38. The squares are calculated from chlorine-36 data, the hexagons from argon-39 data, and the circles from tritium data. *O*, Odessa (ref. 10); S_1, Sikhote–Alin (ref. 10); S_2, Sikhote–Alin (ref. 4); *D*, Canon Diable (ref. 10); *C*, Carbo (ref. 10); *W*, Williamstown (ref. 10); T_1, Treysa (ref. 5); T_2, Treysa (ref. 4)

Treysa. The corrected scheme is shown in Fig. 1. New data on the tritium content of Treysa and Sikhote–Alin are included, although relative cross-sections for helium-3 and tritium production are not well known.

It is apparent that the scheme does not account for the argon-39 data on Treysa, nor for the tritium data on Sikhote–Alin. It has been pointed out[4] that no accepted model of cosmogenic nuclide production can account for the low tritium/argon-39 ratio in these two meteorites. It was suggested that diffusion loss of tritium is responsible for the anomalous value of this ratio. This suggestion is compatible with Fig. 1 for the low value of the tritium content in Sikhote–Alin, but not for the Treysa data (Sikhote–Alin is of the coarsest octahedrite structure, while Treysa is a fine octahedrite).

It is emphasized that Fig. 1 is merely an observation of an apparent correlation between radioactivity and rare-gas measurements. Future data may show the correlation to be coincidental or due to errors in measurement, in which case the scheme will be rejected. However, the low tritium/argon-39 ratio in Treysa may be due to an excess of argon-39. It has been noted[2] that, if one attempts to organize the rare-gas data for all the measured iron meteorites, particular ratios in particular meteorites seem to be anomalous. It was suggested that these anomalous values were due to secondary reactions on trace elements. For example, the excessively high value of the neon-21/argon-38 ratio in Tucson was tentatively ascribed to the ^{24}Mg$(n,\alpha)^{21}$Ne reaction, a larger amount of magnesium being present in this meteorite than in others. Similarly, the ^{39}K$(n,p)^{39}$Ar and the ^{40}Ca$(n,2p)^{39}$Ar reactions would lead to an excess of argon-39, and it is suggested that an excess of these elements in this meteorite may be the reason for the variation of the Treysa data from the proposed scheme.

In iron meteorites the argon-38/argon-36 ratio is generally about 1·6. In stone meteorites, with an iron/calcium/potassium ratio of about 100 : 6·5 : 0·4, the argon-38/argon-36 ratio reaches approximately 2, due primarily to the calcium isotopes. In Treysa this ratio varies from 1·52 to 1·75. This implies maximum calcium and potassium abundances relative to iron in Treysa of about 100 : 2 : 0·2. This limit does not prohibit the postulated argon-39 effect. Stoenner and Zahringer[6] have reported that the potassium content of iron meteorites may be extremely inhomogeneous; this may be a factor in the disparate values for argon-39 content of Treysa reported in refs. 4 and 5. Then a more nearly correct value for the exposure age of Treysa might be obtained by taking the nuclide production-rate from Fig. 1 than by using the argon-39 data.

Table 1. COSMIC RAY AGE OF THE TREYSA METEORITE, IN UNITS OF 10^9 YR.

Source of data	^{3}He–^{3}H	^{38}Ar–^{39}Ar	^{39}Ar–^{45}Sc	Only rare gases	^{3}He–^{3}He/^{38}Ar (Fig. 1)
Ref. 4	1·0 (- -)	0·35 (0·45)			1·1
Ref. 4 (^{38}Ar) Ref. 5 (^{39}Ar)		0·25			
Ref. 4 (^{3}H, ^{39}Ar) Ref. 9 (^{3}He, ^{38}Ar)	1·4	0·49			
Ref. 5 (^{39}Ar) Ref. 9 (^{38}Ar)		0·35			
Ref. 5			1·2 (0·6)		
Ref. 9				(0·5 – 2·0)	1·5

Table 2. COSMIC RAY AGE OF THE SIKHOTE–ALIN METEORITE, IN UNITS OF 10^9 YR.

Source of data	^{38}Ar–^{39}Ar	^{36}Ar–^{36}Cl	^{3}He–^{3}He/^{38}Ar (Fig. 1)
Ref. 4	0·22 (0·42)		0·17
Ref. 10 (^{36}Cl) Ref. 7 (rare gases)		0·17	0·15
Ref. 11		(0·12)	

In Tables 1 and 2 the exposure ages for Treysa and Sikhote–Alin are calculated again, using all available raw data on rare gas, tritium, scandium-45 and chlorine-36 measurements. The ages in parentheses are those deduced in the original papers. The differences arise because of the following considerations: (1) Fireman and DeFelice assumed an argon-38/argon-39 cross-section ratio of 1·5; recent data indicate a nearly constant ratio of about 2 from 0·43 × 10^9 to 6 × 10^9 eV. They estimated a helium-3/argon-38 ratio of 20 for all iron meteorites; according to their estimate of the helium-3/helium-4 ratio, to Schaeffer and Zahringer[7], and to Vinogradov *et al.*[8], this should actually be about 26 in their sample of Sikhote–Alin. (2) Wanke and Vilcsek[5] used a value for the cross-section of argon-39 equal to 5 mb. Recent measurements indicate a value of 6·5 mb. at 0·7 × 10^9 eV. rising to 12·4 mb. at 6 × 10^9 eV.; a value of 10 mb. was used here.

The spread in the ages listed in Tables 1 and 2 reflects both the different experimental values for measurements of the same nuclide in the same meteorite by different investigators, and the uncertainty in the assumptions necessary in proceeding from the measurements to calculations of the exposure age. It is a measure of the errors involved, and would indicate that calculations, leading to such quantities as cosmic ray intensities from these data, contain an element of uncertainty. The ages proposed as 'best' are 0·16 × 10⁹ yr. for Sikhote–Alin and, for Treysa, 1·3 × 10⁹ yr. if Fig. 1 is used to estimate the nuclide production rate; 0·35 × 10⁹ yr. if the argon-39 data are accepted as indicating the primary flux.

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IMPULSE LOADING OF ROCK TARGETS TO PRODUCE FRACTURE PATTERNS SIMILAR TO NATURALLY OCCURRING RADIAL DYKE SYSTEMS

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DURING investigations of the impulse loading of rock and earth materials, a striking similarity was noted between fractures induced by impulsive loads in some rock types and the great dyke swarms occasionally encountered in volcanic provinces.

The impulse loading of a rock material can be accomplished in many ways. A simple method of delivering large amounts of energy to a small target area is to strike the target with a jet derived from a lined-cavity shaped charge. Such a jet-target impact creates a narrow empty hole in the rock target, referred to generally as a penetration. Under suitable conditions of target confinement, the penetration is accompanied by fractures filled or intruded by metal and metal oxides from the shaped-charge jet.

For fracture studies a jet was desired the metal fragments and oxidation products of which would contrast boldly in appearance with the target material. A light greyish-white limestone target rock was selected, previous experimental work[1] having indicated that limestone was an ideal target rock for fracture research. Thus, cast iron was chosen as the jet material because this metal appears dark in a light target, and in addition yields a dark grey-black oxide upon high-velocity impact. Such a colour contrast is especially important for fracture work. Those fractures definitely opened during and immediately following penetration are filled with liner metal and oxides, and are easily distinguishable from cracks created by handling the fractured target during its sectioning and study. Reliance on coloured fracture fillings means, however, that fractures that never were filled by metal and oxides are indistinguishable from both pre- and post-penetration cracks in the target.

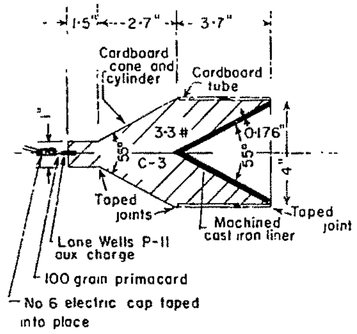
To achieve high projectile velocities and obtain a large amount of target response to impact, heavy cast-iron liners were fired into limestone targets, with use of the simple lined-cavity shaped-charge design illustrated in Fig. 1. This charge design produces a 1-in.-diam. jet of small, sharp, cast-iron particles. The front tip of this heavy jet travels at a velocity of about 21,800 ft. per sec. (velocity across a course of 1·088 ft. extent; course located 2 ft. in front of the shaped charge). Fig. 2 is a flash X-ray photograph (exposure time 1 µsec.) of a cast-iron jet from a shaped charge of the type shown in Fig. 1.

The targets used for this work were composed of relatively fine-grained, crinoidal Pennsylvanian limestone (Madera). Table 1 tabulates the physical properties of the target. Rock targets for impulsive-

Table 1. PROPERTIES OF THE MADERA LIMESTONE TARGET ROCK

Type of load	Sample cross-section (sq. in.)	Breaking strength (lb./sq. in.)
Tensile	0·05	500
Compressive	0·51	6,800

	Average	Larger grains mm. diameter	Smaller grains mm. diameter	Cement areas mm. diameter
Grain size	0·90	0·83	0·13	1·7



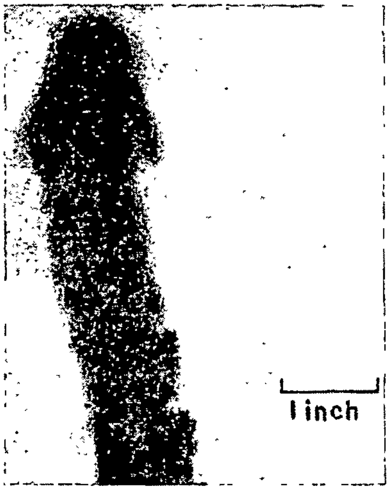


Fig. 1 (left). Design of the lined-cavity shaped charge used to form the fracture patterns shown in Figs. 3 and 4

Fig. 2 (right). A cast-iron jet derived from a charge of the design shown in Fig. 1 (exposure time 1 µsec.)

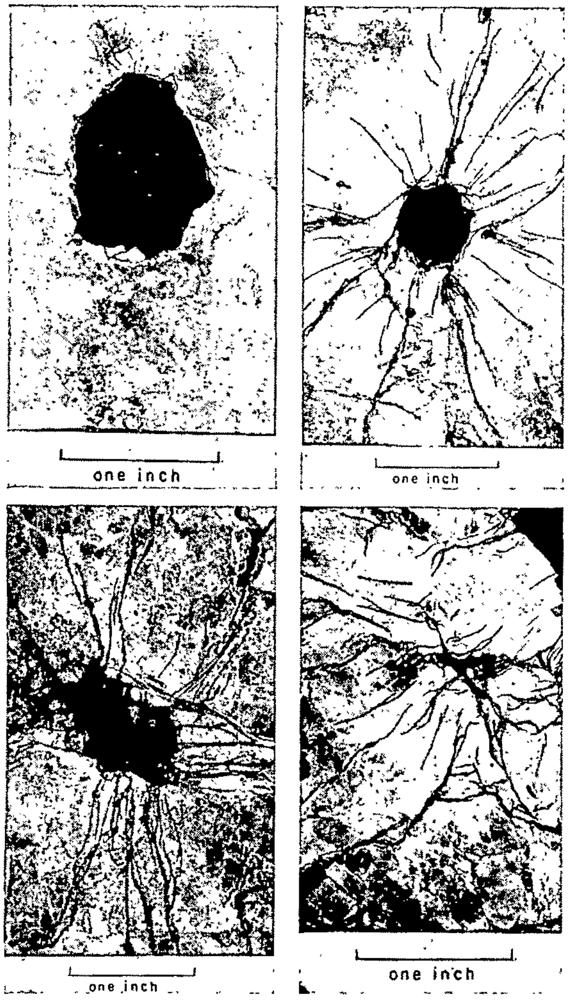


Fig. 3. (upper left) Result of firing a jet into a massive, uncracked limestone target; (upper right) the radial fracture pattern formed as a result of firing a jet into a cracked limestone target; (lower left) the typical fracture swarms formed at right angles to the walls of an angular penetration in a cracked limestone target; (lower right) the fracture complex formed beneath a penetration made in a cracked limestone target.

load investigations of the present type must not be permitted to break into a few large discrete fragments but must be kept essentially unbroken. To prevent fragmentation in a brittle target with such a low tensile strength as that of rock requires very massive targets or very heavy confinement. Massive limestone outcrops in place in the Earth's crust were used for the experiments reported in this article, making confinement laterally and into the target virtually infinite. Targets were able to expand only locally through the closing or tightening of pre-penetration cracks and joints, and perhaps to a minor extent through local decreases in porosity. Experiments so far suggest strongly that the presence of pre-penetration cracks is essential to formation of fractures intruded by jet metal and oxides. Where such cracks are lacking, no significant metal- or oxide-intruded fracture patterns developed in the targets.

Following the loading of the target to create a fracture system, a routine procedure was followed to prepare the target for study. Melted paraffin was poured into the target and allowed to solidify in order to preserve the detail of the penetration walls and help maintain the coherence of the fractured target. The paraffin was added, immediately following penetration of the target, while the target was still slightly warm. (Interestingly, rock penetrations are not hot but only mildly warm.) Next, a percussion-type rock drill was used to drill a double ring of holes surrounding the area of penetration and fracturing. Generally, in view of the type of jet used for penetration, a 1-ft. radius was used. The holes were drilled as close together as possible and slightly deeper than the expected depth of penetration. Following drilling the intervening rock was removed by hand to leave a cylindrical plug of rock containing the fractured area to be examined. Several holes were then drilled into the plug of rock at its point of attachment to the main rock outcrop, and the entire cylindrical plug was broken free as a single piece of rock. This was taken to the laboratory, where it was cut with a power hacksaw into a convenient rectangular block, mounted in plastic, and sawed into ¼-in.-thick slices. The slices were made at right angles to the axis of the penetration, permitting the development of a rather complete three-dimensional picture of the target fracturing that accompanied the jet penetration.

Jet firings into limestone have so far illustrated three basic fracture patterns when the fractures are cut by a plane perpendicular to the axis of the penetration. These are: (1) penetration with essentially no fracturing; (2) radial patterns around a circular or elliptical penetration; (3) patterns with the fractures at right angles to the walls of a penetration having a rectangular or angular cross-section. No metal- or oxide-filled fractures occur in truly massive targets. Given a limestone with no pre-penetration cracks, and thus being unable to expand slightly about the penetration, no metal- and oxide-filled fractures will form. Simple metal- and oxide-filled radial fracture systems are found surrounding penetrations that occur in previously cracked

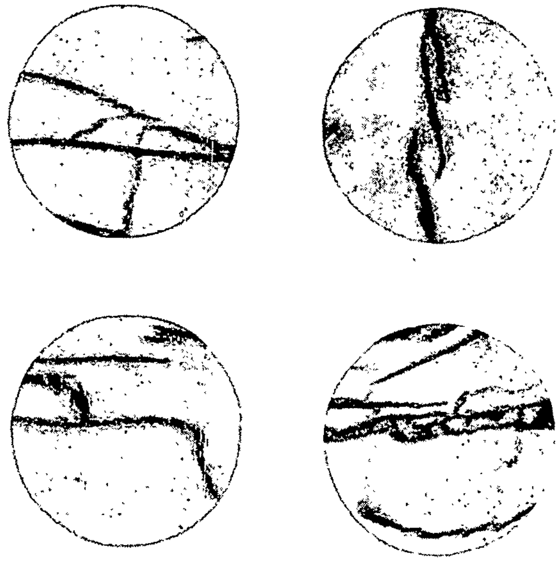


Fig. 4. Fine structure of individual metal- and oxide-filled fractures in a limestone target. The upper left photograph shows the microscopic en echelon character of macroscopically linear fractures; the upper right photograph shows macroscopic en echelon fracture alignment; the lower left photograph shows the typical abrupt junctions between fractures; the lower right photograph shows the rubble-filled character typical of relatively wide fractures

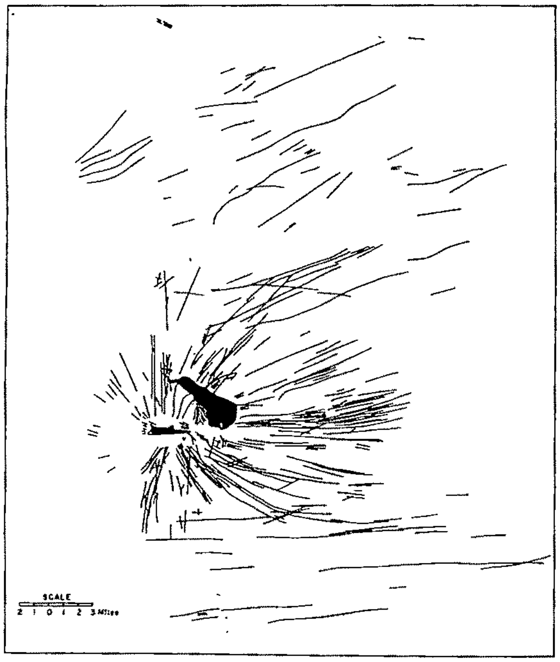


Fig. 5. The dyke swarms of the Spanish Peaks area in Colorado (western United States). Dark areas are intrusive centres, dark lines intrusive dykes. (Adapted from Knopf, ref. 2)

targets. In the case of a target with a pre-penetration crack cutting the penetration at an angle, fracturing begins on the side nearer the crack. In rocks with cracks scattered about the target area, relatively symmetrical fracture patterns are formed during the penetration process. Penetrations tend to become rather angular and irregular in outline towards their bottoms, instead of circular or elliptical. In the bottom areas the fractures resulting from the penetration process appear as swarms at right angles to the walls of the penetration. Although not examined in detail, the evidence at hand suggests that the fracturing immediately below a penetration is very complex. Fig. 3 illustrates the three types of fracture systems, as well as a section showing the complexity at the bottom of a penetration. Fig. 4 presents the fine structure of individual fractures.

Fig. 5 shows the great dyke swarm of the Spanish Peaks area in the western United States. A comparison of this figure with fracture patterns of Fig. 3 exhibits obvious similarity between the natural and artificial fracture-intrusion patterns.

In view of the foregoing similarities in fracture and intrusion patterns and the ease of formation of fracture patterns in rock models, I am continuing my work on the impulse loading of rock and earth materials.

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ACTIVATED NATURAL CRYSTALS

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SUCCESSFUL results have been obtained with tritium and carbon-14[1] and also in experiments with lead[2], in which it was found that when a substance is placed in contact with a source in the form of a solid-labelled compound, the active substance can be found after some time at a distance from the source that cannot be explained on the basis of ordinary diffusion. These results suggested more general experiments to test the phenomena with other active sources and different kinds of substances for activation, particularly crystals.

The sources used were : calcium-45, carbon-14, phosphorus-32 and sulphur-35, prepared using materials supplied by the Radiochemical Centre, Amersham. The activity of each source was, at the beginning of the experiments, about 250 μc. As for the crystals, the inactivity of which was previously checked, in almost all the experiments slices were used about 2–3 mm. thick, cut in different directions ; in several cases it was also possible to make measurements of differently orientated slices cut from the same crystal. The specimens were : apatite (perpendicular to the z-axis and parallel to the same) ; calcite (perpendicular to the optic axis and parallel to a rhombic face) ; celestine (perpendicular to the x-axis and parallel to the same); gypsum (parallel to cleavage) ; pyrite (parallel to a cube face and parallel to a dodecahedral face).

The activation was produced by applying the slice of the crystal to the source for a time ranging from a few days to several weeks. At the end of the exposure the slice of crystal was taken off and counted on both faces, in some cases—when the activity was high—at a Geiger end-window counter ; in other cases at a gas-flow counter. In all cases activity was evident ; much stronger on the 'contact surface', that is, the face that had been kept in contact with the source, than on the 'opposite surface'.

The measurement of these activities was repeated at different times after the end of the exposure (several weeks and also several months). In all experiments done until now, activity was detected.

Besides this, the activated crystals were autoradiographed using Ilford G_5 nuclear emulsions ; a photographic effect was always noticed. Generally, the crystals were activated by exposure to a source, the active element of which was of the same chemical kind as some of the elements contained in the molecules of the crystal (apatite to calcium-45 and to phosphorus-32, calcite to calcium-45 and to carbon-14, gypsum to calcium-45 and to sulphur-35, celestine and pyrite to sulphur-35).

But an attempt was also made to expose a crystal to a source the active element of which was not of the same chemical kind as any of the components of the crystal molecule, but chemically allied to one of them, namely, celestine and calcium-45 ; in this case the effect was rather strong. A further attempt was made by exposing crystals to a source the active element of which was not of the same chemical kind as any component of the molecule nor allied to one of them, namely, gypsum and carbon-14 ; also in this case an activation effect, although a weak one, was noticed.

In order to check if the activation of the opposite surface was actually due to the presence of active atoms and to obviate the effect of possible secondary radiation from the contact surface, the following procedure was adopted : two superimposed slices (cut from the same crystal and equally orientated) were exposed together ; after removal from the source, the slice farther away was tested separately. In each case this showed a weak activity, and this could only be due to the presence of active atoms at a distance from the source corresponding to the opposite surface.

To check if the activity noticed at the opposite face of the activated crystal was due to active atoms having migrated from the contact surface through the crystal or along its surface, and to find out if, and how, the activity passed from the contact surface into the crystal, several crystals were carefully counted again, then weighed, ground on one face, weighed and counted again ; this process was repeated several times and also on the opposite face. Care was taken during each grinding to renew frequently the material used for grinding and to clean thoroughly each ground surface before counting. This technique of grinding has been used in some work on ordinary diffusion[3], but the effect described here is different. In fact, the activity decreased with increasing depth from each surface, but the greatest decrease was noticeable only after the first grinding ; after this, the rate of decrease was much lower and always less, the activity reaching a rather uniform value. This appears in Figs. 1 and 2, which relate to examples that illustrate the general result of experiments of this kind, and refer to the activity measured on both faces of slices of calcite cut perpendicular to the optic axis and cut parallel to a rhombic face.

These activities were measured some months after the end of the exposure and the measurements of the ground surfaces correspond to counts done as soon as grinding had been finished. The activity of the last two ground surfaces of each sample, after a lapse of time, appeared to have increased (probably lattice disorders had occurred during grinding).

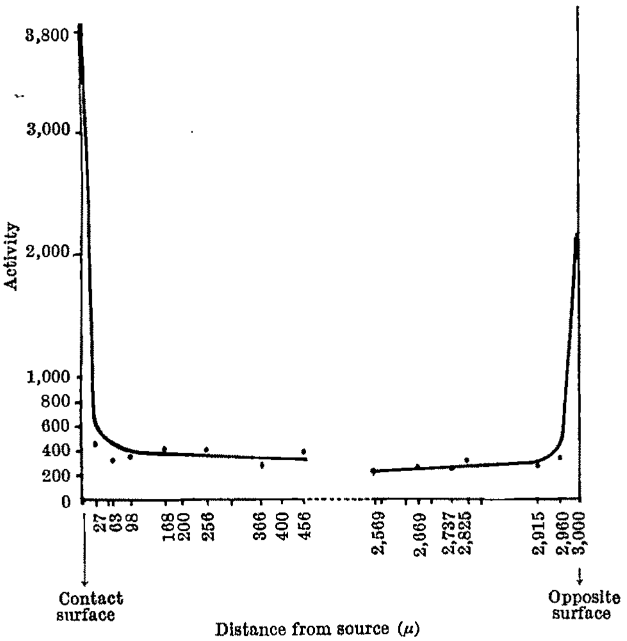

Fig. 1. Calcite cut perpendicular to the optic axis. Distance from source represents distance from contact surface. Activity is net counting-rate ± *S.D.*

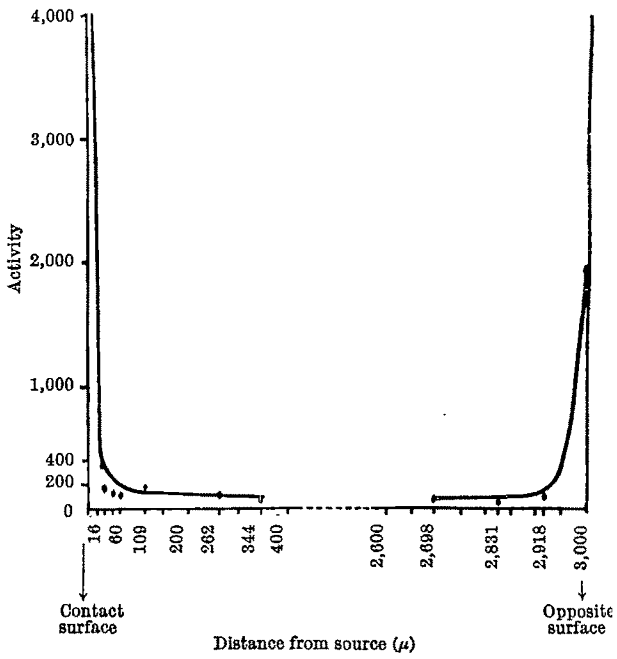

Fig. 2. Calcite cut parallel to a rhombic face. Distance from source represents distance from contact surface. Activity is net counting-rate ± *S.D.*

The result of the experiment demonstrates that the active substance penetrated into the crystal ; at the same time the particularly high activity noticed at both external surfaces of the slices demonstrates that some active substance also migrated along the surface. The magnitude of this effect, calculated on the basis of the activity in the region where it appeared to be more or less sufficiently uniform, assuming that the activity measured corresponds to the radiation emitted from a depth of 150 microns (about three-quarters the range of the beta radiation from calcium-45 in calcite) and correcting for self-absorption, was: 0·0064 μc./gm. (in the first sample with calcite perpendicular) and 0·0022 μc./gm. (in the second sample with calcite parallel).

The difference cannot be ascribed to the different directions alone, since geometry and exposure time were also different.

It has been demonstrated that natural crystals can be activated by simple contact with a radioactive source. It appears that the active atoms migrate and that this effect may be considered as the combination of two effects : (1) a migration through the material in depth (bulk effect) ; (2) a migration along the surface (surface effect). This effect may be explained by the fact that the radioactive substance present in the source produces an excited state : (*a*) in the source itself by means of the radiation emitted and of the recoil of the emitting atoms ; (*b*) in the contacting substances by means of the radiation up to its full range (a region that can be called 'range layer').

Consequently, in the source and in the contacting substances, up to the range layer, breakage of bonds, ionization and knock-on and knock-off of atoms may occur. These facts explain the possibility of atom exchange between neighbouring molecules and, in solids, the production of lattice defects (vacancies and interstitials). This, together with the possibility of intergranular migration, opens the way to a further migration beyond the range layer, which differs from ordinary diffusion, because the concentration at the origin, instead of being constant, increases with time.

A theoretical treatment of these processes, done in collaboration with someone else, leads to conclusions in agreement with the experimental results and will be published separately. Obviously, not only the active atoms migrate, but also all kinds of atoms and ions that have undergone collision, and this may occur from source to contacting substance as well as vice versa.

Therefore, the active element observed in the active crystals by counting will be mixed most probably with the inactive carrier in the same proportion as in the source itself, so that it may be expected that, until saturation is reached, the magnitude of the effect may be proportional to the square of the specific activity of the source.

The effect here described differs from ordinary diffusion, not only because it is explained differently, but also because the experimental conditions are completely different, as neither particularly adherent contact nor heating is required. Further differences concern its magnitude and the two kinds of migration.

The experiments done up to now are not sufficient to give a complete picture of the effect on crystals, but are sufficient to indicate some lines of research : (*a*) The dependence of the effect on the chemical constitution of the source and of the activated substance, as well as on the lattice conditions ; also dependence on crystallographic structure. (*b*) The explanation of some concentric arcs which were noticed on some autoradiographs. I shall be pleased to hear from others who have done experiments of this kind.

This research has been carried on in the Department of Radiotherapeutics of the University of Cambridge. I thank Prof. J. S. Mitchell for hospitality and advice; also Dr. N. F. M. Henry, of the Department of Mineralogy, who kindly read the manuscript of this paper. Also, I thank the Consiglio Naz. le dello Ricerche, which gave grants for my stay in England.

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PATTERNS OF PREFERENCE IN EQUIPROBABLE SITUATIONS

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We have previously[1] distinguished two types of gambling situation. In one, the player's skill may, if he so decides, affect his success or failure ; in the other, his skill (or what he believes to be his skill) is confined to making the choice, and once his choice is made, he is unable to do anything to affect the outcome. This second type is the characteristic lottery situation and the one with which we are now concerned. The earlier work was limited to isolated choice situations and not properly systematized. This defect may now be remedied by introducing a new method of representation or 'model'.

All variations of the sorts of choice we propose to study may be represented by assigning suitable values to the symbols :

$$ {}^{r}_{s}D^{w}_{l} $$

where D is the number of draws which the subject can make from a given source (s); w is the number of winning tickets ; l is the number of non-winning tickets; s is the number of sources of tickets ; and r indicates that a ticket drawn from a given source is replaced at random before the next draw.

The experimental procedure required the subjects to choose one of two (or more) possibilities of winning a prize. For example, they could choose to draw once from a box containing ten tickets including one winning ticket, *or* from a box containing a hundred tickets including ten winning tickets : ${}_{1}1^{1}_{9}$ or ${}_{1}1^{10}_{90}$. The instructions varied appropriately from one situation to another. More than six hundred subjects, young and old, took part in these experiments.

Let us now consider the results of the systematic experimental variations in turn :

(i) *Effect of varying 's'* (that is, number of sources or boxes from which the tickets were to be drawn).

The subjects were presented with the following four alternatives:

$$ {}^{r}_{1}10^{10}_{90} \text{ or } {}^{r}_{s}10^{10}_{90} \qquad (1, a) $$

where s took the values 2, 5 or 10.

$$ {}^{r}_{1}10^{10}_{990} \text{ or } {}_{10}10^{10}_{990} \qquad (1, b) $$

$$ {}_{1}1^{1}_{9} \text{ or } {}_{2}1^{1}_{4} \qquad (1, c) $$

$$ {}^{r}_{1}10^{10}_{90} \text{ or } {}_{20}10^{10}_{40} \qquad (1, d) $$

In a further experiment subjects ranked the following situations in order of preference :

$$ {}^{r}_{1}10^{10}_{90},\ {}^{r}_{5}10^{10}_{90},\ {}_{10}10^{10}_{90},\ {}_{20}10^{10}_{40} \qquad (2) $$

The general result was to prefer the alternative with fewer sources. In 1,041 choices, some 68 per cent preferred the alternative in which the ticket was to be drawn from a single source ($p < 0{\cdot}01$).

(ii) *Effect of varying w and l* (that is, the number of winning and non-winning tickets).

Three situations were examined, when p, the mathematical probability of drawing the winning ticket, was 0·1, 0·5 and 0·9 respectively, namely:

$$ {}_{1}1^{w_i}_{l_j} \text{ or } {}_{1}1^{10}_{90} $$

where i, j take the paired values 1,9; 2,18 or 5,45

$$ {}_{1}1^{w_i}_{l_j} \text{ or } {}_{1}1^{50}_{50} $$

where i, j take the paired values 5,5; 10,10 or 25,25 and

$$ {}_{1}1^{w_i}_{l_j} \text{ or } {}_{1}1^{90}_{10} $$

where i, j take the paired values 9,1; 18,2 or 45,5

A preference (statistically significant) was displayed for choosing from the larger total of tickets ($w+l$), when p was 0·1, and from the smaller total when p was 0·5 or 0·9.

(iii) *Effect of varying D* (that is, the number of draws).

The principal choice situations here were :

$$_{1}1_{l_j}^{w_i} \text{ or } _{1}10_{99}^{1}$$

where i, j took the paired values 1,9 or 10,90.

The preferences for one or other alternative seemed to be unstable from group to group except when the number of draws was counterbalanced by a larger value of w, in which case there was a tendency to prefer the latter.

(iv) *Effect of varying the mathematical probability of drawing the winning ticket as well as the value of the prize, proportionately.*

Here the choice was short odds ($p = 0{\cdot}1$) of winning a prize (box of chocolates) or long odds ($p = 0{\cdot}01$) of winning ten boxes. The preference was clearly in favour of the shorter odds. When shillings were substituted for chocolates in another experiment, the preferences for short odds virtually disappeared.

(v) *Points of subjective equivalence of w, l and D.*

From additional data provided by our subjects, it was possible to determine the mathematical probability of drawing a winning ticket when values of w, l and D in the rejected alternative were changed by the subject so as to render the latter as attractive as the chosen alternative. The inference is justified that winning tickets, non-winning tickets and draws are not subjectively valued equally when each is adjusted for equivalence to either of the others in the accepted alternative. Thus the psychological probability attached to draws is less than that attached to winning or non-winning tickets; and assessments of l are more realistic than assessments of w or D.

(vi) *The problem of transitivity of preference.*

When our subjects' preferences are studied over sets of different choices, some 15 per cent appear to be intransitive in the strict sense. In fact, however, the psychological value or 'utility' of any alternative is not constant but may vary according to the alternative with which it is juxtaposed. It follows, therefore, that the use of a notation which suggests invariance (A is preferred to B, B to C, therefore A to C) would be a misapplication of the principle of transitivity. Thus, for example, $_{1}1_{9}^{1}$ may be preferred to $_{1}1_{90}^{10}$ because of a smaller l; and $_{1}1_{90}^{10}$ may be preferred to $_{1}10_{99}^{1}$ mainly because of a larger w. But $_{1}10_{99}^{1}$ may still be preferred to $_{1}1_{9}^{1}$ because of the larger D.

General conclusions.

The notation we have introduced enables the experimenter to represent a great variety of situations and also facilitates the interpretation of the results.

Two generalizations may be hazarded :

$$ {}_{s}^{r}D_{l_i}^{w_i} \text{ is preferred to } {}_{ns}^{r}D_{l_j}^{w_j} \qquad (1)$$

$$\text{when } l_i > w_i,\ l_j > w_j,\ l_i \geqslant l_j$$

$$ {}_{s}D_{l}^{w} \text{ is preferred to } {}_{s}D_{nl}^{nw} \qquad (2)$$

$$\text{when } p > 0{\cdot}1$$

These may be explained on the ground that when the chances of success are smaller than the chances of failure, the majority of our subjects concentrate on the possibility of winning. They prefer, therefore, to rely on a concentration of winning tickets in fewer sources; and they prefer a large aggregate of winning tickets to a small aggregate of non-winning tickets.

When, however, the chances of success are equal to or greater than the chances of failure, most of our subjects concentrate on the alternative with an absolutely small number of non-winning tickets. They prefer to rely on a dilution of the non-winning tickets in a larger number of sources, and they prefer a small aggregate of non-winning tickets to a large aggregate of winning tickets.

When $w = l$, the choice situation clearly discriminates between two types of person: one with a desire to maximize the chances of success and the other with a desire to minimize the chances of failure. This discrimination may prove to have important diagnostic value in clinical psychology and studies of 'personality'.

In general, the preferences seem to be based neither on the relative values of D, w and l nor on their absolute values, but on some combination of relative and absolute values. This is consistent with the results of our earlier quantitative studies of meaning[2]. Such preferences may reflect a combination of relative and absolute psychological phenomena which have hitherto been regarded as autonomous (for example, in Weber's law and the perceptual constancies). This and other theoretical questions of choice in equiprobable situations will be discussed in a full report of the investigation to appear elsewhere[3].

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A PHYSICAL THEORY OF CARCINOGENESIS BY AROMATIC HYDROCARBONS*

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A NUMBER of aromatic hydrocarbons can induce cancer when injected into or painted on to the skin of a mouse. These compounds, which are also carcinogenic in other sites and in other animals, have been called primary carcinogens[1] to distinguish them from the secondary carcinogens, such as the 4-aminostilbenes and azo-compounds, which are more specific in their biological activity, and which probably act through metabolites.

It is observed that there is a significant correlation between the carcinogenicity index and the energy E_1 of the 1st excited π-singlet state of the molecule[2]. An analysis of data[3] for 66 hydrocarbons and 1 : 2-benzanthracene derivatives is shown in Fig. 1. It is found that 22 of the 23 carcinogens considered have $E_1 = 3{\cdot}04 - 3{\cdot}20$ eV. (24,800 – 25,950

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* Paper read at a meeting of the British Biophysical Society, London, December 1960.

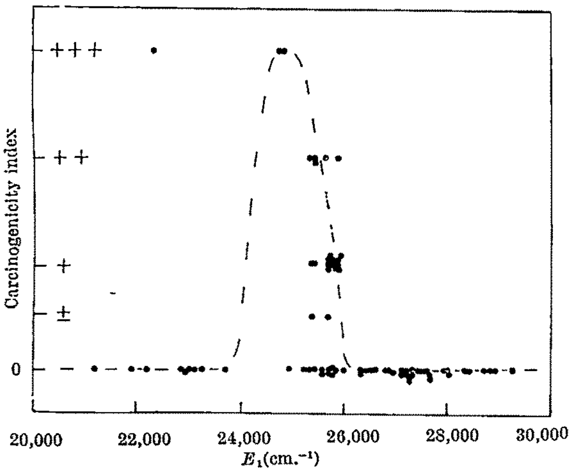

Fig. 1. Polycyclic hydrocarbons and 1 : 2-benzanthracene derivatives. Carcinogenicity index versus energy E_1 of 1st π-electronic singlet state

cm.$^{-1}$). On the other hand, 12 of the 43 non-carcinogens considered also have E_1 in this 'active' region. The degree of correlation indicates that E_1 is related to the carcinogenicity, but that additional factors, such as protein affinity, may also enter.

The form of Fig. 1 suggests a resonance between the carcinogen molecule and some cellular constituent. A common intermolecular resonance effect observed in aromatic molecular systems is dipole–dipole transfer of π-excitation energy, the theory of which has been formulated by Förster[4]. The rate parameter for dipole–dipole energy transfer is determined by the overlap resonance integral :

$$J = \int f_s(\lambda)\, \varepsilon_A(\lambda)\, \lambda^4 \mathrm{d}\lambda \qquad (1)$$

where $f_s(\lambda)$ is the quantum intensity of fluorescence emission of the sensitizer molecules, and $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor molecules at wave-length λ.

E_1 is the edge of the 1st singlet π-electron absorption band, which has a mean energy $\bar{E} \simeq E_1 + 0{\cdot}5$ eV. Hence if the carcinogenicity is plotted against $\bar{E}$ a similar curve to Fig. 1 is obtained, but with its peak at $\bar{E} \simeq 3{\cdot}54$ eV., corresponding to a mean absorption wave-length $\bar{\lambda} \simeq 350$ mμ. The one important cellular constituent known to emit efficiently in this spectral region appears to be the essential amino-acid, tryptophan, the emission spectrum[5] of which with a peak at $\lambda = 348$ mμ is shown in Fig. 2.

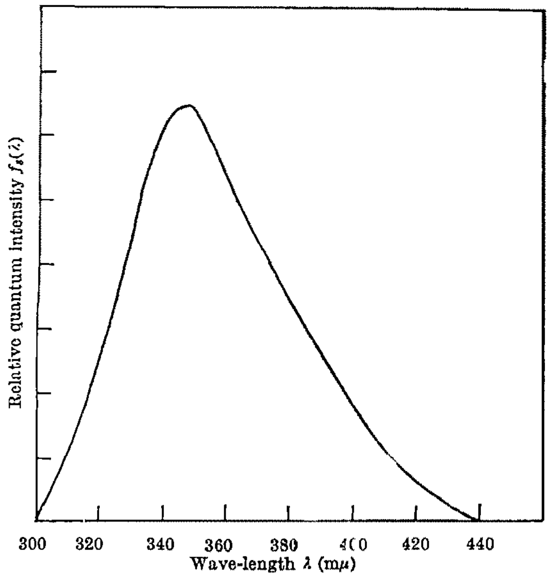

Fig. 2. Fluorescence spectrum of tryptophan

To test the hypothesis that carcinogenesis may be due to dipole–dipole resonance between tryptophan and the carcinogen molecule, as suggested by the above considerations, the overlap integral J has been evaluated, with tryptophan as the sensitizer and the hydrocarbon as the acceptor, for the polycyclic hydrocarbons and 1 : 2-benzanthracene derivatives the absorption spectra[6] of which are known. The results for the methyl derivatives of 1 : 2-benzanthracene are shown in Fig. 3, in which the carcinogenicity index (mean of the Pullman–Pullman[7] indices for painting and injection) is plotted against J, measured in arbitrary units.

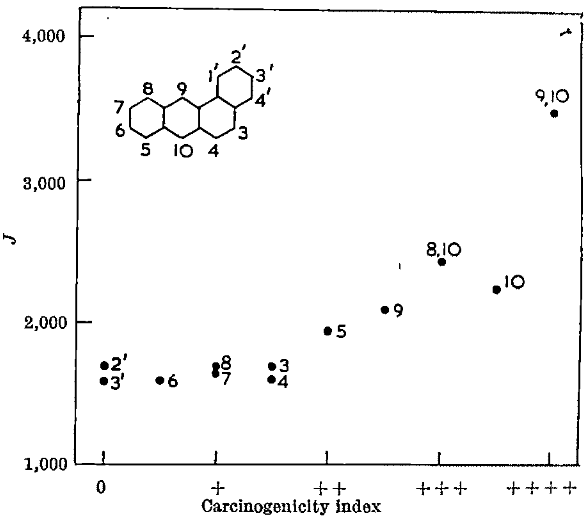

Fig. 3. Monomethyl and dimethyl derivatives of 1 : 2-benzanthracene. Tryptophan overlap integral J versus carcinogenicity index

The methyl 1 : 2-benzanthracenes, which vary markedly in biological activity, have always proved the most challenging and critical group of compounds in previous attempts to correlate some physico-chemical property with carcinogenicity. Pullman and Pullman[7] agree that their electronic theory fails to account for several differences within this group, and they enumerate many other physical and chemical properties that show much less satisfactory correlations. Considering (*a*) the uncertainties in the carcinogenicity data, (*b*) that no allowance has been made for differences between $\varepsilon_A(\lambda)$ and $f_s(\lambda)$ in solution and in the cellular environment, and (*c*) that no account has been taken of the protein affinity of the hydrocarbon, the monotonic relation between J and the carcinogenicity index shown in Fig. 3 is striking. The structurally related potent carcinogens, cholanthrene (+ + + +) and 20-methylcholanthrene (+ + + +), also have high values of $J = 2{,}250$ and $J = 2{,}200$ respectively, consistent with those of the methyl-1 : 2-benzanthracenes.

Heidelberger and Moldenhauer[8] have shown experimentally for a number of aromatic hydrocarbons that the formation of a stable complex with mouse-skin proteins is a necessary, though not a sufficient, condition for carcinogenicity. The outstanding exception to a simple correlation between protein binding and carcinogenic activity is 1 : 2 : 3 : 4-dibenzanthracene, which although non-carcinogenic is as tightly bound as the potent carcinogen, 3 : 4-benzpyrene. The absorption spectrum $\varepsilon_A(\lambda)$ and the 'tryptophan overlap' spectrum $f_s(\lambda)\ \varepsilon_A(\lambda)\lambda^4$ of these two compounds are compared in Figs. 4 and 5. J is only 1,200 for 1 : 2 : 3 : 4-dibenzanthracene, compared with $J = 6{,}200$ for 3 : 4-benzpyrene, showing the major difference in their dipole–dipole resonance with tryptophan.

The values of J and carcinogenicity index for other polycyclic hydrocarbons are listed in Table 1. All the carcinogens have high values of J, with the sole exception of the weak carcinogen 3 : 4-benzphenanthrene, while the non-carcinogens have usually low values of J. Some of the larger non-carcinogenic molecules (for example, pentacene, pentaphene), which have E_1 well below the active region, have anomalously large values of J due to the overlap of their 2nd π-singlet absorption band with the tryptophan emission. If only the overlap with the 1st π-singlet absorption band of the hydrocarbon is considered, and the corresponding overlap integrals J_1 are evaluated, these anomalies disappear. The use of J_1 rather than J appears justified, since the tryptophan emission occurs from the 1st excited π-singlet state which may be expected to resonate with the corresponding excited state of the hydrocarbon. The polarizations of the 1st and 2nd excited states are orthogonal, and it is suggested that the hydrocarbon molecules are bound to the proteins with their axes and planes approximately parallel to those of the tryptophan molecules. Such alignment would greatly enhance the resonance with the 1st excited state, and exclude resonance with the 2nd excited state.

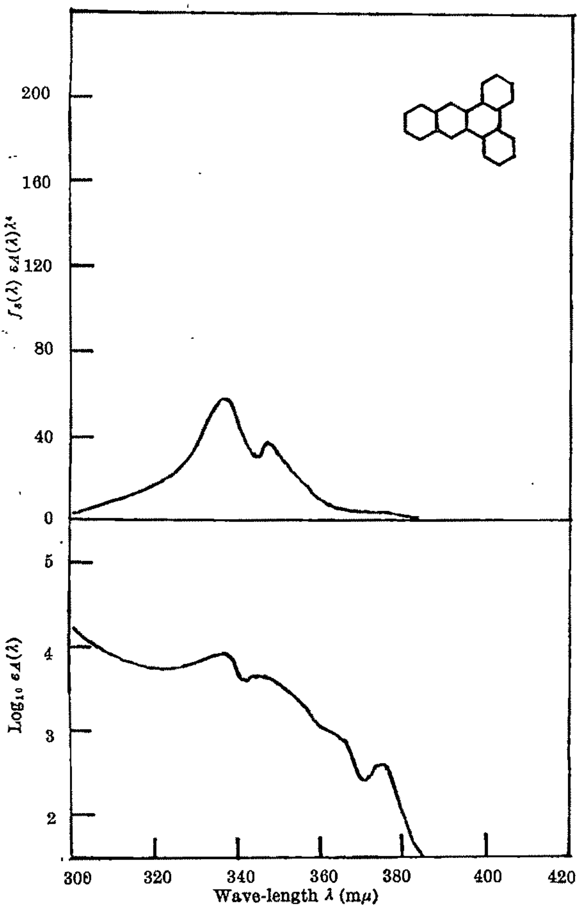

Fig. 4. 1 : 2 : 3 : 4-dibenzanthracene. Absorption ($\log_{10} \varepsilon_A$) and 'tryptophan overlap' ($f_s(\lambda)\ \varepsilon_A(\lambda)\lambda^4$) spectra

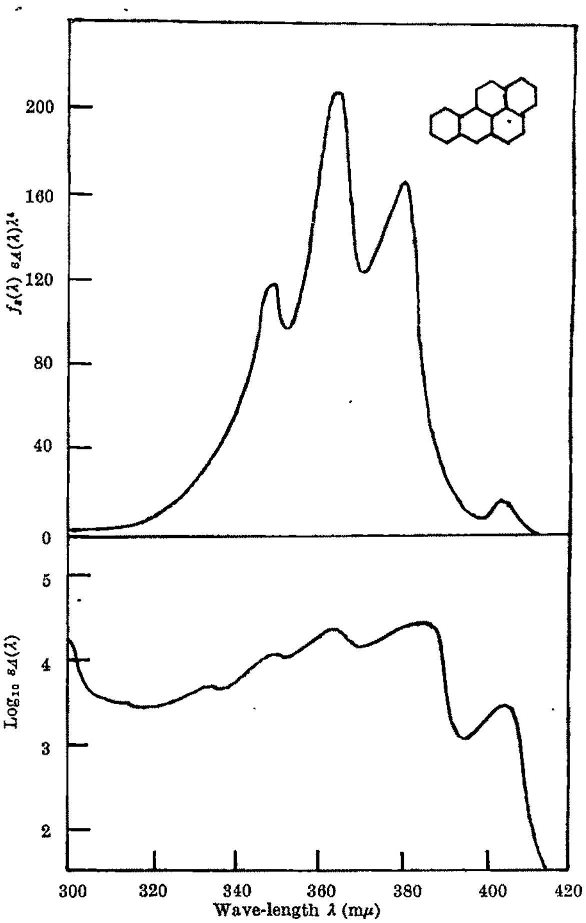

Fig. 5. 3 : 4-benzpyrene. Absorption ($\log_{10} \varepsilon_A$) and 'tryptophan overlap' ($f_s(\lambda)\ \varepsilon_A(\lambda)\lambda^4$) spectra

On the basis of the close correlation observed between J_1 and carcinogenicity, it is proposed that a primary carcinogen should satisfy two fundamental conditions : (1) it should form a bound complex with protein ; (2) its first singlet π-electron absorption band should overlap strongly the emission spectrum of tryptophan, yielding a high value of J_1. Neither of these conditions requires that the primary carcinogen should necessarily undergo any chemical

Table 1. POLYCYCLIC HYDROCARBONS : TRYPTOPHAN RESONANCE INTEGRALS AND CARCINOGENICITY

	J	J_1	Carcinogenicity
Benzene	0	0	0
Naphthalene	0	0	0
Phenanthrene	0	0	0
1 : 2-benzpyrene	1,750	~200	0
Picene	1,600	~260	0
3 : 4-benzphenanthrene	400	400	+
Chrysene	500	500	0
1 : 2 : 3 : 4-dibenzanthracene	1,200	~1,000	0
1 : 2-benzanthracene	1,350	1,350	?
Anthracene	1,550	1,550	?
1 : 2 : 7 : 8-dibenzanthracene	2,950	~1,900	+
1 : 2 : 5 : 6-dibenzanthracene	3,350	~2,400	++
3 : 4 : 8 : 9-dibenzpyrene	3,600	~2,500	++++
1 : 2 : 3 : 4-dibenzpyrene	5,700	~3,300	+++
3 : 4-benzpyrene	6,200	~6,000	++++
Pentacene	2,600	0	0
Pentaphene	7,750	~100	0

change, thus distinguishing the present theory from alternative biochemical theories of carcinogenesis. This is consistent with the observations of Peacock, Beck and Anderson[9], who found that 3:4-benzpyrene persisted in its original form in mouse skin tissue over a period of 4 months, during which tumour initiation occurred. They concluded that the chance of inducing a tumour varied directly as the persistence of unchanged benzpyrene at the site of injection.

The proposed theory is consistent with the 'protein-deletion' hypothesis of carcinogenesis, favoured by Haddow[10], which considers that the interaction between carcinogen and protein leads to the loss of function of proteins involved as enzymes essential for the control of growth. The results indicate that the deleted proteins are those containing tryptophan. This is consistent with biochemical observations[11] that mouse tumour tissues are about 20 per cent deficient in tryptophan content compared with normal 'controlled' tissues.

Perhaps the most interesting aspect of the theory is that it indicates that the tryptophan molecules are in their 1st excited π-singlet state at some stage in the normal biochemical process, and that it is the loss of this excitation energy by resonance transfer to the carcinogen which hinders or prevents the protein synthesis.

Note added in proof. Other cellular constituents, notably the DNA and RNA purines, guanine and adenine, also have π-excitation states at ~ 4 eV., and fluoresce weakly at λ ~ 350 mμ. The postulated π-excitation, which is similar to that observed in bioluminescent systems, where it is produced by oxidation, might therefore be associated with the genetic and biosynthetic processes.

Further studies of complex formation and of energy transfer between molecules of biological interest are being made to elucidate more fully the mechanisms of carcinogenesis and other biological processes.

I wish to acknowledge the assistance of Dr. F. H. Read, who evaluated the overlap integrals.

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NATURE OF THE 'INFECTIVE RIBONUCLEIC ACID COMPONENT' IN CELLS INFECTED WITH ENCEPHALOMYOCARDITIS VIRUS AND THE 'VIROSOME' CONCEPT

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PHENOL extraction at 4° C. of ascites tumour cells infected with murine encephalomyocarditis virus yields infective ribonucleic acid (RNA), although the latter cannot be extracted from the purified virus by similar treatment[1]. Evidence has been presented that the RNA component in infected cells is a virus precursor[2] synthesized in the nuclei[3] during the eclipse phase; it is not deposited with the virus when crude virus preparations are centrifuged[1], but apart from this little is known of its nature. The nature of this component has been investigated by treatment with ribonuclease and papain and reported here.

Infective RNA treated with ribonuclease did not give rise to virus when added to susceptible cells, although untreated RNA did so[1]. Ribonuclease treatment (1 μgm./ml.) of cells infected with RNA greatly reduced the number of cells releasing plaque-forming virus, even when the enzyme treatment was delayed until 2 hr. after infection (Table 1). On the other hand, ribonuclease treatment of cells infected with murine encephalomyocarditis virus did not reduce the number of cells releasing virus if the enzyme were added at 1 hr. or at 4 hr. after infection (Table 1). This suggests that in the RNA component susceptible to phenol extraction and synthesized between 1 hr. and 4 hr. after infection[2], the RNA itself is not 'free', or in any way accessible to the action of ribonuclease.

This suggestion was further supported by experiments in which a series of concentrations of the enzyme was added to cells 1 hr. before infection, and the infected cells then left to synthesize virus while still in contact with the enzyme. Yields of infective RNA and of infective virus after one cycle of virus growth were determined at each enzyme concentration, as was the number of cells killed by virus (Table 2). Ribonuclease did not significantly decrease the amount of virus produced or the number of cells killed; the infectivity of RNA preparations was only affected at enzyme concentrations equal to or greater than 100 μgm./ml. Phenol extraction may not remove all ribonuclease from preparations containing such a high concentration of enzyme, so

Table 1. EFFECT OF RIBONUCLEASE ON CELLS INFECTED WITH RNA OR WITH VIRUS

Cells treated with:	Cells/ml. forming plaques when tested at the stated time after infection 1 hr.	2 hr.	4 hr.
RNA*	$2\cdot3 \times 10^3$	$1\cdot5 \times 10^3$	—
RNA* then ribonuclease†	$2\cdot0 \times 10$	$7\cdot0 \times 10$	—
Virus‡	$4\cdot8 \times 10^5$	—	$1\cdot2 \times 10^6$
Virus‡ then ribonuclease†	$7\cdot0 \times 10^5$	—	$9\cdot5 \times 10^6$

* Cells 5×10^6/ml.; RNA 5×10^4 plaque-forming units/ml. Number of cells releasing virus calculated as the difference in plaque titre between the whole culture and the supernatant after centrifuging at 500*g* for 5 min.

† Cells incubated with 1 μgm./ml. crystalline ribonuclease for 15 min. at room temperature.

‡ Cells 5×10^6/ml.; virus 5×10^6 plaque-forming units/ml. Number of cells releasing virus estimated by direct plating of cells after antiserum treatment and washing.

Table 2. EFFECT OF RIBONUCLEASE PRESENT DURING VIRUS GROWTH

	Ribonuclease concentration (μgm./ml.) 0	0·1	1	10	100	1,000
Virus yield (plaque-forming units/ml. × 10^8)	2·9	5·5	4·2	4·1	7·5	4·0
Cell death (per cent stained by eosin)	78	92	93	71	74	86
RNA infectivity (LD_{50}/ml. × 100)	150	150	100	150	1·5	0·3

Cells (1 × 10^8/ml.) left for 1 hr. at room temperature with ribonuclease, and then infected with 3 plaque-forming units/cell of murine encephalomyocarditis virus. After 5 min. more at room temperature, the cells were diluted in Earle's saline and kept in suspension culture for 17 hr. The concentrations of enzyme were thus reduced after infection to one-fifth of those shown, although the absolute amounts of infected cells and enzyme remained constant.

that at least some of the loss of infectivity may have been caused by residual ribonuclease attacking RNA 'freed' by phenol extraction. However, even if the effect of the enzyme at high concentration is on the RNA component itself, the latter must be at least 100 times more resistant to the action of ribonuclease than 'free' RNA.

These experiments did not exclude the possibility that ribonuclease cannot penetrate the cell or nuclear membrane, so that the RNA component in its intracellular location is protected from enzyme attack. This seems unlikely, however, since ribonuclease can apparently overcome these barriers in cells infected by RNA preparations. Such protection was also excluded experimentally by homogenizing and fractionating cells 3 hr. after infection with virus and then incubating each fraction with 5 μgm./ml. ribonuclease for 30 min. at room temperature. RNA was extracted from the fractions using cold phenol and titrated in mice (Table 3). No significant change in the infectivity of RNA from any fraction could be detected following enzyme treatment.

Table 3. RESISTANCE OF FRACTIONS OF INFECTED CELLS TO RIBONUCLEASE

RNA preparation from :	Infectivity of RNA (LD_{50}/ml.) Untreated control	Ribonuclease treated
Whole cells	501	1,000
Nuclei	39·8	39·4
Mitochondria	42·2	39·8
Microsomes + sap	14·7	28·8

Cells were infected with 4 plaque-forming units/cell of murine encephalomyocarditis virus, incubated for 3 hr. at 37° C., lysed by osmotic stock (Martin, E. M., Coote, J., and Work, T. S., unpublished work), homogenized, and fractionated by centrifugation. A sample of each fraction was incubated with 5 μgm./ml. crystalline ribonuclease for 30 min. at room temperature. RNA was prepared from treated and control samples and tested for infectivity.

The resistance of the RNA component to ribonuclease can be partly overcome by preliminary incubation with a proteolytic enzyme, papain (Table 4). When the 10,000g deposit of lysed-infected cells (nuclei and mitochondria, containing most of the RNA component 3 hr. after infection[3]) was exposed to papain concentrations up to 10 μgm./ml., the RNA which could be extracted was not rendered less infective. However, ribonuclease treatment following the papain reduced the infectivity of RNA by one logarithmic unit. RNA extracted from fractions treated with 100 μgm./ml. papain followed by 5 μgm./ml. ribonuclease totally lacked infectivity; but this concentration of papain by itself considerably reduced the amount of infective RNA which could be recovered. Thus it would seem that the RNA component contains a protein which is combined in such a way that it is susceptible to the action of papain, but normally protects the RNA from the action of ribonuclease.

Table 4. EFFECT OF PAPAIN AND RIBONUCLEASE ON THE RNA COMPONENT

Papain concentration (μgm.)	Infectivity of RNA (LD_{50}/ml.) Untreated control	Treated with ribonuclease
0	4,200	—
1	4,200	68
5	6,800	680
10	4,900	68
100	19	0

Cells were lysed by osmotic shock 3 hr. after infection with 4 plaque-forming units/cell of murine encephalomyocarditis virus. The lysate was centrifuged at 10,000g for 15 min. and the deposit was suspended in cysteine–versene-citrate buffer pH 7·5. Papain was added to give the concentrations shown and the samples were incubated at 37° C. for 30 min. The pH was then adjusted to 8·0 and ribonuclease was added to a sample to give a concentration of 5 μgm./ml.; an untreated sample served as a control. After 30 min. at room temperature, the samples were frozen. RNA was extracted and tested for infectivity.

The RNA component in cells infected with murine encephalomyocarditis, therefore, probably consists of ribonucleoprotein particles, which represent the 'vegetative' form in which the virus is present during the eclipse phase. These ribonucleoprotein particles are smaller or less dense than the virus[1], or the microsomes of the infected cell, and we propose to call them 'virosomes' by analogy with ribosomes. RNA extracted from virosomes is infective, but since little plaque-forming material can be recovered from infected cells during the time when large numbers of virosomes are present, the particles themselves may have a low probability of initiating infection.

Franklin, Wecker and Henry[4] also showed that ribonuclease treatment (10 μgm./ml.) of a homogenate of mouse brain infected with mouse encephalomyelitis virus did not reduce the infectivity of the RNA afterwards extracted by phenol at 4° C. They concluded that the sole source of infective RNA from infected tissues was the virus particle, based on the fact that infective RNA could be obtained from purified virus using hot phenol and the assumption that only the virus particle is resistant to ribonuclease. Our results, however, suggest that some virus precursors may be resistant to ribonuclease. Moreover, theoretical difficulties occur if their conclusions are extended to other viruses which, like murine encephalomyocarditis, lack a stage sensitive to ribonuclease in multiplication. If RNA becomes infective only after incorporation into mature virus, it should be impossible to extract infective RNA from infected cells before mature virus particles are present. Since there is an increase in the quantity of infective RNA extracted during the first four hours after infection with murine encephalomyocarditis virus, it would, therefore, be necessary to postulate an increase in the number of mature infectious particles present during this time, and one would expect that this increase would be detectable by other means. There is good experimental evidence that a true eclipse phase occurs in the multiplication of murine encephalomyocarditis virus[5] and that the source of infective RNA is not the virus particle[1].

The eclipse phase, infectivity of extracted RNA and ribonuclease resistance of 'vegetative' virus, can be reconciled by the concept of the virosome. The infectivity of virus is then a property of its RNA, but in the infected cell this is present during the eclipse phase in small ribonucleoprotein particles, virosomes. The function of the virosomal protein is unknown; it may be involved in synthesis of virosomal RNA or simply be required to protect or stabilize the RNA. This protein presumably lacks the specific surface structure, which, in the mature virus particle, is necessary for adsorption or penetration of the cell, while the ribonucleoprotein particle may be too large to be taken up like a molecule of RNA; in this case, the virosome would not be infective and an eclipse phase would be observed. After eclipse, the virosome is coated with surface protein and acquires the properties of the infective particle. This extra protein may also make the particle more resistant to phenol extraction, so that it is necessary to use hot phenol to obtain an equivalent yield of infective RNA.

In the case of viruses with a ribonuclease-sensitive stage in multiplication[6], the RNA in the 'vegetative' state may be either bound or free, since some ribonucleoproteins are sensitive to the action of ribonuclease[7]. If these ribonuclease-sensitive materials were shown by some other means to consist of easily dissociated ribonucleoprotein particles, they too could be called virosomes, providing they contained infective RNA but themselves had a low probability of initiating infection. In the test for infectivity of ribonucleoprotein particles, it would be essential to exclude that due to spontaneously liberated RNA. Therefore, virosomes may not be confined to virus systems without a ribonuclease-sensitive stage in multiplication.

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HÆMOGLOBIN F: ITS SIGNIFICANCE IN THE GENETICS AND EVOLUTION OF HÆMOGLOBIN

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THE recent hypothesis of Ingram and Stretton[1] visualizes the thalassæmias as true hæmoglobinopathies characterized by amino-acid substitutions in either the α- or the β-chain of the hæmoglobin molecule, which remain electrophoretically 'silent' because they do not alter the net electric charge but manifest themselves by depressing the rate of synthesis of hæmoglobin. The theory covers the genetic mechanisms controlling the structure and production of α- and β-chains and thus of the major adult hæmoglobins, but does not specifically account for the γ-chains of fœtal hæmoglobin, for the occurrence of which under normal and pathological conditions no satisfactory genetic hypothesis has yet been offered.

We wish to extend the speculation so as to account for the formation of hæmoglobin F and to present a unifying concept which relates the biochemical and genetic facts concerning hæmoglobin synthesis in the course of normal development as well as in the thalassæmias and the hæmoglobinopathies to basic control by the two pairs of genes known at present as α- and β-genes. Our thesis is that the production of γ-chains is under the control of the same pair of genes which determines the formation of β-chains, once the maturation of the fœtus has reached the point where adult hæmoglobin can be made. We are aware that this concept involves seemingly radical departures from present notions concerning the effects of the genetic coding mechanism on protein synthesis. On the other hand, the hypothesis seems capable of reconciling an extraordinary number of as yet poorly explained observations of diverse nature, clinical, hæmatological, biochemical, genetic and evolutionary, which form a body of evidence no fundamental theory can ignore.

The concept arose from the study of Caucasian pedigrees in which two distinct thalassæmia genes could be identified in heterozygotes by their divergent effects on the minor components hæmoglobins A_2 and F (ref. 2). One gene produced the now familiar pattern of an elevated A_2 fraction[3] with normal or barely elevated values for hæmoglobin F, the other was consistently associated with normal values for A_2 but exceptionally high levels of hæmoglobin F, ranging from 4 to 12 per cent. The presence of the two variant genes in one person had resulted in the classical picture of homozygosity, Cooley's anæmia, that is, the genes 'interacted'.

It is now known that both hæmoglobins A_2 and F possess α-chains[4,5]. The elevation of either component in a thalassæmic heterozygote thus militates against a depression of α-chain formation and indicates that both thalassæmia variants observed by us were of the β-variety, involving substitutions in a β-chain. The difference between hæmoglobins F and A_2 resides in the polypeptides complementary to the α-chains, the former possessing γ-chains whereas the latter has now been shown to contain so-called δ-chains[4]. Since the primary effect of β-genes should be limited to substitution and depression of synthesis of β-chains proper, the question arose how in these β-thalassæmias the variant β-genes could mediate differentially another effect, namely, the elevation of a γ-compound in one case and a δ-compound in the other. Clearly a simple compensatory mechanism involving independent effects of hypothetical γ- or δ-genes was not adequate.

The simplest possible assumption seemed to be that the character or site of the substitution in the β-chain itself determined the nature of the by-product, γ or δ, accumulating in consequence of a 'block' in the metabolic pathway of β-chain synthesis. Depending on the nature of the block caused by one or the other substitution, one or the other alternate pathway was being utilized and the resultant γ- or δ-chains (that is, potential β-chains which failed to achieve normal structure) combined with available α-chains to form either hæmoglobins F or A_2. The two thalassæmia genes might accordingly be written $\beta^{X\gamma}$ (or β^{XF}) and $\beta^{X\delta}$ (or β^{XA_2}) to characterize the two substitutions.

In this view, γ- and δ-chains become primitive or altered β-chains, the production of which would thus be controlled by β-genes. Regarded as primitive β-chains, the γ-chains of the alkali-resistant hæmoglobin F of the fœtus should then also be controlled by the genes which in later life determine the production of β-chains. On this hypothesis, the 'switch-over, from hæmoglobin *F* to hæmoglobin A in the normal fœtus can be viewed as a modulation of a single genetic effect, induced in the course of maturation, an event for which many precedents are known in the behaviour of enzymes and hormones. At a given point in ontogeny a previously inactive enzyme may become active, opening up a new metabolic pathway, and the same gene which earlier induces synthesis of γ-chains now expresses its complete structure and induces β-chain formation.

The implication that γ- and β- (as well as δ-) chains have large parts of their structure in common is in keeping with the results of 'fingerprinting' and amino-acid analyses of human hæmoglobins[6]. The further implication that a common pathway is utilized in the synthesis of ultimately divergent products requires the assumption that protein molecules can be assembled in a step-wise manner, a possibility not yet excluded by the known facts concerning the template action of the genic substrate DNA. This assumption is not necessarily in conflict with the basic concept of fixed genetic templates. It merely stipulates that the same complement of genetic material present in the fertilized ovum as in the fully developed individual may exert different effects under different conditions of internal or external environment.

The consequences of this hypothesis as applied to normal and abnormal human hæmoglobin synthesis can now be examined.

If γ-chains represent an early and β-chains a later expression of the activity of β-genes, it becomes possible to visualize how the same erythrocytes can contain both hæmoglobins F and A. As the final metabolic system matures, β-chains can be made in cells already containing a complement of 'primitive β', that is, γ-chains. Increasingly the production of newly synthesized hæmoglobin will entail the use of the ultimate pathway. The gradations observed in the acid-resistant staining of hæmoglobin of neonatal erythrocytes[7] correspond to the proportion of γ- and β-chains in each individual cell. The γ-pathway would seem to be a simpler one which always remains open. Synthesis of β-chains is relatively more complex, though once established it is the more efficient mechanism ; but a residual minimum of utilization of the γ-pathway always persists, hence the minute amount of hæmoglobin F in the normal adult.

It is more difficult to speculate on the biochemical and genetic basis for the production of the δ-chains of hæmoglobin A_2. Stated in a general way, our original observations indicate that primary control by β-genes influences the rate of production of δ-chains and possibly the activity of δ-genes if such exist. One might visualize the utilization of a common pathway which would permit the hypothetical δ-genes to complete manufacturing δ-chains only if the sequence of amino-acids up to the 'branching-off point' under the primary control of β-genes were normal, and one might then use the purely operational term, 'secondary genes', for δ-genes. For the purposes of our main hypothesis pertaining to the genetics of hæmoglobin F and its evolutionary meaning, it is immaterial whether such a relationship or one of several alternatives is assumed. The crucial point remains the actual observation that certain substitutions in a β-chain appear somehow to regulate the production of both γ- and δ-chains.

The assumption that the formation of γ-chains utilizes a simpler pathway than that of β-chains can be interpreted in terms of evolutionary theory. The alkali-resistant adult hæmoglobin of lower animals may be indicative of a γ-chain component reflecting an earlier evolutionary stage, and the final development of a β-chain type of hæmoglobin may represent a relatively late achievement of higher species brought about by a mutation of a 'γ-gene' to a 'β-gene'. If this were true, the transient occurrence of a γ-chain type, hæmoglobin F, in the human fœtus would be truly part of the recapitulation of phylogeny. An even more speculative possibility is suggested by the ontogenetic stability of the α-chains, which may be a still older evolutionary species of polypeptides utilized for hæmoglobin formation. The development of the γ–β type might then represent itself a mutation of an original α-gene, and one is tempted to coin the terms palæo-, meso- and neo-hæmoglobins.

As visualized by Ingram and Stretton, the many amino-acid substitutions theoretically possible in either the α- or the β-chain of the hæmoglobin molecule allow for many variants of either type of thalassæmia. The substitution may be more drastic than those leading to a mere increase of γ- or δ-residues and may result in the production of identifiably anomalous compounds such as hæmoglobin Lepore[8], which appears to be the expression of still another β-thalassæmia gene. It is of interest that in thalassæmic heterozygotes with hæmoglobin Lepore, as in the heterozygotes with the $\beta^{X\gamma}$ type of gene observed by us, the A_2 fraction was not increased, suggesting that this particular block likewise could not be associated with increased utilization of the normal δ-pathway. The combination of the Lepore trait with an 'ordinary' thalassæmia gene of type $\beta^{X\delta}$ resulted in thalassæmia major, as might be expected in an individual lacking the genetic mechanism for the production of normal β-chains altogether.

It would appear that in the presence of a severe block in β-chain synthesis, γ-chains are most readily made, presumably because the pathway utilized for their production is always open—as if the block were 'distal' to the 'branching-off point'. Thus, hæmoglobin F accumulates in all situations in which there is severe interference with the synthesis of both β-chains, whether the gene combination produces two identical or merely similar substitutions $(\beta^{X\delta})_2$ ('classical' thalassæmia), $(\beta^{X\gamma}\beta^{X\delta})$ (the homozygote observed by us), $(\beta^{X\delta}\beta^{X\,Lepore})$ (Lepore-thalassæmia), and probably in other variants yet to be found. The large concentrations of hæmoglobin F in thalassæmic homozygotes as well as the great individual variations reported from case to case (20–90 per cent) become understandable when viewed as the result of blocks of varying severity in the synthesis of β-chains.

The genetic basis for the large amounts of hæmoglobin F found in carriers of the so-called 'High F' gene is obviously very different from that involved in thalassæmia. Such persons show no hæmatological abnormality but exhibit 20–40 per cent hæmoglobin F (ref. 9). The gene is transmitted in a simple Mendelian manner and appears to be allelic to the genes for hæmoglobin S, C (ref. 10) and others already known to represent β-chain mutations[1]. The high percentage of hæmoglobin F presumably reflects not secondary accumulation of γ-chains resulting from a block in the synthesis of an altered β-chain but a 'back mutation' of a β-gene, making it assume the more primitive DNA structure coding for γ-chains as postulated in the evolutionary theory above—a molecular atavism as it were. The existence of such a mutation does, in fact, strengthen the evolutionary hypothesis.

It now seems possible to grade the various substitutions in the β-chain on the basis of the severity of the resultant blocks. Evidently the thalassæmias represent the most drastic of the many possible substitutions, since γ- and/or δ-chains accumulate, at least to a small degree, even in the heterozygous state. Synthesis of hæmoglobin is so severely reduced that the individual erythrocytes cannot fill themselves with pigment and become hypochromic–microcytic. By contrast, the substitutions involved in the sickling mutation and in hæmoglobins C, E, G and F (when produced by the 'high F' gene) are less drastic, that is, they reduce the rate of synthesis of the abnormal β-chains to a lesser degree. No γ- or δ-chain residues accumulate and no hæmatological effects are observed in the simple heterozygous condition. However, the occurrence of target cells in some of these states suggests less-than-optimal filling of the erythrocytes with hæmoglobin, in keeping with the reduced proportion of the abnormal hæmoglobin as compared to hæmoglobin A in heterozygotes ; but the reduction in total cellular hæmoglobin synthesis is not great enough to lead to measurable microcytosis. Some substitutions may not appreciably affect the rate of synthesis.

One might thus construct a tentative 'map' of the polypeptide substitutions based on the relative severity of the resultant blocks (and not to be construed in any strict 'geographical' sense) as follows : $\beta^{X\gamma} \longrightarrow \beta^{X\,Lepore} \longrightarrow \beta^{X\delta} \longrightarrow \beta^{S}$, β^{E} and $\beta^{C} \longrightarrow \beta^{D}$, β^{F} and $\beta^{G} \longrightarrow \beta^{A}$.

Homozygosity should result in increased production of hæmoglobin F in proportion to the severity of the resultant block. The thalassæmias, representing the most drastic substitutions, show the highest proportion of hæmoglobin F. Thalassæmia major is also the severest of the clinical syndromes, with severe anæmia and hæmoglobin deficit within the red cells.

Homozygosity for the sickling gene is complicated by the catastrophic effects which the physical state of hæmoglobin S in deoxygenated cells can produce on the survival of erythrocytes. Here the additional stress created by the rapid rate of hæmolysis may enhance the effect of a mutation which, in terms of its primary effect on the rate of hæmoglobin synthesis, would appear to be relatively mild, judged from the heterozygous state. Thus the demands made on enzymatic processes for the synthesis of the abnormal β-chains may be inordinately great, and part of the available substrate is forced into the less demanding γ-pathway. This may explain the presence of variable and usually modest amounts of hæmoglobin F in homozygotes for the *S*-gene, and the absence of hæmoglobin F increases in homozygotes for the *C*-gene, which causes a substitution at exactly the same point in the molecule.

When a β-thalassæmia gene is combined with another β-chain mutant such as *S*, *C* or *E*, the affected individual is unable to make any normal β-chains. In such double heterozygotes, hæmoglobin F may be expected to accumulate to an extent determined by the nature of the substitutions. The interaction effect apparent in the high proportion of the abnormal hæmoglobin S, C or E in persons who possess only one gene for S, C or E reflects the fact that the thalassæmic substitution is much more drastic than any of the other mutations ; or, in other words, that the rate of synthesis of the thalassæmic β-chain is much more depressed than that of the β-chain carrying an S, C or E substitution. The relatively large increases in hæmoglobin F observed in all these interaction syndromes may thus be attributed chiefly to the drastic depression of that β-chain which carries the thalassæmic substitution and is channelled principally if not exclusively into the γ-pathway.

Combinations of two mutant β-genes other than thalassæmia and causing less severe substitution effects might be expected to result in smaller, if any, increases in hæmoglobin F and in less severe anæmias. Sickle cell–hæmoglobin C disease may serve as an example. No increases in hæmoglobin F seem to have been reported. The approximately equal proportions of hæmoglobin S and hæmoglobin C in this condition indicate the equivalence of the substitution effects caused by the *S* and *C* genes (as might perhaps be expected from the similarity of the mutations themselves).

In α-thalassæmias the production of hæmoglobin F—or for that matter of hæmoglobin A_2—should not be enhanced, since these compounds contain α-chains. In non-interacting combinations with sickling or hæmoglobin C genes, α-thalassæmia genes seem to be involved. The proportion of hæmoglobin S or C in such cases is no greater than expected in simple heterozygotes, the inheritance is non-allelic, the proportion of hæmoglobin F is not increased and the values for both hæmoglobin F and hæmoglobin A_2 in the thalassæmic relatives are low or at most normal[11]. Available space does not permit a discussion of the implications of our hypothesis for the group of α-thalassæmias. It may be pointed out, however, that the transient appearance of Bart's hæmoglobin, a γ-tetramere[12], followed by the appearance of hæmoglobin H, a β-tetramere[13], in H-thalassæmia, which has been designated on other grounds as an α-thalassæmia[14], is in keeping with the assumption that the same genes successively induce synthesis of γ- and β-chains.

Small elevations of hæmoglobin F have been observed in a variety of unrelated hæmatological conditions. In the light of the hypothesis presented, this phenomenon may be interpreted as the result of stress leading to maximal utilization of enzyme systems required for β-chain synthesis, with ultimate development of a deficit necessitating the increased use of the alternate (γ) pathway. Such a mechanism was invoked above in order to account for the relatively high concentrations of hæmoglobin F in sickling homozygotes as compared to homozygotes for hæmoglobin C or E genes.

The hypothesis presented here, though based on apparently crucial observations, extends speculatively to many assumptions. It is presented because it seems to fit most observations concerning the structure and genetics of human hæmoglobins and because it can be tested in a variety of ways, experimental and clinical.

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COMPLEMENT-FIXING BLOOD GROUP ANTIBODIES WITH SPECIAL REFERENCE TO THE NATURE OF ANTI-Le^a

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CERTAIN blood group antibodies are complement fixing, that is, they unite with homologous red cells, complement is fixed and lysis may ensue. In many instances, however, lysis does not result but the aggregate gives a positive antiglobulin test; it is only when the antibody is in high concentration that lysis occurs.

Dacie *et al.*[1] showed that the antiglobulin reagent united not with the antibody but with absorbed complement. In this article E represents erythrocytes, A antibody, and C' complement. In the combination EAC', therefore, antiglobin serum unites with C'. Later, Mollison[2] showed that EA, although not lysed by human complement, were lysed by certain animal complements, for example, guinea pig, rabbit.

Blood group antibodies that are complement fixing are cold incomplete anti-H, anti-Lewis (anti-Le^a and anti-Le^b), anti-Jk^a and anti-Jk^b, some examples of anti-Fy^a and anti-K, and certain antibodies from cases of cold acquired hæmolytic anæmia; these latter have been studied[3,4] and found to be γ_1-globulins. Although cold incomplete anti-H is undoubtedly the commonest antibody, unfortunately, as Dacie[5] originally showed, it does not unite with the red cells in the absence of complement and it is impossible, therefore, to prepare EA alone. One of the most useful antibodies to work with is anti-Le^a and $Le(a+)$ erythrocytes, particularly since anti-Le^a is a fairly common antibody. The work described in this article was done using this system.

Stratton[6] showed that EA could be prepared using this system, and originally it was found that the sensitized EA gave a negative antiglobulin test. It was the combination EAC' which, as previously described, gave a positive antiglobulin test. It was also possible to prepare an eluate from EA which would unite with $Le(a+)$ cells producing more EA; these EA may not themselves give a positive antiglobulin test but would, nevertheless, react with C', forming EAC'. Antibody could not be eluted from EAC'.

The components of C' which are fixed when C' reacts with EA are shown in Table 1. $C'2$ is completely fixed; this, however, gives no clue as to the nature of the C' component on EAC' which unites with the antiglobulin serum. This problem was tackled by three groups of workers in somewhat different ways[7–9], but they all came to the same conclusion—the important component was $C'4$. Jenkins *et al.*[9] demonstrated that the active fraction of the antiglobulin serum was anti-β_1.

Table 1. Fixation of C' Components by EA

	$C'1$ (per cent)	$C'2$ (per cent)	$C'4$ (per cent)
Normal serum	100	100	100
Absorbed with normal cells (E)	93	80	80
Absorbed with EA	73	0	48

This is a valuable system for studying the action of complement on the sensitized cell EA, since in addition to lysis a new sensitive method is available for detecting the absorption of C' by EA, namely, the antiglobulin test. Le^a group specific polysaccharide (provided by Prof. W. T. J. Morgan) can be utilized in experiments on sensitized cells. Le^a substance when mixed with EA in a suitable concentration will prevent the treated EA fixing C'; but it has no effect on the reaction between EAC' and antiglobulin serum. This, among other things, is another means of determining C' fixation[10]. In this system the binding power of complement on the antigen–antibody combination can be demonstrated.

As a preliminary to further investigations on the effect of C' on antibody in EA it was decided to try to determine the nature of the antibody. Fudenberg, Kunkel and Franklin[11], in a study of high molecular weight antibodies, pointed out that 5–10 per cent of the total γ-globulin of normal serum consists of faster-moving $19S$ component of molecular weight about a million. The other 90–95 per cent consists of $7S$ of a molecular weight of 150,000. It is known that mobility and weight are not necessarily associated, but, for convenience, in this article the two groups are referred to as γ_1- and γ_2-globulins. These workers found certain antibodies belonged to the first, or γ_1-group, for example, often complete antibodies or agglutinins, and others, such as incomplete Rh antibodies, to the second group. The main difficulty in this work is that most anti-Le^a C' fixing antibodies are of low activity and the amount of serum protein present as antibody is probably very small indeed. Many of the normal physical methods applicable to their study cannot be used and indirect methods have been employed from which tentative conclusions are drawn.

Work involving electrophoresis of anti-Le^a antiserum on polyvinyl particle blocks[12] showed that antibody activity was present in the γ-globulin fraction of the serum. The euglobulin fraction of the serum (prepared at pH 6·4) in certain instances contained all the antibody activity. It was thought possible, therefore, that the antibody belonged to the γ_1-globulin group. Deutsch and Morton[13] showed that sulphydryl compounds, such as mercaptoethanol, dissociate macroglobulins, and Grubb[14] applied this to study the effect of this treatment on sera containing blood group antibodies. Using essentially similar methods anti-*Le*^{a} sera so treated would not make *EA* or *EAC′* when mixed with *Le*(a+) erythrocytes, even after the addition of *C′*. This is what would have been expected if the antibodies were heavy-weight γ-globulins.

Many different anti-*Le*^{a} antisera were used to prepare *EA*. All the examples of *EA* originally prepared were un-agglutinated by routine or commercial antiglobulin sera, or even by those prepared following immunization of rabbits with euglobulin fractions of normal human serum. Nevertheless, that such an antiglobulin serum was united with *EA* was suggested by its ability to impair or abolish human *C′* fixation by *EA* pre-treated with antiglobulin serum. Afterwards, potent anti-*Le*^{a} sera were found from which *EA* could be prepared which were agglutinated by anti-euglobulin antisera (Table 2).

Experiments were done involving the titration of sensitized cells *EA* with antiglobulin sera, together with tests of the inhibiting effect of γ_1- and γ_2-globulin preparations on the ability of anti-γ_1 serum to agglutinate sensitized cells. Prof. N. Martin provided anti-γ_1 and γ_1-globulin, and the γ_2-globulin preparation used was free from heavy component. *EA* were prepared using several different anti-*Le*^{a}, and the *Rh* sensitized cells were coated with antibody considered to be a γ_2-globulin (Tables 2 and 3). Additional tests[12] also showed that α- and β_1-globulins were non-inhibitory. Table 4 shows that there is a correlation between the titre of the antiglobulin serum with the *EA* and the extent of their lysis by absorbed guinea pig serum. This suggests that the differences of antiglobulin titre may be due to differing amounts of antibody on the *EA*. These results (Tables 2, 3 and 4) suggest that the antibody on *EA* is a γ_1-globulin.

Further experiments on the effect of *Le*^{a} substance and mercaptoethanol on the sensitized erythrocytes, *EA* themselves, provided additional information. If a 10 per cent suspension of *EA* (iii, Table 2) was mixed with an equal volume of 2×10^{-5} solution of *Le*^{a} substance at 37° C., the *EA* were not then agglutinated by the antiglobulin serum. Secondly, treatment of *EA* with 0·2 *M* mercaptoethanol at room temperature for 2 hr. resulted in a negative antiglobulin test and the titration score[15] fell from 15 to 0, but if *Rh*-sensitized cells were similarly treated the titration score was reduced only from 26 to 23. The mechanism of these effects is not entirely clear, but it does suggest that normally the antiglobulin serum is united with the absorbed antibody. This is important because it is possible that serum proteins, other than antibody, may be absorbed on to *EA* at the time of their preparation (cf. Vaughan[16]). The second group of experiments will be more fully described elsewhere.

In reaching the conclusion that these *C′* fixing anti-*Le*^{a} antibodies are heavy-weight γ-globulins, it must be borne in mind that the γ_1-globulins used in inhibition tests and for the preparation of antibodies are from cases of macroglobulinæmia and, therefore, are pathological and not normal serum proteins.

Table 2. TITRE OF ANTIGLOBULIN SERA WITH VARIOUS SENSITIZED CELLS

Test cells*	Antiglobulin titre Anti-euglobulin	Anti-γ_1
EA i	0	16
ii	1	16
iii	8	128
iv	16	512
Rh-sensitized cells	64	1,024

* *EA* prepared with different anti-*Le*^{a} i–iv.

Table 3. INHIBITION TESTS

A Substances added to equal vol. of anti-γ_1 dil. 1/20	*B* Antiglobulin test mixtures from column *A* *EA* (iv)*	*Rh*-sens.*
Saline	+ + + + +	+ + + + +
γ_1-Globulin dil. 1/400	Neg.	+ + + + +
γ_2-Globulin dil. 1/25,000	+ + + + +	Neg.
γ_2-Globulin dil. 1/1,000	+ + + +	Neg.
γ_2-Globulin dil. 1/100	Neg.	Neg.

* As in Table 2.

Table 4. RELATIONSHIP BETWEEN ANTIGLOBULIN TITRE AND LYSIS OF *EA*

Test cells*	Antiglobulin test titre	Lysis with absorbed guinea pig serum (per cent)
EA i	0	0
ii	2	0
iii	8	40
iv	16	70
E	0	0

* As in Table 2.

Practical applications of these observations spring to mind. An antibody, anti-γ_1, active against the antibody is available, together with another, anti-β_1 (ref. 9), against absorbed complement; these facilitate the study of the action of *C′* on *EA* (ref. 10). Wiener *et al.*[17] have used a system involving the neutralization of antiglobulin serum for the detection of human serum globulins. The results shown in Table 3 may be taken to illustrate the known cross-reactions between γ_1- and γ_2-globulins or their impurity. Purified protein preparations prepared by chemical methods must always be regarded as impure when considered for immunological work and, similarly, an antibody prepared in animals is not merely an antibody against a single protein but also contains a spectrum of antibodies. Thus far, the system has been used only for the assessment of the relative purity of serum fractions supposedly predominantly either γ_1- or γ_2-globulin.

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EFFECTS OF IONIZING RADIATIONS ON RICE PLANTS

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THE effects of ionizing radiations on various plants have been reported by many investigators since the early work of Stadler[1,2]. Hence, there exists a wealth of information on the radiophytological responses. The results of plant reactions during the first year of study on radiation induction of useful mutations in a rice crop are presented here and discussed with the view of enlarging our present knowledge of radiobiological effects.

Five varieties of Burmese rice (*Oryza sativa* var. *indica*)—*A*36–3, *B*401, *C*28–16, *D*25–4 and *Xq*–4—were each irradiated both with 25 kr. and 35 kr. of X-rays at the Brookhaven National Laboratory, after exposure to a relative humidity of approximately 60 per cent for about 2 weeks. Nearly 3 weeks after this processing, some of the irradiated seeds were germinated here on moist filter-papers in Petri dishes on May 11, that is, about one month in advance of normal sowing season which usually falls due in mid-June. The remaining lot set aside at room temperature was likewise sprouted on June 15 to study the storage effect, if any.

The sprouts were transferred from dishes to earthen pots during 3–6 days following germination. Water was supplied regularly and close observations were made daily. All were uprooted, measured, photographed, and transplanted in pots when about 32 days old. Seedling fatality was expressed as the number of dead seedlings (per cent of germinants).

Tillerings and heights of transplants were recorded weekly. About 2 weeks before floral emergence, both treated and untreated transplants were properly isolated in linen bags in case of cross-pollination in the irradiated (*R*1) generation. This preventive measure was necessary to ensure that mutants to be screened in generation *R*2 were due to radiation only. Seed fertility was determined by counting the total number of both filled and unfilled grains in each treatment, and then calculating the number of filled grains per hundred of that total number. The susceptibility value of rice plants to false smut disease due to *Ustilaginoidea virens* (Cke.) Tak. was computed as the number of infected grains as a percentage of all filled grains in the treatment.

Four other varieties of Burmese rice—*A*28–81, *B*24–92, *C*33–18 and *D*17–88—after bombardment with thermal neutron fluxes of $6{\cdot}92 \times 10^{8}$/cm.^{2}/sec. and $6{\cdot}70 \times 10^{8}$/cm.^{2}/sec. for 15 and 20 hr., respectively, on July 13 at Brookhaven were germinated, as described, on August 12. Transplantation was completed on September 14, and transplants in the neutron-treated (*N*1) generation were also isolated.

As shown in Table 1, there was practically no difference in germination of rice grains either among varieties or treatments with X-rays or thermal neutrons. On the other hand, Mashima and Kawai[3] reported a decrease in germination-rate of rice grains as a result of exposure to both X-rays and thermal neutrons. Development of sprouting seeds apparently slowed down directly the X-ray dosages increased. During the initial stages of growth in the nurseries, *R*1 seedlings looked like lean, medium-dwarfs with relatively thick, narrow, and dark green leaves, displaying varying degrees of foliar convolution and restricted growth. Only one albina-type mutant showed up among sixty-three 25-kr. members of *B*401, but it did not survive transplantation. Other such chlorophyll-defective mutants as those with chlorotic/yellowish stripes along margins or mid-ribs of leaves were seen on two *C*28–16, two *D*25–4, and one *Xq*–4 members of the 25-kr. treatment.

Fatality and retardation in height of 32-day old rice seedlings generally increased as the X-ray dosages increased (Table 1). Some differences of intervarietal responses were also apparent. Matsuo *et al.*[4] made similar observations on mortality and inhibition of seedling height. However, *B*401 seedlings were taller at a higher dose-rate than at the lower dose-rate. Such an apparent stimulation at a higher dose-rate, and a direct relationship between chronic gamma-ray dosage and degree of growth inhibition in *Vicia faba* were also reported by Sparrow and Gunkel[5]. Ouang and Chang[6] recorded about 10 per cent increase over the control in height of *X*1 (*R*1) plants. A departure from direct proportionality between reduction in height of maize seedling and radiation doses at certain dosages of X-rays or thermal neutrons had been demonstrated by Schwartz[7], though. Moutschen[8] noted the same with barley coleoptiles, the develop-

Table 1. RICE GRAIN GERMINATION, SEEDLING FATALITY, SEEDLING HEIGHT, AND RANGE OF SEEDLING HEIGHTS IN *R*1 AND *N*1 GENERATIONS RAISED ON MAY 11, 1959

Variety	Dosage	Average germination (per cent)	Average fatality (per cent)	Average seedling height (cm.)	Range of seedling height (cm.)
*A*36–3	0 hr. (control)	100	4	59·59	41·3–71
	25 kr.	98	14·2	37·80	21–57·5
	35 kr.	97	76·3	27·47	8·8–42·4
*B*401	0 hr.	98	5·1	56·77	30–69·5
	25 kr.	100	37	30·21	16–46·5
	35 kr.	99	93	37·61	27·9–46·3
*C*28–16	0 hr.	99	3	39·70	25–52
	25 kr.	98	10·2	25·70	18–40·5
	35 kr.	98	74·5	20·80	10–47
*D*25–4	0 hr.	97	5·2	48·98	28–69
	25 kr.	95	33·6	27·89	19–56·5
	35 kr.	97	93	23·80	11–43
Xq–4	0 hr.	100	3	49·46	30·6–68·4
	25 kr.	100	22	29·16	12·7–39
	35 kr.	97	70·1	23·56	9·4–39·1
*A*28–81	0 hr. (control)	96·5	—	43·36	41–46
	15 hr.	97·5	—	38·14	32–46
	20 hr.	98	—	37·72	34–40
*B*24–92	0 hr.	99	—	44·62	42–46
	15 hr.	98	—	41·85	40–47
	20 hr.	98·5	—	45·2	40–50
*C*33–18	0 hr.	95	—	37·94	34–44
	15 hr.	97	—	36·4	33–40
	20 hr.	97	—	37·12	33–42
*D*17–88	0 hr.	94·5	—	41	37–45
	15 hr.	90	—	40·44	35–45
	20 hr.	94·5	—	41·56	36–48

kr., kiloroentgens of X-rays ; hr., hours of exposure to thermal neutrons ment of which depends on both cell-division and cell-elongation. According to Schwartz and Bay[9], seedling growth is determined simultaneously by mitotic inhibition and intensity of chromosomal damage.

As observed by Ehrenberg and Nybom[10] and Caldecott[11] in barley, and Matsuo *et al.*[4] and Yamaguchi[12] in rice, both wide and narrow ranges of seedling heights were obtained ; this suggested larger and smaller deviations around mean values in the X-ray and thermal neutron treatments (Table 1), respectively. Contrary to the results of Matsuo *et al.*[4] and Huang[13] on the progressive inhibition of seedling height consequent on increasing bombardment with thermal neutrons, Table 1 indicates no appreciable disparity in height among the treatments.

Table 2 suggests that about one-month delay in sowing clearly enhanced the fatality of seedlings from grains irradiated with X-rays, but did not apparently affect germination of almost all strains tested. The storage effect, as it is termed, was also exhibited in barley seeds, and depended on a number of factors (Caldecott[14]).

Differences in height among treatments may persist in transplants of certain varieties for some weeks. Later these may narrow down close enough to suggest a recovery from the setback in growth at the early stages. Success in attaining the height of the control at the time of flowering of *R*1 wheat plants was also observed by Bora[15]. The stunting effects of X-rays are probably due to gene mutation[6].

Tillering in some strains may vary directly or inversely with the X-ray doses. Yet, most of the tillers in the treated plants were either late or poorly developed and unproductive. The early differences in the numbers of tillers between treatments tended to drop and the number became nearly equal to the control later in the season owing mainly to the death of underdeveloped tillers in certain strains. Natarajan *et al.*[16] and Bora[15] obtained a decrease as well as an increase in the number of tillers in wheat plants, respectively, whereas Ouang and Chang[6] reported both inhibitory and stimulatory effects of X-rays on tillering in rice plants.

Table 3 summarizes the number of panicles per plant, grains per panicle, and percentages of grain fertility and susceptibility to false smut disease, and dates of heading. There was a significant gain in the number of panicles per plant at 35-kr. dose-rate as compared to controls; the exceptions are probably irresponsive *B*401, highly responsive *C*28–16, and adversely affected *Xq*–4 at both levels of X-rays, while such a difference between controls and 25-kr. treatments was mostly lacking ; but nevertheless, the panicles of *R*1 plants usually had less grains than those of their respective controls. Essentially, no considerable divergence in grain or panicle numbers was apparent among the thermal neutron treatments. Grain fertility was greatly reduced though not always in progression with the dosages administered. Ouang and Chang[6] and Matsuo[4], however, found a direct relation between the grain sterility and the dosages. As reported[13], the depression of grain fertility seemed to be more severe in thermal neutron than in X-ray treatments. Grain sterility is attributed to chromosomal damage or rearrangement[17,18]. The resistance to false smut disease of all varieties studied remained almost unimpaired at the levels of X-rays used. Most of the *R*1 plants of 25-kr. series came to heading 2–5 days later than the controls. Ouang and Chang[6] noticed earliness as well as lateness in heading of treated rice plants while Huang[13] reported a distinct delay in flowering.

Table 2. RICE SEED GERMINATION AND SEEDLING FATALITY IN *R*1 GENERATION RAISED ON JUNE 15, 1959

Variety	Dosage	Germination (per cent)	Fatality
*A*36–3	0 hr.	98·8	—
	25 kr.	99·1	37·5
	35 kr.	98·9	82
*B*401	0 hr.	97·9	—
	25 kr.	97·3	95·6
	35 kr.	98·8	100
*C*28–16	0 hr.	99·5	—
	25 kr.	98·47	16
	35 kr.	94·99	100
*D*25–4	0 hr.	93	—
	25 kr.	89	41·5
	35 kr.	85	100
Xq–4	0 hr.	99·1	—
	25 kr.	99·1	39
	35 kr.	95·9	87·1

—, data not taken

Table 3. NUMBER OF PANICLES AND GRAINS, PERCENTAGES OF GRAIN FERTILITY AND SUSCEPTIBILITY TO FALSE SMUT, AND DATE OF HEADING

Variety	Dosage	Average No. of panicles per plant	Average No. of grains per panicle	Average grain fertility (per cent)	False smut suscept-ibility (per cent)	Date of heading (days)
*A*36–3	0 hr.	13	140	55	0	—
	25 kr.	13	116	8	0·1	†4
	35 kr.	16	111	3	0	—
*B*401	0 hr.	18	119	56	0	—
	25 kr.	18	111	6	0·001	†2
	35 kr.	17	116	16	0	—
*C*28–16	0 hr.	10	180	65	0	—
	25 kr.	14	115	5	0·11	†3–4
	35 kr.	13	124	13	0	—
*D*25–14	0 hr.	17	150	45	0·3	—
	25 kr.	16	136	5	0	†0 1
	35 kr.	20	119	1	0	
Xq–4	0 hr.	13	164	51	0·06	
	25 kr.	9	120	11	0·09	†3 5
	35 kr.	9	115	10	0·09	
*A*28–81	0 hr.	8	97	6	—	
	15 hr.	9	100	3	—	
	20 hr.	7	100	3	—	
*B*24–92	0 hr.	13	103	5	—	
	15 hr.	8	100	<1	—	
	20 hr.	7	99	<1	—	
*C*33–18	0 hr.	6	92	15	—	
	15 hr.	6	98	<1	—	
	20 hr.	5	99	<1	—	

†, later than control ; kr., kiloroentgens of X-ray ; hr., hours of exposure to thermal neutrons ; 0 hr., control ; —, data not taken

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LETTERS TO THE EDITORS

GEOPHYSICS

Origin of Stone and Iron Meteorites

THE question arises whether meteorites in general have had a common origin, and in particular whether the stone meteorites have had an origin different from that of the iron meteorites. One line of experimentation bearing on this problem is the determination of cosmic-ray exposure ages of meteorites. If the total content of a stable cosmogenic nuclide is measured, and if its rate of production can be determined, then the length of exposure to the radiation is known.

But the rate of production of a stable nuclide must be estimated either from purely theoretical considerations or from measurements on a radioactive nuclide. The theoretical arguments are probably not too reliable at this stage, and the best estimates seem to be based on radioactivity measurements. For example, the tritium decay-rate at the meteorite's time of fall gives directly the tritium production-rate, and if the cross-section ratio $\sigma_t/\sigma_{^3\mathrm{He}}$ is known, the helium-3 production-rate can be calculated. Then from measured amounts of helium-3 an age can be determined.

A table of such meteorite ages has recently been compiled by Urey[1]. The helium-3 measurements were carried out by a number of investigators, using either mass spectrometry or activation analysis. The production-rate was calculated on the basis of five tritium measurements in different stone meteorites. Since all the values were approximately equal, it was concluded that the helium-3 production-rate is invariant and equal to 10^{-8} stand. c.c./gm./million years. The production in iron meteorites was chosen as half this value.

These calculations lead to a general trend of ages, as shown in Fig. 1. A discrepancy can be seen to exist between the ages of the stone meteorites and those of the iron meteorites. On this basis it was suggested[1] that the stones and the irons may have had a different origin and history. The irons presumably coming from the asteroidal belt, perhaps the stones come from the surface of the Moon. This would be in line with shorter exposure ages for the stones.

But several refinements which should be made to such an estimation of exposure ages lead to perhaps not negligible adjustments. In particular, one should try to estimate better the difference in helium production-rates in iron and stone meteorites, the dependence on depth of helium production, and the effect of space erosion. Some preliminary experimentation has been done on at least the first two of these effects.

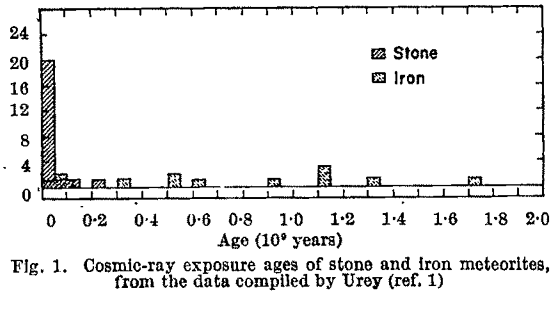

Fig. 1. Cosmic-ray exposure ages of stone and iron meteorites, from the data compiled by Urey (ref. 1)

Aluminium may be considered to be an element of Z nearly equal to the average for stone meteorites, and $\sigma_{^3\mathrm{He}}$ for an iron target has been found to be greater than for an aluminium target[2], increasing to a factor of three at bombarding energies of several BeV. (Schaeffer, O. A., and Fisher, D. E., unpublished results). When one applies this correction to Urey's data, and when data on seven other iron meteorites that have been recently measured[3] are included, the discrepancy in ages between the stones and the irons is largely removed. However, more cross-section data are needed for those elements which actually make up the bulk of stone meteorites.

It is not clear whether the dependence on depth of nuclide production will be important in stone meteorites, although in irons it has been shown[4] that a production-rate taking this factor into account may vary by up to an order of magnitude from estimations ignoring the effect. Eberhardt and Hess[5] assume that the effect will be negligible in stones, because of their rather small size. But many of the stones are of size comparable to several irons in which a serious depth effect has been estimated. Further, Öpik has pointed out that stone meteorites have a high probability of shattering on contact with the Earth's atmosphere, so that small fragments found on the Earth may actually have been in a large meteorite in space[6]. If a depth-effect exists, one would expect to find differences in content of both stable and radioactive nuclides within the same meteorite, and differences in content of radioactive nuclides in different meteorites, such as are found in irons. Begemann *et al.*[7] found the same tritium content in two pieces of Norton County stone meteorite, but Fireman and DeFelice[8] found a value half as much in still another piece. Fireman and DeFelice also measured the argon-39 in two pieces of Norton County and found a 30 per cent variation. Eberhardt and Hess[5] measured the helium-3 in four stone meteorites that have been investigated by other workers, and in three of them obtained significantly different values, two of them differing by greater than a factor of ten. Some recent tritium measurements in different stone meteorites suggest a range of values[9]. The argon-39 contents of two stone meteorites were measured[8] and a factor of four difference was found. From these considerations, then, it would appear that a serious dependence on depth may exist in stones.

A scheme for estimating the depth-dependent production-rate of stable nuclides in iron meteorites, based on argon-39, chlorine-36, and rare gas ratio measurements, has been presented[3,4]. It is not so easy, however, to estimate this effect in individual stone meteorites. The rare gas ratios are not expected to vary uniformly with depth, due to radiogenic helium-4 and to a varying chemical composition which will affect neon and argon production. Approximations to the upper and lower limit of helium-3 production-rates in stones can be estimated,

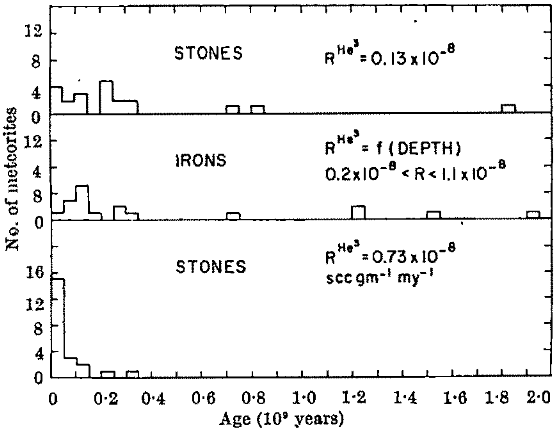

Fig. 2. Depth-dependent cosmic ray exposure ages for stone and iron meteorites. $R^{3\text{He}}$ = helium-3 production-rate in standard c.c./gm. of meteorite per million years

however, from the upper and lower limits in the iron meteorites[4] and from the relative cross-sections in iron and aluminium targets. The ages thus calculated are shown in Fig. 2. In this figure ages have been calculated only for those iron meteorites for which all the rare gases have been determined. A production-rate in stones of $0{\cdot}7 \times 10^{-8}$ stand. c.c./gm./m.y. may be compared to that of $1{\cdot}0 \times 10^{-8}$ obtained by Urey on the basis of tritium measurements. Ages have also been calculated[3] for the irons on the basis of their neon-21 and argon-38 contents, and on measured values for the ratios $\sigma_{^3\text{He}}/\sigma_{^{21}\text{Ne}}$ and $\sigma_{^3\text{He}}/\sigma_{^{38}\text{Ar}}$ in iron targets. These ages agree well with the helium-3 ages.

The age distribution for the stone meteorites, with $R^{3\text{He}} = 0{\cdot}7 \times 10^{-8}$ peak at a lower value than does the iron age distribution. There are several stones with ages of approximately 10–20 million years, and none older than 400 million years, whereas some irons are older than a billion years. However, when $R^{3\text{He}}$ reaches $0{\cdot}13 \times 10^{-8}$ in stones, the stone age distribution is no longer different from the iron's. As the depth effect becomes more important in stones, then, their age distribution shifts more towards that of the irons. It should also be noted that a slightly younger age for the stone meteorites is reconcilable with a longer mean life against collision destruction in the asteroidal belt for irons than for stones. From Fig. 2 it does not seem possible to say that there is an age difference greater than can be accounted for by this consideration.

Only a very general statement can be made at this time on the effect of space erosion. However, it should be expected that the combined wearing effect of dust, gas, ions, and electrons in interplanetary space might be quite different for irons than for stones. Fireman and DeFelice[8] have shown that the effect may be quite large, and may be more than two orders of magnitude greater for the softer stones than for irons. This would lead to a shorter apparent age for the stones.

Until more information is forthcoming, then, on more direct radioactive-stable nuclide determinations (such as argon-38/argon-39, as well as hydrogen-3/helium-3), one is not justified in postulating separate origins for the stone and iron meteorites on the basis of their cosmic-ray exposure ages.

I am grateful to Dr. O. A. Schaeffer of Brookhaven National Laboratory for his criticism. Part of this work was done while I was at Brookhaven National Laboratory.

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Enhancement of Night-Sky Molecular Nitrogen Emission in Polar Cap Absorption Events

IT has long been accepted that auroral activity is associated with magnetic disturbances. Observations inside the auroral zone show the relationship to be rather complex; bright auroral displays are often observed during quiet magnetic conditions, and even in the greatest of magnetic storms visual auroræ are not always observed. On the other hand, well-exposed auroral spectra are always obtained during these storms. The source of this emission lies in extensive glows, often above the threshold of vision, but of such low contrast that observers normally do not recognize or report them. Changes in the colour of the night sky can be observed on colour film exposed during such storms. The forbidden atomic oxygen lines and the first negative, first positive and second positive bands of molecular nitrogen are all enhanced in these glows.

Since the beginning of the International Geophysical Year, patrol spectrographs have monitored the night sky emissions in the Antarctic and Arctic. The zenith intensity of the first negative band of ionized molecular nitrogen at 3914 Å. has been determined during some of the major magnetic storms, at stations situated inside the auroral zone.

The relative intensity of the band at 3914 Å. is plotted on a logarithmic scale in Fig. 1. Curve 1*a* is the intensity variation during May 1959, at South Pole Station, Antarctica, and curve 1*b* is the intensity variation during November 1960, at Thule, Greenland. On May 10, 1959, a flare on the Sun was followed about 6 hr. later by the start of a polar cap absorption event. Soon after the polar cap absorption began, the intensity at 3914 Å. began to increase steadily, reaching a maximum, some 100 times above normal minimum night-time levels, about 24 hr. later, coincident with the peak of activity of the associated major magnetic storm. The intensity then fell steadily, returning to its normal night-time minimum values some 48 hr. after the peak.

In November 1960, two similar events occurred following flares on the Sun, and the sequence of events at Thule is almost identical to the behaviour observed at South Pole Station in 1959. The times of the flares, polar cap absorption commencements and the sudden commencements of the magnetic storms are shown in Fig. 1.

The intensity of the first and second positive bands of molecular nitrogen show the same behaviour as the first negative bands.

In the three large polar cap absorption events, in July 1959, the same behaviour was observed at Scott Base, Antarctica. These observations cover six polar cap absorption events, indicating that this behaviour is not unusual.

Comparison with all-sky camera films shows that most of this energy does not arise from visual auroral forms, since, during many of the exposures at the height of the storms, no auroral forms could be seen in or near the zenith.

The onset of the increased nitrogen emission is closely associated with the beginning of the polar cap absorption. Throughout this absorption the *D*-region ion density is well above its normal level, which implies that a large flux of ionizing and exciting particles is penetrating down to heights of 65 km. and lower[1].

At Thule the observations begin and end when the Sun is illuminating the atmosphere down to 100 km. in the zenith. During normal days the emission at 3914 Å. shows considerable twilight enhancement (shown in Fig. 1*b* by dotted lines which are exposures during which the solar depression is less than 18°). During the great storm enhancements no twilight enhancement is evident, suggesting the emitting region is below 100 km. The storm enhancement is so great that the normal twilight enhancement at these times would not be apparent. At Pole Station, in Fig. 1*a*, the Sun is at a constant solar depression of approximately 18°, so no twilight effects occur.

The forbidden OI lines at 6300 and 5577 Å. do exhibit enhancements during the main phase of the magnetic storm, but they do not exhibit the steady changes over a period of days like the molecular nitrogen emissions. The Vegard–Kaplan bands of nitrogen are not observed in these spectra. These emissions are all from metastable states which would be collisionally deactivated below 100 km. The molecular oxygen bands are also present in the spectra. These factors suggest that the source of emission is near or below 100 km.

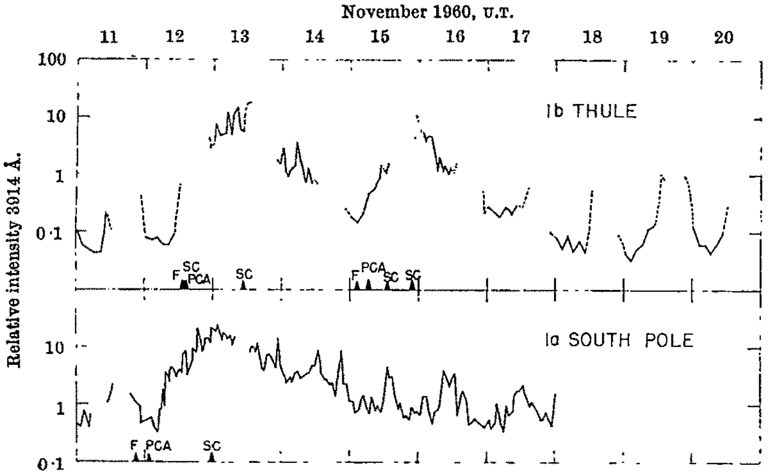

Fig. 1. The relative intensity in the zenith of 3914 Å. radiation at South Pole Station from May 10–16, 1959, in Fig. 1*a* and at Thule from November 11–20, 1960, in Fig. 1*b*. Times of major flares, polar cap absorption commencements and magnetic storm sudden commencements are indicated

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ASTROPHYSICS

Origin of Cosmic Rays

ALTHOUGH cosmic rays have been known for more than forty years, their origin has not been satisfactorily elucidated. At present, it is believed that primary cosmic rays comprise nuclei of heavy elements ≈ 1 per cent apart from protons and α-particles. The presence of cosmically rare elements, for example, lithium, beryllium and boron in cosmic rays is of considerable importance for any theory of their origin. The other characteristic of cosmic rays is the energy spectrum which is of the form:

$$I(E) = KE^{-\gamma} \qquad (1)$$

where I is the intensity of a given kind of particles and E is the total energy of a proton or nucleon. Here γ varies between 1·9 and 3·0, depending on the energy range. Their energy lies between 5×10^{8} and 10^{18} eV. They also show in general the isotropy. The small anisotropy is, however, generated by the local sources and the local magnetic fields.

It is generally believed that the acceleration of ions in astronomical objects is caused by the interaction of the electric charge of ions with the local magnetic fields and not from nuclear or particle decay. Several acceleration mechanisms for the cosmic ray particle have been discussed in the literature, but none of them satisfactorily takes account of the whole range of the energy spectrum. I am of the opinion that not a single mechanism is involved, but a multiple process depending on the environment. Alfvén[1] has discussed the possibility of solar origin for the cosmic radiation. The theory is in good accord with most of the observed phenomenon, for example, the variations of cosmic rays in relation to various solar features, etc. However, it needs revision which has been deferred for future publication. On the other hand, Fermi[2] developed the theory of acceleration of cosmic dust through interstellar space. The cosmic particles are accelerated in interstellar space by moving magnetic fields. The longer the particle moves in interstellar magnetic fields, the greater will be its energy. From elementary considerations, he obtained the energy spectrum of the form of equation 1. However, this mechanism can accelerate only those particles the energy of which exceeds a minimal 'injection' energy (E_{inj}), which for protons in interstellar space is $\approx 10^8$ eV. and for iron nuclei, 5×10^9 eV./nucleon. Further, Alfvén's theory[1] cannot account for the particles of energy $> 10^{15}$ eV. Thus there must be some sources inside the galaxy which somehow generate particles of energy exceeding E_{inj}. The particles carrying great energies abandon their sources and are released into interstellar space, where they move in galactic magnetic fields for hundreds of millions of years and become accelerated by Fermi's mechanism.

The solar flare of February 23, 1956, is of considerable interest. During this flare, particles of the energy-range 10^9–$2{\cdot}4 \times 10^{10}$ eV. were observed. Simpson[3] found the total energy of the flare to be well in excess of 10^{32} ergs. These flares may be much more frequent in red dwarf stars as compared with the Sun and may in fact be the source of cosmic ray generation. In particular, the flare stars of spectral type *dM3e* (*UV* Ceti) and later, are very numerous. Perenago, as quoted by Shklovsky[4], believes that red emission dwarfs constitute a considerable fraction of the entire population of red dwarfs, the most abundant stars in the galaxy. It is possible that essentially all red emission dwarfs are flare stars to some extent. During the flare of *UV* Ceti stars, which lasts for several minutes, the luminosity rises by a factor of about ten. The flare occurs from once to several times a week. Thus the flare phenomenon on such stars is of the same general character as on the Sun, but will be much more energetic. Shklovsky[4] found that the power of optical radiation in a red-dwarf flare will be some 300 times as great as for such large solar flares as that of February 23, 1956. Thus the total energy of the red dwarf flare will be well in excess of 3×10^{35} ergs.

According to Kuiper[5], 81 per cent of the main sequence stars are *M*-dwarfs. The total number of stars of a galaxy are $\approx 2 \times 10^{11}$ and about 95 per cent of all the stars in our galaxy belong to the main sequence. Thus the total number of *M*-dwarfs in our galaxy will be $1{\cdot}6 \times 10^{11}$. Let us now assume that 10 per cent of the *M*-dwarfs are emission stars accompanied by a flare and that the flare event occurs on the average once in a year. The total release of energy by all the *M*-dwarf emission stars will be $\approx 1{\cdot}5 \times 10^{38}$ ergs/sec. This estimate may be an order of magnitude or two less because of the conservative approximation. Shklovsky[6] found that about 10^{39} ergs/sec. are lost in the galaxy as a result of nuclear collisions with interstellar gas. Thus I conclude that red dwarfs will supply sufficient energy to maintain dynamic equilibrium of cosmic rays in our galaxy. The hypothesis is further supported by the presence of heavy nuclei in cosmic rays since the synthesis of elements is advanced in the stellar system.

I am grateful to the University Grants Commission, India, for the award of a senior fellowship.

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METEOROLOGY

Numerical Retracing of Atmospheric Events

For obvious practical reasons, all numerical integrations of the equations of atmospheric motion published so far have been made forward in time. To be useful, such numerical forecasts must be started from a position of uncertainty and extend up to, or beyond, the most clear-cut stage of the development in question. But the numerical approach can also be regarded as a method of experimenting with the atmosphere, and in that context the above procedure seems far from ideal. It would then be more logical to start from some well-defined point separating the initial and mature stages and to integrate forward and backward in time, possibly using different models for the two stages. Since the origins of atmospheric disturbances are often inconspicuous on the synoptic scale or obscured by lack of observations, the processes during the developing stage, and hence the results of the backward integration, would in general have the principal interest in this type of experimental work.

At first sight, a backward projection of atmospheric events seems to require the reversal of the large-scale flow—a major dynamical problem. However, the structure of numerical integration procedures offers a very simple means of reversing the direction of the time co-ordinate itself. All the procedures suggested so far make use of rates of changes, or 'tendencies', which are assumed constant during each time-step and are added to the values of the corresponding variables at time t to obtain their values at time $t + \Delta t$. A reversal of the time-direction therefore requires merely that the tendencies be subtracted instead of added.

Owing to the use of non-centred differences, the subtraction of tendencies will not ensure a complete reversal of events; but the discrepancies between the results of forward and backward integrations evidently arise from comparable errors in both computations. Other elements of irreversibility are introduced by finite difference approximations, smoothing operations and boundary processes.

The last of these require brief discussion. Charney, Fjørtoft, and von Neumann have shown[1] that conditions must be prescribed in detail for boundary points with inflow. Mathematical arguments were presented recently[2] in favour of relaxing this condition, but it seems likely[3] that the prerequisites for this are not generally fulfilled. Backward integrations can be expected to illuminate this point further, since the reversal of flow implied in the reversal of time should make it necessary to reverse also the boundary condition.

We have tested the arguments presented here by means of the barotropic model applied to 500-mb. charts for the Australian region. The first calculation took an existing situation 24 hr. forward in time and then brought it back to the starting point. There was a loss of minor details, but all major features were fully restored. Computational instability developed during the retracing calculation at the outflow boundary, and vanished when the boundary condition was reversed. This supports the validity of the classical argument[1], and was further confirmed by the second calculation.

In this a marked upper depression which had developed unexpectedly over western Bass Strait was traced backward for 24 hr. to a region of the Great Australian Bight, where the available observations could not have shown its existence. The calculation also indicated the presence of a depression over the southern Tasman Sea, where the analysis previously had shown only an indefinite trough.

These results suggest that the retracing of large-scale flow developments may also have practical utility. Even with the best of present-day networks, forecasts remain affected by the uncertainties of regions lacking observations. The backward integration procedure can be used to make the analysis in such regions dynamically consistent with that for the network area. This approach will be explored in detail when we test the performance of the barotropic and other models in the Australian region.

We are indebted to the Bureau of Meteorology for the 500-mb. charts used in this work, and to Dr. F. Loewe and Dr. F. A. Berson for helpful comments.

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Small-Scale Turbulent Diffusion in the Atmosphere

Clarenburg and Tang[1] recently published a communication under this title in which they compared observations of smoke cloud spreading made by Frenkiel and Katz[2] with the relationships t, and $(t + 3)^{(2-n)/2}$, where t is time after generation of the burst, and n describes the shape of the one-point Lagrangian velocity autocorrelation; n was assigned the value 0·26, following Sutton[3]. Before the conclusion by these authors about the results of this comparison can be accepted, two points must be made:

(1) The authors base their computation of the spreading of smoke puffs on Sutton's well-known diffusion theory. It was pointed out in 1946, by M. I. Yudine[4], who referred to Richardson's[5] work and to the paper by Obukhov[6], that Sutton's treatment describes average dispersion, computed with reference to a fixed space-point or axis; but it does not describe relative diffusion of particles about a centre of gravity which is itself at liberty to move with the turbulence, as is the case with a smoke puff. The point has been emphasized and clarified in papers by Brier[7], and Batchelor[8–10], and many others. Relative diffusion depends on factors additional to the single-point, Lagrangian velocity correlation and hence cannot be described correctly by means of the parameter n. The additional factors governing relative diffusion are: (*a*) the initial size of the smoke puff; (b) multi-point Lagrangian correlations. Arguing from Obukhov's dimensional theory of turbulence, Batchelor deduced that the dispersion of a smoke puff should be proportional to a power of t less than unity at first, and afterwards to $t^{3/2}$.

(2) Clarenburg and Tang compared their diffusion theory with Frenkiel and Katz's excellent series of observations of the diameters of nineteen smoke puffs. The experiments were made on two successive days, and consisted of 7–20 photographic puff observations per experiment, made at one-second intervals. When a smoke puff spreads, at first its visible diameter increases; but later a maximum is reached, followed by a rapid decrease to zero. This maximum diameter seems actually to have been recorded[2] for at least eight of Frenkiel and Katz's smoke puff observations; runs number 6, 7, 13, 16, 18, 20, 9 and 11. The observed puff diameter is clearly not the same quantity as the (root mean square) dispersion of diffusion theory. The dispersion increases with time indefinitely. It is for this reason that other workers, starting with Roberts[11] (and including Frenkiel and Katz) have interpreted the visible smoke puff outline as a line-of-sight integrated smoke concentration value. According to this interpretation, it was shown by Gifford[12] that Frenkiel and Katz's smoke puff data (among others) support the predictions of the theory of relative diffusion. The observed puff dispersion proceeds at first according to a power of time less than unity, as long as the influence of the initial puff size is still dominant. But after about 10 sec. (for these puffs), the dispersion, but not the puff diameter, is found to obey a $t^{3/2}$ law for the remainder of the puff's visible lifetime.

The high correlations of smoke puff diameter observations with the assumed dispersion law $(t + 3)^{0.87}$, obtained by Clarenburg and Tang, stem from two separate, unrelated effects, neither of which has been considered by them: (*a*) At first, smoke puff diameters do increase in proportion to a power of time less than unity; this initially slow spreading does not, contrary to Clarenburg and Tang's assumption, reflect the degree of turbulence present, or the shape of the one-point Lagrangian autocorrelation function. Instead, it reflects primarily the influence of the initial puff size, according to the prediction of the theory of relative diffusion. (*b*) Later, when the influence of the initial puff size decreases to zero, relative diffusion proceeds at an accelerating rate, that is, according to a power of time greater than unity. The puff diameters, on the other hand, reach a maximum and then actually decrease with time. This is, of course, a purely optical effect, and does not reflect any corresponding decrease in the real turbulent diffusion-rate. In fact, the higher the real turbulent diffusion, the more marked this latter effect should be.

For the above reasons, it is difficult to agree with the conclusion by these authors that their theoretical results are in good agreement with these experimental data.

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PHYSICS

Velocity of Light emitted by a Moving Source

THE theory of special relativity is based on two main postulates, namely, the principle of relativity and the principle of the constancy of the velocity of light. If it is assumed that Maxwell's equations are correct and obey the principle of relativity, then the principle of the constancy of the velocity of light follows. The experimental evidence usually quoted in favour of the second principle is de Sitter's analysis of double stars. However, the principle has not been confirmed by experiments carried out in the laboratory using terrestrial sources. The need for such an experiment has been stressed recently by Dingle[1]. If the velocity of light is to have the same numerical value in all inertial frames then the velocity of light must be independent of the velocity of the source of light.

Generally, π^0-mesons decay into two γ-rays each having energy ~ 60 MeV. if the π^0-mesons decay when they are at rest relative to the laboratory. If the π^0-mesons are moving relative to the laboratory when they decay, then the source of the γ-rays is moving relative to the laboratory. It is known that this motion changes the energy and frequency of the γ-rays corresponding to the Doppler effect. If the principle of the constancy of the velocity of light were not correct then the velocity of the γ-rays from π^0-decay should vary depending on the velocity of the π^0-mesons relative to the laboratory when they decayed. Hence, if the velocity of the γ-rays arising from π^0-decay were measured, then it should afford a direct laboratory test of the principle of the constancy of the velocity of light. Let the π^0-mesons be produced in nuclear interactions inside the crystal of one scintillation counter by charged primaries (for example, protons $\sim 10^9$ eV. from an accelerator). The ionization produced in the crystal gives rise to fluorescence which is converted into an electrical signal by a photomultiplier. Any π^0-mesons produced will decay in a time $< 10^{-14}$ sec. so that the time of production of the γ-rays can be taken to be the time of entry of the primary particle. Let one of the γ-rays produce an electron pair in a second scintillation counter spatially separated from the first. The ionization produced by the electrons causes fluorescence in the crystal which is converted into an electrical impulse by the second photomultiplier. The time delay between the two electrical pulses can be measured electronically by several methods, for example, the first pulse could be used to trigger an oscilloscope and the position of the second pulse noted. With a separation of one metre the time delay would be $\sim 3 \cdot 3 \times 10^{-9}$ sec. if c were equal to 3×10^{10} cm./sec. If the velocity of the γ-rays depended on the velocity of the parent π^0-mesons then this time delay should vary depending on the velocities of the π^0-mesons. The main experimental difficulty would be to be sure that the coincidences observed would be due to the above events. As a check, the scintillation counters could be placed inside a large cloud chamber so that it could be checked that only one primary entered the first scintillation counter and that an electron pair was actually produced in the second counter. From the energy and direction of the electron pair, as observed in the cloud chamber, one could calculate an approximate value for the velocity of the parent π^0-meson.

It is probable that some other nuclear or meson process might prove more convenient to use in practice. A possibility might be to use a K-meson beam. Sometimes a K-meson emits π^0-mesons which afterwards decay into γ-rays. If the K-mesons were passed through a series of scintillation counters the positions and time of decay of the K-meson could be determined sufficiently accurately. The γ-ray detector could be placed off axis at a distance from the K-meson beam and the time delay measured.

The fact that in principle the experiment could be performed is of considerable interest even if it proved impossible to carry it out in practice. It has often been suggested that a direct experimental check of the principle of the constancy of the velocity of light is impossible since one would have to assume it to be true in order to synchronize the spatially separated clocks. In the above experiment one would not use two clocks but two crystals and photomultipliers, which would feed electrical signals into an apparatus which measures time intervals and which need not move before and during the experiment. When the events occurred, electrical signals would be sent along electrical cables to the timing machine and the delay in the transmission of the information could be allowed for once and for all by placing the two scintillation counters close together, and sending charged particles through both crystals and adjusting the lengths of the connecting cables until the pulses from both counters arrived simultaneously at the timer. The crystals and photomultipliers could then be moved apart without changing the length of any connecting leads. The delay in the receipt of information after the events would remain independent of the separation of the detectors if the velocity of electromagnetic disturbances in the connecting cables is independent of the directions and orientations of the connecting cables and is not changed when the cables are moved about. The usual method of synchronizing spatially separated clocks, as discussed in the text-books, is to use light signals which are assumed to travel in straight lines. In the present case the signals from the events can travel in curved paths such that the delay remains independent of the separation of the crystals and photomultipliers; the actual timing mechanism is not moved. In fact, in order to check the second principle one need not adjust the zero as described above, but one need only observe whether or not the time difference between the pulses from the two counters is the same whatever the velocity of the parent π^0-mesons relative to the laboratory when they decay.

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Absolute Measurement of Average Energy lost by Very Soft X-rays per Ion Pair in Air

FIG. 1 gives a survey of measurements made so far of W, the average energy lost by photons and particles per ion pair in air. At energies exceeding 10 keV. W is practically constant. At less than 10 keV. a tendency to higher values of W is indicated. The fairly large discrepancies between some of the results, especially at low energies, caused us to carry out an absolute measurement of W with very soft X-rays[1].

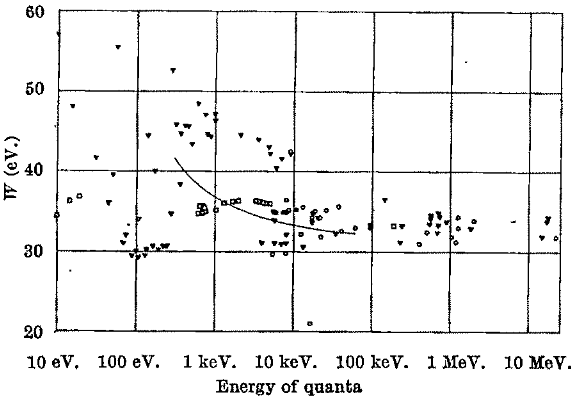

Fig. 1. W as a function of the energy. W for heavy particles is plotted in terms of the energy of electrons having the same velocity. ○, Photons ; ▽, electrons ; □, heavy particles

The measuring method is based on the equation, $W = nE_q/z$. The number n of monochromatic X-ray quanta of the energy E_q, produced by the filter difference method[2] has been measured by a total absorbing cæsium iodide scintillation counter. The crystal was enclosed in a container with a window of two aluminium foils, each 6·4μ thick, and mounted on the multiplier. The absolute counting was proved in preparatory experiments ; the readings are independent (*a*) of the place of absorption in the crystal and (*b*) of the voltage across the electrodes of the photomultiplier in a certain range. The number z of ion pairs in air produced by the quantity of energy nE_q has been measured by a total absorbing standard ionization chamber[3]. The required corrections have been calculated and determined experimentally.

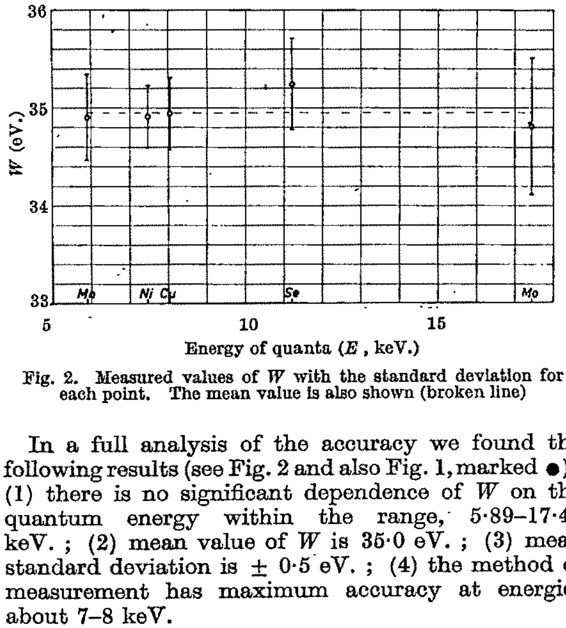

Fig. 2. Measured values of W with the standard deviation for each point. The mean value is also shown (broken line)

In a full analysis of the accuracy we found the following results (see Fig. 2 and also Fig. 1, marked ●) : (1) there is no significant dependence of W on the quantum energy within the range, 5·89–17·44 keV. ; (2) mean value of W is 35·0 eV. ; (3) mean standard deviation is ± 0·5 eV. ; (4) the method of measurement has maximum accuracy at energies about 7–8 keV.

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Similarity in Behaviour between Gas Bubbles in Liquid and Fluidized Solid Systems

Davidson *et al.*[1] have noted a marked similarity between the behaviour of gas bubbles injected into beds of solids which were gently fluidized and gas bubbles rising in a liquid. Within the narrow range of main fluidizing air velocity used for beds of glass beads, silver sand and swede seeds, the velocity of rise of bubbles, U, was found to depend only on the volume of the bubble, V, and agreed with the semi-empirical formula :

$$U = 0{\cdot}792\ V^{1/6}g^{1/2} \qquad (1)$$

derived by Davies and Taylor[2] for spherical capped gas bubbles in an inviscid liquid.

In this Department, using a γ-radiation absorption technique, the variation in voidage has been assessed when a measured stream of gas was injected into a similarly gently fluidized bed of 0·8 mm. diameter ballotini. The injection nozzle was clear of the base of the bed. It was found by calibration that change in observed count-rate was, to a first approximation, proportional to the change in voidage in the radiation beam over the voidage variation encountered. Thus it was possible to estimate an effective void residence time along the path of the radiation beam. By scanning the face of the column it was possible to measure an average projection of the voidage contours within the column and from this to measure directly the breadth of the injected disturbance—the bubble diameter. It was assumed that the bubbles were spherical on formation at the nozzle and then rose with the velocity given by equation 1 ; no allowance was made for interaction between bubbles in a chain. The bubble frequency to give the measured void residence time was calculated and Fig. 1 shows the relationship between this frequency and the rate of gas injection for two nozzle diameters (0·16 in. and 0·096 in.).

The general form of Fig. 1 shows that the bubble frequency, within the general scatter of results, is approximately constant at 22 per sec. for the higher injected gas-flow rates and that below injection-rates of 50 c.c./sec. there is a marked tendency for this frequency to rise. This is closely in accord with the results published by Calderbank[3] in a review of the behaviour of bubbles formed when air is injected into water through similar sized orifices. Thus these

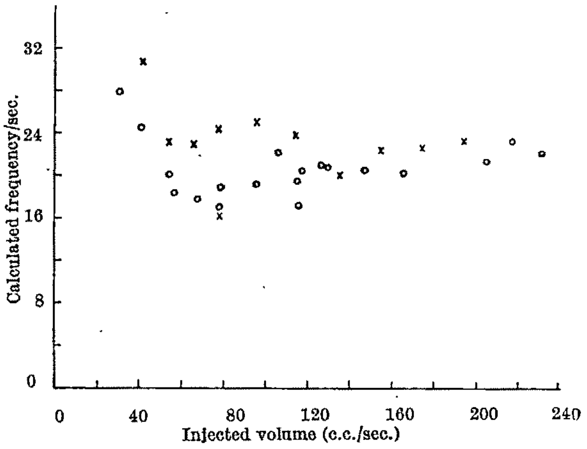

Fig. 1. Relationship between injected gas volume and calculated bubble frequency. ○, 0·16 in. diam. nozzle ; ×, 0·096 in. diam. nozzle

observations also show a similarity in behaviour between gas bubbles in liquid and fluidized solids systems even though the actual mechanism of bubble formation in these two systems must obviously be quite different.

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Nucleation in Agitated Solutions

Mechanical disturbances are known to speed the onset of nucleation in supersaturated solutions. Early workers[1] demonstrated that severe mechanical shock could induce nucleation in metastable solutions at temperatures well above the so-called 'metastable limit', and more recent work[2] has shown that mechanical stirring can cause rapid nucleation in unseeded solutions which will not normally form nuclei under quiescent conditions. Published work to date has not given any indication of the effect of varying the intensity of agitation on the tendency of an unseeded solution to form nuclei under any given set of conditions, but it is generally assumed that more vigorous agitation increases this tendency.

Investigations carried out in these laboratories on nucleation and growth conditions in a pilot-scale Oslo crystallizer operating with unseeded solutions of ammonium dihydrogen phosphate have confirmed that the critical supersaturation, defined here as the degree of supersaturation at which nuclei are first observed in a solution being cooled at a constant rate, is dependent on the rate of circulation of the liquor, that is, on the degree of turbulence. Less supersaturation is required for nucleation to occur at the higher rates of flow.

It was observed, however, that at low rates of circulation, represented by Reynolds numbers of about 700–1,400 in a column of 3 in. diameter, the degree of supersaturation necessary for nucleation was not apparently reduced by increasing the turbulence. Unfortunately, variations in the value of the critical supersaturation were here of the same order as the experimental error. Accordingly, recourse was made to a smaller apparatus where conditions were more easily controlled. A glass *T*-stirrer was used in a small baffled beaker. The solutions of ammonium dihydrogen phosphate were covered with a thick layer of liquid paraffin and cured for about 30 min. at 10 deg. C. above their saturation temperatures before each run. Accidental inoculation by dust particles was thereby largely eliminated and reproducible results were obtained.

Whereas gentle stirring causes nucleation in solutions which are otherwise stable, and sufficiently vigorous stirring creates an even greater tendency to form nuclei, the transition may not be continuous ; no simple relationship between the critical supersaturation and the intensity of agitation has been found. In general, a plot of critical supersaturation against rate of agitation is not a single smooth curve ; in fact, a portion of the curve may have a reversed slope (see Fig. 1) indicating a region where an increase in the agitation reduces the tendency to nucleate. So far as is known, this is the first experimental evidence of such a phenomenon.

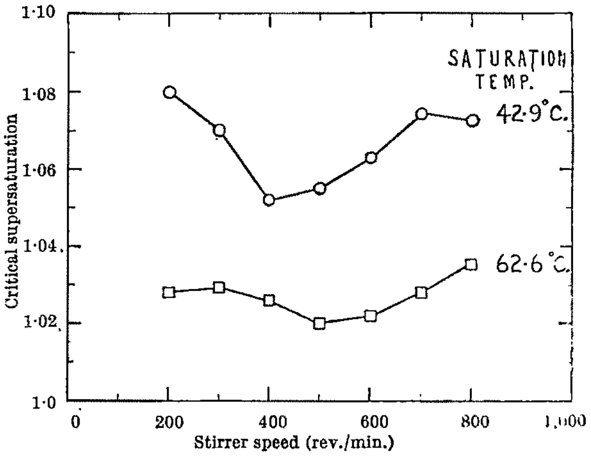

Fig. 1. Critical supersaturation (solution concentration/saturation concentration) versus stirrer speed for aqueous solutions of ammonium dihydrogen phosphate

Nucleation in liquids has hitherto been considered as consisting of two stages : a diffusional transport step which is aided by agitation, and a surface reaction step when growth of the sub-nucleus occurs, the latter not being influenced by agitation. It would now appear that a third effect may also operate, which impedes the growth of, or disrupts, the sub-nuclei, and that this effect may predominate in certain circumstances.

The work, which so far has been confined to aqueous solutions of ammonium dihydrogen phosphate with saturation temperatures in the range 40–70° C., is being extended to cover a wider range of concentration and other solutes, to determine whether this effect is of general occurrence or specific to a limited range of conditions, and to attempt a theoretical explanation.

One of us (K. D. R.) is grateful to the Department of Scientific and Industrial Research for a studentship, during which this work is being carried out.

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Origin of the Word 'Neutron'

The word 'neutron' has been attributed to Rutherford by Glasson[1] and to W. D. Harkins[2] by Glasstone[3]. It appears likely that it was not used by either Rutherford or Hawkins before about 1920. In both cases, the neutron was a hypothetical combination of a hydrogen nucleus (also called a 'positive electron' or 'proton') and an ordinary negative electron.

I wish to point out that William Sutherland used the word in almost exactly the same sense in two papers published in 1902, in the first of which there is the passage[4]:

"As it would be convenient to have symbols different from the operational + and − to distinguish the two sorts of electrons, I propose to use the musical types ♯ for the + electron and ♭ for the − electron, and ♮ for the neutron, which consists of the ♯ and ♭ united to form that electric doublet which gives to the æther its electric and magnetic properties."

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Phenomena occurring at Electrically Stressed Metallic Surfaces in Vacuum

CERTAIN hypotheses put forward to explain the mechanism of electrical breakdown of high vacuum, between metallic electrodes, rely on the removal from the electrode surfaces of aggregates of material[1,2]. Such aggregates have become known as 'clumps'. By means of radioactive layers on the electrodes or spectroscopic analysis, material transfer during the pre-breakdown phase has been shown to occur[3-6]. Breakdown has been initiated by the introduction of particles of electrode material in the gap[7]. The occurrence of points and pits on the electrodes has been confirmed by examining the electrodes microscopically before and after voltage stressing, while actual growth and tearing off of small points several μ in length has been photographed during an increase in voltage[8,9]. The latter observations showed that the points appeared to grow only on the anode, a fact supported by an observation that indicated the breakdown voltage was proportional to the tensile strength of the anode[10].

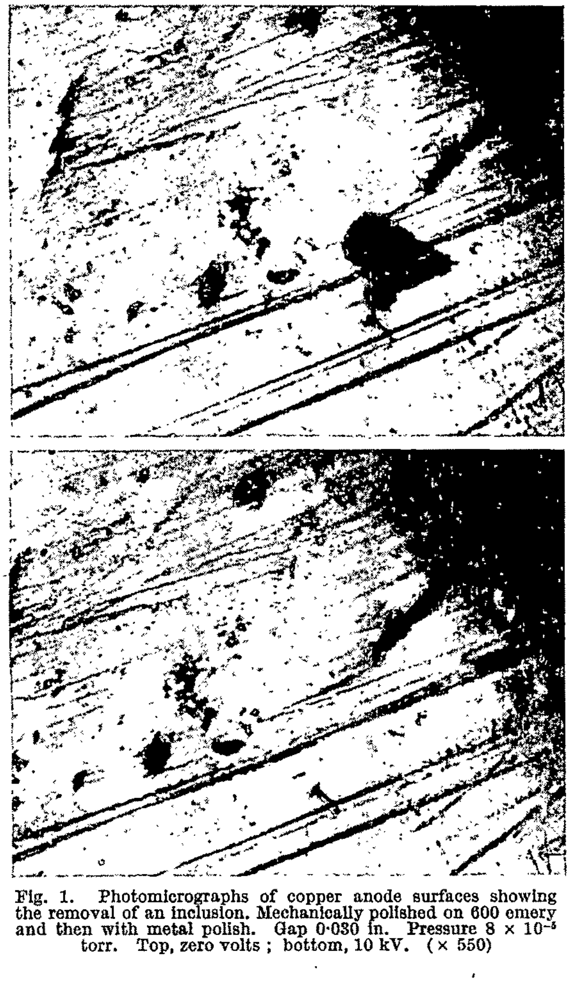

Fig. 1. Photomicrographs of copper anode surfaces showing the removal of an inclusion. Mechanically polished on 600 emery and then with metal polish. Gap 0·030 in. Pressure 8×10^{-5} torr. Top, zero volts; bottom, 10 kV. (× 550)

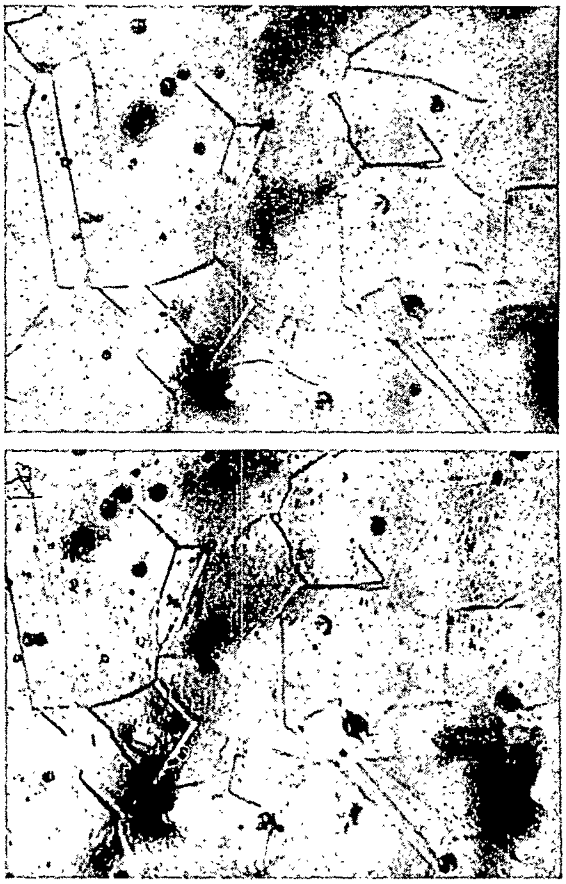

Fig. 2. Photomicrographs of electrolytically polished copper anode surfaces showing the appearance of 'spots'. Gap 0·015 in. Pressure, 8×10^{-5} torr. Top, zero volts; bottom, 24 kV. (× 550)

The actual observation of the growth of points was the first direct visual evidence that such multi-atom clumps were pulled out of the surface and transported across the gap. An apparatus was designed, before the foregoing results were known to us, in which the surface could be observed normally and with the voltage applied. It consisted essentially of an anode and cathode mounted in a continuously pumped vacuum cell. The cathode was in the form of a copper disk in the centre of which was a hole with bevelled sides, the diameter of the hole opposite the anode being 0·34 in. Located behind the cathode and in line with the hole was an 8-mm. objective lens, of 0·5 N.A., which fitted into a Vickers microscope. The anode was in the form of a 45° copper cone, the top of which was ground flat so that it just fitted the field of vision through the microscope. Analysis showed the copper to be about 99·5 per cent copper and 0·3 per cent oxygen with normal commercial impurities. Direct voltages were used and the breakdown current was limited by a 2-megohm series resistor. The pressure lay between 5×10^{-5} and 9×10^{-5} torr. Before some tests the anode was polished with emery cloth down to 600 grade and then with 'Silvo' and finally washed in water, alcohol, and ether; in other cases it was electrolytically polished.

Some results of a preliminary series of tests are now reported as they confirm other investigators' results and give further information. With the gap set at 0·03 a large inclusion 2×10^{-3} cm. in diameter was seen to disappear from the anode at about 10 kV. or a 'stress' of $1·3 \times 10^5$ V./cm. Its removal did not cause breakdown. The effect is shown in Fig. 1, and although scratch marks can be seen from where the inclusion vanished they appear to be less intense than on either side of the inclusion. Thus it may well have been a piece of embedded cleaning material. Razin[9] also reports a clump tens of microns in size, being pulled off at a stress of 2×10^5 V./cm.

In certain cases as the voltage was increased a different type of phenomenon took place in that 'spots' were seen to appear on the anode surface, as shown in Fig. 2. In the case shown the anode had been electrolytically polished, the gap was 0·015 in. and the spots arose at 24 kV. This corresponds to a stress of $6·3 \times 10^6$ V./cm. With the mechanically polished surfaces described earlier the spots arose at a stress of about 4×10^5 V./cm. The size of the spots is about $1·5 \times 10^{-4}$ cm. diameter, which compares well with clump sizes estimated from the Slivkov hypothesis[2] of 5×10^{-5} cm. and with the smaller marks observed by Schwabe[8] of 2×10^{-4} to 5×10^{-4} cm. Cranberg[1] postulated the clump size to lie between 10^{-2} and 10^{-6} cm. The number of spots per square centimetre of electrode surface is of the order of 10^6. In several runs it took a further increase of voltage to cause breakdown but in others breakdown was immediate.

We wish to acknowledge the assistance of Dr. J. D. Meakin and Mr. R. A. Owen, Department of Metallurgy.

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GEOLOGY and MINERALOGY

Age of the Orogeny and Granites in South-West England

The Dartmoor and related granites form one of the key points in the revised geological time-scales of Profs. A. Holmes[1] and J. L. Kulp[2]. Therefore, their stratigraphic relationships need to be determined as precisely as the field evidence will allow. Kulp *et al.*[3] write that "while the Dartmoor granite is almost certainly post-Lower Westphalian, Lower Stephanian strata in Somerset are affected by the same fold system and it is therefore probable that the granites are of latest Stephanian or Permian age". It is by no means generally accepted, however, that the fold system in Devon and Cornwall is of the same age as that in the Somerset coalfield[4,5]. Indeed, Holmes[1] adopts a suggestion from Owen that the granites may have been emplaced in Upper Westphalian times and that the Pennant Sandstone of South Wales "may be the product of the rapid erosion of the rising Cornubian chains"[6]. A brief discussion of the available evidence therefore seems desirable.

The granites invade a folded and faulted stratigraphical succession in which, *at the present level of erosion*, the youngest sediments so far recognized by fossils are referable to the Upper Gastrioceras Zone of the Lower Westphalian[7–9]. It is likely, however, that a considerable tectonic thickness of deformed Palæozoic strata (perhaps many thousands of feet in the Dartmoor region) was originally present above the structures now seen[10] bordering the exposed granites. It therefore seems reasonable to suppose that even younger Westphalian, and perhaps even Stephanian sediments could well have been present in the deformed mass into which the granites were emplaced. Prentice's sedimentological conclusions from the Bideford area[11] support this view.

It appears that only unaltered Palæozoic rocks from the upper part of this deformed mass were being eroded in basal New Red Sandstone times[12]. Later New Red sediments contain contact-metamorphosed Palæozoic rocks, while the Lower Cretaceous Wealden sediments of southern England are thought to provide "the earliest evidence of the uncovering and denudation of the [Dartmoor] granite itself"[13].

It therefore seems preferable on the present evidence to regard the main orogenic movements in Devon and Cornwall as being of essentially the same age as those in the nearby Somerset coalfield, which are known to affect Lower Stephanian strata[14,15]. I support the assignment of the granites to the Permian[2] rather than to the latter part of the Carboniferous period[1]. The position of these granites in the geological time-scale will presumably affect the length of time to be allotted to the Carboniferous period, a matter of some importance in Carboniferous stratigraphy.

Thanks are due to Prof. A. Holmes for discussion in correspondence and to Prof. P. Allen, Prof. J. Sutton and Dr. R.St.J. Lambert for their comments.

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Attempted Low-Temperature Syntheses of Kaolin Minerals

Although kaolin minerals appear to be formed in Nature under acidic conditions at relatively low temperatures and low water-vapour pressures, their synthesis in the laboratory under similar conditions has been found exceptionally difficult. It appears that a proper choice of many variables is essential. In previous investigations[1,2], individual kaolinite crystals have been obtained by ageing mixed alumina and silica gels for 2 months at 100° C. and under *p*H conditions around 4·5, but the crystalline yield was extremely poor and the kaolinite could be proved only by single-crystal, electron diffraction methods; X-ray diffraction failed to detect the small yield. It seems that the ready polymerization of silica, with aluminium incorporated in the structure, inhibited the crystallization of a layer structure. Similar difficulties are not encountered in forming hydrous magnesian silicates.

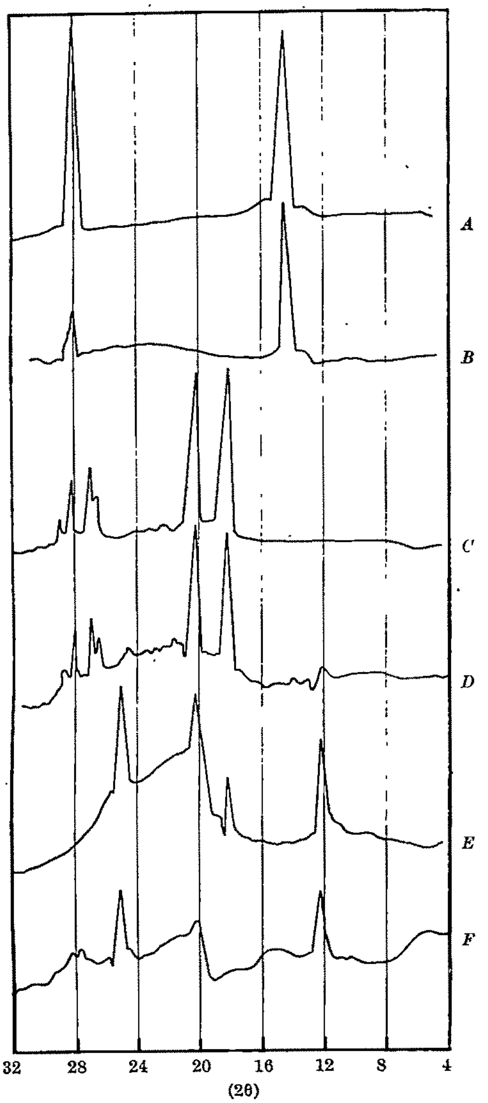

Fig. 1. X-ray diffractometer patterns; copper $K\alpha$ radiation. *A*, boehmite; *B*, boehmite obtained from gibbsite in sealed tube at 150° C.; *C*, gibbsite; *D* and *E*, gibbsite partially transformed to kaolin mineral; *F*, gibbsite wholly transformed to kaolin mineral

In the present experiments, gibbsite, $Al(OH)_3$, has been used as a source of alumina, together with silica either as a gel, 'Ludox', or in solution as ethyl silicate. The experiments have been conducted in sealed glass tubes at 150° C. and about 5 atm. water-vapour pressure, and with vigorous end-over-end agitation. The *p*H was controlled mainly by $N/10$ hydrochloric acid, but in experiments lasting 1–3 weeks the *p*H value often varied and a better control is desirable.

The results are illustrated in Fig. 1 by diffractometer diagrams. When the final *p*H exceeds 1·9, gibbsite is the only crystalline phase observed (cf. diagram *C*). When the *p*H remains below 1·5, a kaolin mineral replaces the gibbsite (cf. diagrams *D*, *E*, *F*). Electron micrographs show plate-like forms and also rod-like forms having some resemblance to halloysite. Reversible swelling and shrinkage behaviour is readily followed by X-ray diffraction, and each cycle of operations seems to improve the crystal structure. Whereas gibbsite in the absence of silica becomes transformed to boehmite at 150° C. under the conditions of the experiments (cf. diagrams *A* and *B*; also ref. 3), in the presence of silica the formation of boehmite is completely inhibited. Probably the instability of the gibbsite structure facilitates its reaction with silica to develop an alumino-silicate layer structure.

Further experiments are in progress in which temperature and *p*H will be controlled with more precision. This investigation forms part of Project 55, sponsored by the American Petroleum Institute.

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CRYSTALLOGRAPHY

Spiral Formation on Natural Crystalline Sulphur

Recently, observations of the growth spirals suggested by Frank's theory[1] have been reported on many different substances.

This communication records some interesting observations of growth spirals originating from screw dislocations on the (001) faces of natural sulphur crystals. The observations were made by means of an ordinary metallurgical microscope, using bright-field illumination. The heights of the steps were measured using interferometric techniques[2] and the values found range from 5000 Å. to 100 Å., showing that growth-promoting dislocations are of multiple strength.

Most of the faces investigated showed one spiral only, covering the whole area, which seems to prove that sulphur crystals must be extremely perfect.

Fig. 1 shows a spiral originating from a right-handed dislocation. Besides this elementary type of spiral, several other more complicated growth patterns, for

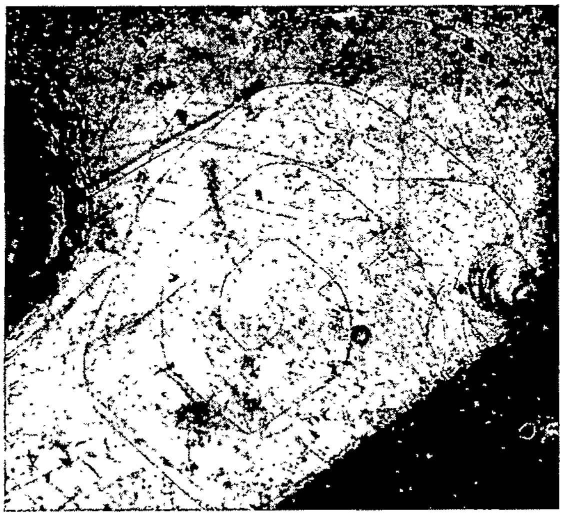

Fig. 1 (× 90)

two, three or larger numbers of dislocations ending on crystal faces, have been photographed.

The observed density of dislocations varies widely on different specimens, ranging from a few to a maximum of approximately 10^4 per sq. cm. On any one crystal they are predominantly of the same hand.

A more detailed account of these observations will be submitted elsewhere.

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Growth Spirals on Synthetic Magnetoplumbite Crystals

In connexion with a study of crystal growth from lead oxide and lead oxide–lead fluoride melts, we have recently observed growth spirals on (0001) faces of tabular magnetoplumbite ($PbFe_{12}O_{19}$) crystals. The spirals on crystals obtained from lead oxide–lead fluoride melts are polygonal, relatively open, and show concave growth fronts as well as reversed curvatures near the outer edges of the crystals. The step heights appear to be small multiples of the unit cell dimension in the *c* direction. The spirals on crystals grown from lead oxide melts are polygonized to various degrees, are more tightly wound, and exhibit greater step heights.

The magnetoplumbite crystals showing well-defined spirals were located on the top surfaces of melts from which yttrium–iron garnet crystals were grown by procedures approximating those suggested by Nielsen[1,2]. The spirals were observed with a petrographic microscope, using bright-field illumination. Fig. 1 shows a typical growth spiral on the surface of a crystal still attached to the top surface of a solidified lead oxide–lead fluoride melt. The small particles covering the crystal surface and decorating the step are believed to be flux components that solidified when the crucible was removed from the furnace (at about 1,000° C.). These particles were removed without otherwise altering the surface, by treating the surface with dilute nitric acid. Step heights were measured by a thin-film interferometric technique of the type described by Tolanski and Omar[3]. The value obtained for the height of the step near the origin of this spiral was 48 Å. The unit cell dimensions of a typical crystal (exhibiting a growth spiral) were determined to be $a = 5{\cdot}91$ Å., and $c = 23{\cdot}1$ Å., and the spiral was shown to be on the (0001) surface by X-ray methods. The step height (and Burgers vector of the screw dislocation(s) responsible for the spiral) on the surface of this crystal is approximately twice the unit cell dimension in the *c* direction. Dissociation of the spiral into two steps can be seen in the reversed curvature region (see below). The step height on a second spiral of similar appearance was determined to be about 68 Å., or approximately three times the unit cell dimension in the *c* direction. The displacement of interference fringes across the spiral step on many of the crystals was observable, but too small to measure. It is likely that these steps were a unit cell in height.

An interesting feature of the spiral shown in Fig. 1 is the partial reversal in curvature. A uniform supersaturation across the crystal face should lead to an equilibrium growth spiral that is approximately Archimedian in shape[4]. In the presence of concentration gradients, however, the shape of the spiral will be altered. An increase in supersaturation between the centre and the edge of a crystal face frequently occurs during crystal growth, and the suggestion has been made by Amelinckx[5] that a suitable gradient of this type may result in a complete reversal of spiral curvature. The spirals on magnetoplumbite crystals grown from lead oxide–lead fluoride melts have a shape that suggests a radial increase in supersaturation just sufficient to produce a reversal in curvature from about one-half of the radius to the edge of the face. The effect can be clearly seen in Fig. 2. Both right- and left-handed spirals are present on the surface of this crystal and both types show curvature reversals near the edge of the face. One can also see that the nearly straight segments of the spirals in Figs. 1 and 2 are slightly concave. The concave growth fronts may also be the result of an increase in growth-rate (supersaturation) with increasing distance from the centre of the crystal.

Numerous multiple-step spirals and complex systems of spirals were characteristic of the surface

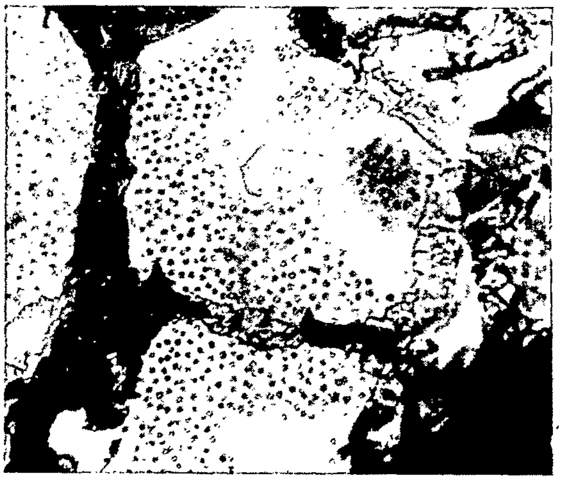

Fig. 1. Growth spiral on a magnetoplumbite crystal grown from a lead oxide–lead fluoride melt. (× c. 273)

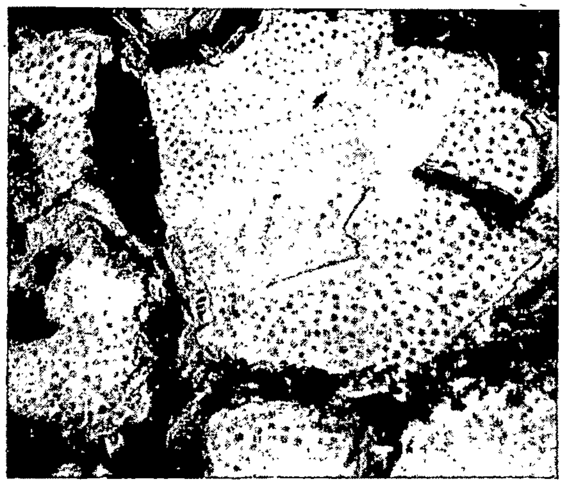

Fig. 2. Right- and left-handed growth spirals on a magnetoplumbite crystal. (× c. 273)

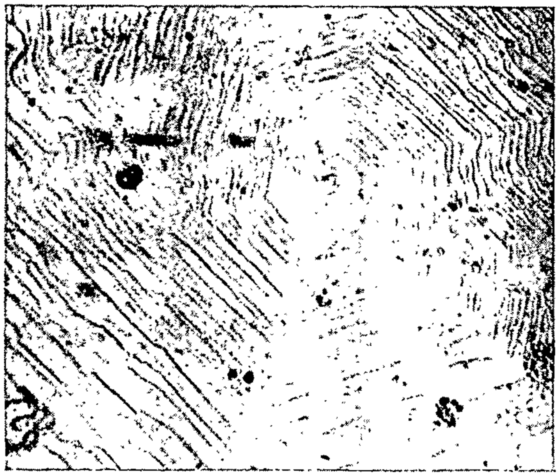

Fig. 3. Multiple-step growth spiral on a magnetoplumbite crystal grown from a lead oxide melt. (× c. 273)

features observed on magnetoplumbite crystals grown from lead oxide melts. Fig. 3 shows a tightly wound, multiple-step spiral on the surface of a crystal grown from a lead oxide melt. A value of 163 Å. (7 unit cells in the c direction) was obtained for the maximum height of a single step on this spiral. At higher magnifications, dissociation of the steps into smaller components was observed in several regions. The fact that these steps are more closely spaced than those observed on the faces of crystals grown from the lead oxide–lead fluoride melts is probably the result of higher supersaturations during growth. Such a result is consistent with the fact that magnetoplumbite is the major phase in the lead oxide system and a minor phase in the lead oxide–lead fluoride system.

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CHEMISTRY

Catalytic Activity of Coloured Alkali Halides

The properties of alkali halides containing colour centres are in some respects similar to those of semi-conductors: it was therefore thought to be worth while to determine whether such alkali halides have any of the catalytic properties of semi-conductors. The oxidation of carbon monoxide was chosen as a test reaction, as this reaction is readily catalysed by semi-conductors at low temperatures[1].

The halides investigated were sodium and potassium chlorides, potassium iodide and potassium bromide; all were of reagent grade purity and were used without further purification. The salts were thoroughly dried and ground, the portion between 150 and 300 mesh being used in the experiments.

Carbon monoxide was prepared by dehydrating formic acid with sulphuric acid, the gas being purified by passage through potassium hydroxide and magnesium perchlorate towers and finally through a liquid-air trap. Cylinder oxygen was passed through the same purification train, and probably contained traces of nitrogen and argon. A stoichiometric mixture of carbon monoxide and oxygen was used in all experiments.

The reaction vessel was a 'Pyrex' cylinder which could be fitted with a vapour-jacket for temperature control. The reactor, which had a volume of approximately 45 ml., was fitted with a thermocouple well, the end of which was always in the salt under investigation. Pressures were measured with both mercury and di-*n*-butyl phthalate manometers.

In a typical experiment, approximately 1 gm. of the ground salt was spread in an even layer over the bottom of the reactor. The reactor was then sealed into the vacuum system and heated to 250° C. for 10 min. at a pressure of less than 10^{-5} mm. mercury. The reactor was then brought to room temperature under vacuum, and dry air admitted to a pressure of 0·1 mm. mercury. The discharge from a Tesla coil was then played over the reactor for several minutes to produce colour centres in the salt. The reactor was then rapidly pumped out to a high vacuum ($<10^{-5}$), brought to the required temperature, and a sample of the carbon monoxide–oxygen mixture admitted. The manometer readings were followed as a function of time and, if the room temperature varied during the run, corrected for changes in the density of the manometric fluid. The manometer readings were also corrected for pressure changes in the non-thermostatted dead space of the reactor if there was any change in room temperature.

Experiments were carried out from 35° C. to 125° C. (above which temperature the colour centres bleached rapidly), with initial gas pressures of 10–250 mm. mercury. Runs were made both in the dark and in the presence of normal laboratory lighting. In no case was any evidence of catalytic activity obtained; although 0·2 per cent reaction in a 3-hr. period could have been detected. At the higher pressures a rapid, poorly reproducible decrease in pressure was observed immediately on admission of the gas. This decrease amounted to approximately 0·5 per cent of the initial pressure. This decrease could not be observed at the lower pressures, presumably because of drainage errors in the oil manometer.

Although the adsorptive properties of alkali halides have not been investigated in detail, it would seem from the work of Tompkins[2] that this pressure decrease might be due to a small adsorption of carbon monoxide. The work of Orr[3] suggests that oxygen adsorption would be negligible in the temperature-range of this work.

Experiments with the same gas mixture, in the same system, using nickel oxide as a catalyst, gave results comparable to those previously reported[1].

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Determination of Very Small Amounts of Hydrogen Peroxide

It has been found that radiation doses of a magnitude as low as 10 r. damage cells in culture and interfere with their multiplication[1,2]. Although the damage is to be ascribed primarily to short-lived radicals produced by the rays rather than to the hydrogen peroxide also made, it is desirable to study the biological effects of small concentrations of this substance.

In this connexion, a very sensitive method for the determination of hydrogen peroxide is needed. The efficiency of ionizing rays in producing chemical species is measured by the *G* value, which is defined as the number of molecules formed per 100 eV. of energy absorbed. Using γ-rays (cobalt-60), *G* values for hydrogen peroxide production of 1·05–1·31 were found at dose-rates of about 3×10^3 r./min.[3-8]. Therefore, a dose of 10 r. will give in 1 ml. water hydrogen peroxide of about 10^{-11} mole ($\sim 3{\cdot}5 \times 10^{-10}$ gm.).

To develop a suitable method, we started from the report by Andreae[9] that scopoletin (6-methoxy-7-hydroxy-1,2-benzopyrone) is oxidized stoichiometrically (1 : 1) by hydrogen peroxide with loss of its fluorescence, provided horse radish peroxidase is present. The fluorescence before and after interaction with peroxide was measured with a Beckman spectrophotometer model *DU*, adjusted for fluorimetry. 10^{-10} mole of hydrogen peroxide could be measured. In the present work the determination was made with a Galvanek-Morrison fluorimeter, mark *V* (Jarrell Ash Co.), as usually employed for the determination of uranium in solid sodium fluoride pills. In this way, the sensitivity of the method has been improved by about one magnitude for the concentration, and by two magnitudes for the quantity of hydrogen peroxide.

The method was tested with hydrogen peroxide solutions. The solvent water had to be very pure to avoid spontaneous decomposition of the solute. Twice-distilled conductivity water was distilled three more times. First, with alkaline potassium permanganate; secondly, with silver nitrate; and finally, without any addition in a quartz apparatus. Hydrogen peroxide ('Perhydrol *p.A.*', Merck) was added to this water to give a solution about 0·2 per cent, and the exact concentration determined by iodometry. This stock solution was stable when stored in silica in darkness. For use it was diluted in steps, and the concentration measured by the scopoletin method after each step.

For the purpose, 0·1 ml. of scopoletin solution, 0·1 ml. of horse-radish peroxidase (C. F. Boehringer) solution (concentration not critical), and 0·1 ml. of hydrogen peroxide solution were mixed. The amounts of scopoletin were chosen so that at most three-quarters were used up by reaction with the hydrogen peroxide. 15 min. after mixing, 0·3 ml. of 0·15 *M* borate buffer (*p*H 10·0) was added, exactly one-half of the mixture transferred to a platinum dish, and the fluorescence measured. To obtain the fluorescence of the intact scopoletin, a blank mixture was made by including 0·1 ml. water instead of the same amount of hydrogen peroxide solution.

In Fig. 1, the logarithm of the number of moles of scopoletin oxidized (S_{ox}) is plotted against the logarithm of the number of moles of hydrogen peroxide as calculated on the basis of the iodometry with the stock solution. Clearly the method is applicable over a large range of concentrations. In Fig. 2, the destruction of the fluorescence in aliquots of one and the same scopoletin solution (containing initially $3{\cdot}12 \times 10^{-11}$ mole) by varying quantities of peroxide is shown, and Fig. 3 contains a similar plot for an initial concentration of $2{\cdot}5 \times 10^{-12}$ mole. It will be noted that the inclinations of the straight lines in the graphs are exactly 45°, as required by the stoichiometry 1 : 1.

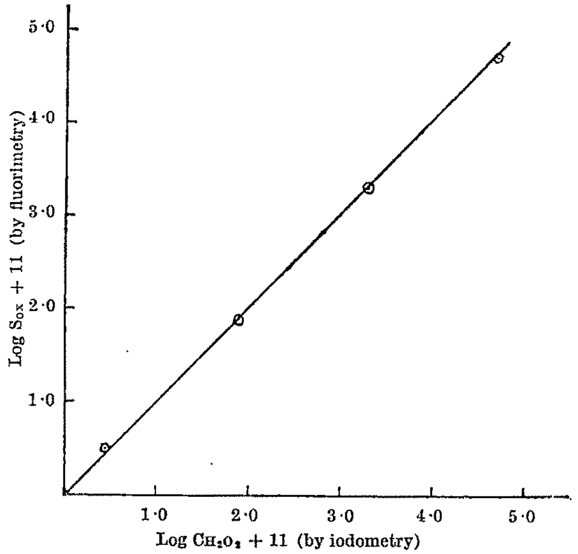

Fig. 1

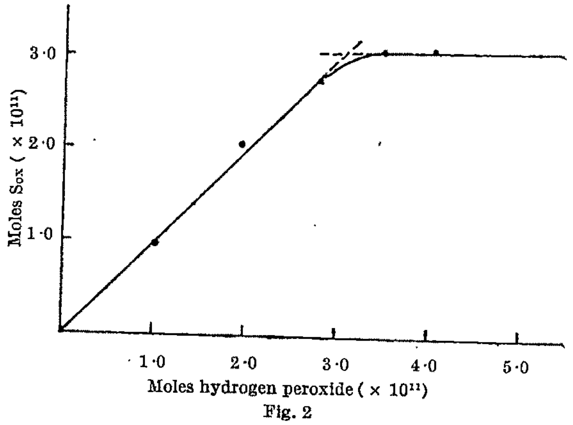

Fig. 2

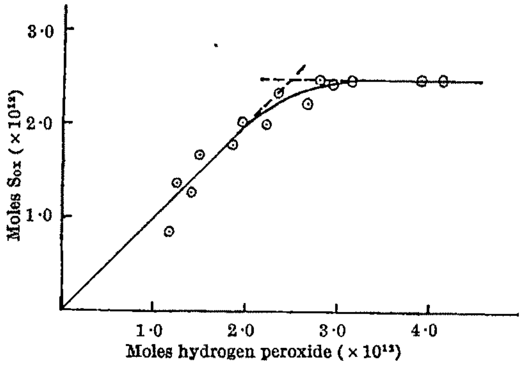

Fig. 3

It appears that in the range covered by Fig. 3 the limit of the method is reached. The scatter of the points in the lowest range is due to the fluctuations of the instrument when it works at its highest sensitivity. By proceeding statistically, that is, by measuring several identical samples in parallel and taking averages, the limit could be pushed down a little further.

In the method as used, the standard error is about 10 per cent in the measurement of $1\cdot5 \times 10^{-12}$ mole hydrogen peroxide, corresponding to a concentration of $1\cdot5 \times 10^{-11}$ mole/ml. in the initial solution, and to a radiation dose to this solution of about 15 r. Presumably even smaller concentrations and doses could be determined if the fluorimeter were adapted to take larger samples, or if the hydrogen peroxide could be diluted to a lesser extent by the reagents before measurement.

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Note added in proof. By using more concentrated reagents, solutions of 5×10^{-12} mole/ml. have now been measured, corresponding to a dose of about 5 r.

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Electron Spin Resonance Line-Widths in Solutions of Copper and Silver Dithiocarbamates

In previous publications the electron spin resonance data of dithiocarbamates of the coinage metals ($[RR'\mathrm{NCS}_2]_2Me$; Me = copper, silver or gold) have been used for a determination of valence states of the metals[1] and in a discussion of the bonding of the metal ions[2]. The copper and silver compounds exhibit very low effective spin-orbit coupling energy with small resonance anisotropy and unusually narrow lines. The small line-width makes it possible to analyse the influence on the width from a number of factors, and we shall briefly report on the effect of temperature, of dissolved oxygen and of different solvents.

In a liquid, where the paramagnetic molecules tumble at a sufficient rate, the position of the lines will mainly be determined by the isotropic parts of the spin Hamiltonian. However, in the cases where the hyperfine structure is anisotropic, the line-width will be dependent on the nuclear magnetic quantum number m_I. A theory for this effect was first given by McConnell[3], who used the results obtained by Bloembergen, Purcell and Pound for the nuclear relaxation. Recently, Kivelson[4] has re-examined the problem starting from the treatment given by Kubo and Tomita. The contributions to the longitudinal relaxation (characteristic time T'_1) and to the transverse relaxation (characteristic time T'_2) are given by Kivelson as :

$$\frac{1}{T'_1} = \frac{4\pi^2}{h^2}\left[\frac{1}{15}(\Delta g\beta B_0)^2 - \frac{1}{5}\Delta g\beta B_0 b m_I - \frac{1}{40}(b m_I)^2 + \frac{7}{40}b^2 I(I+1)\right]\frac{\tau_c}{1 + 4\pi^2\nu^2_0\tau^2_c}$$

$$\frac{1}{T'_2} = \frac{4\pi^2}{h^2}\left[\frac{4}{45}(\Delta g\beta B_0)^2 - \frac{4}{15}\Delta g\beta B_0 b m_I + \frac{1}{8}b m_I)^2 + \frac{3}{40}b^2 I(I+1)\right]\tau_c$$

The paramagnetic molecules are assumed to have axial symmetry. $\Delta g = g_{\parallel} - g_{\perp}$ is the g-value anisotropy and $b = 2(A - B)/3$ is related to the hyperfine structure anisotropy. B_0 and ν_0 are the static magnetic field and the microwave frequency at resonance. Rogers and Pake[5] showed that these formulæ correctly describe the frequency and the m_I dependence of the line-widths of the VO^{++} ion.

We have studied the dependence on temperature of the line-widths of acetone solutions of copper diethyldithiocarbamates ($\Delta g = 0\cdot065$, $b = -0\cdot79 \times 10^{-2}$ cm.$^{-1}$)[2]. The conditions for validity of the formulæ are well fulfilled; for example, $\Delta g/g = 0\cdot03 \ll 1$. The correlation time was estimated using the relation $\tau_c = 4\pi\eta r^3/3kT$ with $r = 3\cdot5$ Å. and where η is the viscosity[6]. ν_0 is about 9,130 Mc./s. Fig. 1*a* shows the variation with temperature of the width (measured between the points of maximum and minimum slope) of the $m_I = +\frac{1}{2}$ line for carefully deoxygenated solutions. In the low-temperature region the agreement with the calculated width ($= (1/2T'_1 + 1/T'_2)/\pi\sqrt{3}$) is good, but the fact that there is a minimum in the line-width at a certain temperature cannot be explained on the basis of anisotropy mechanisms alone. The exchange mechanisms suggested by Pake and Tuttle[7] and further developed by Kivelson[4] might provide an explanation for the increase in line-width with increasing temperature; but as their contribution to the width should be proportional to the concentration they probably do not account for the whole difference between the experimental and calculated widths. Spin-orbit interaction or mechanisms such as discussed by Al'tshuler and Valiev[8] might be responsible for the main part of the high-temperature width. All such phenomena should, however, be independent of m_I and from Fig. 1*b* it is clear that Kivelson's formulæ well predict the difference between the widths of two hyperfine lines. The contributions to the line-width from the quadrupole interaction as calculated by

Kivelson are also in general dependent on m_I, but for only one nucleus with $I = 3/2$, these contributions accidentally are independent of m_I.

Dissolved oxygen has a marked influence on the line-widths of both copper and silver dithiocarbamates. The width increases linearily with increasing partial pressure of oxygen while preserving the area under the absorption curve. From the solubility of oxygen in benzene[9] and the formulæ given by Bloembergen, Purcell and Pound[10] for dipolar broadening, the contribution from oxygen with $p_{O_2} = 760$ mm. mercury is estimated to be of the order of 0·1 gauss. Experimentally the result is about 5 gauss (the resonance of diphenylpicrylhydrazyl in benzene is also broadened by dissolved oxygen to about the same extent). The discrepancy again could be due to exchange effects and measurements at different temperatures should be carried out. However, the variation with temperature of the solubility of oxygen makes such experiments difficult.

There is also a solvent effect in the electron spin resonance spectra both on the widths and on the resonance parameters. The most pronounced effect we found in pyridine, where for $([C_2H_5)_2NCS_2]_2Cu$, the g-value is 2·0567 (in benzene 2·0453) and the hyperfine structure constant is $-0{\cdot}67 \times 10^{-2}$ cm.$^{-1}$ (in benzene $-0{\cdot}74 \times 10^{-2}$ cm.$^{-1}$). Similar solvent effects have been reported by Pake and Sands[11] and by McGarvey[12]. In mixtures of pyridine and benzene the lines have intermediate positions without appreciable broadening (Fig. 2), which shows that the exchange between solvent molecules close to the paramagnetic molecule must occur with a frequency higher than about $0{\cdot}5 \times 10^9$ c./s. This clearly demonstrates the inability of the dithiocarbamates to form complexes with pyridine as has also been demonstrated by chemical methods[13].

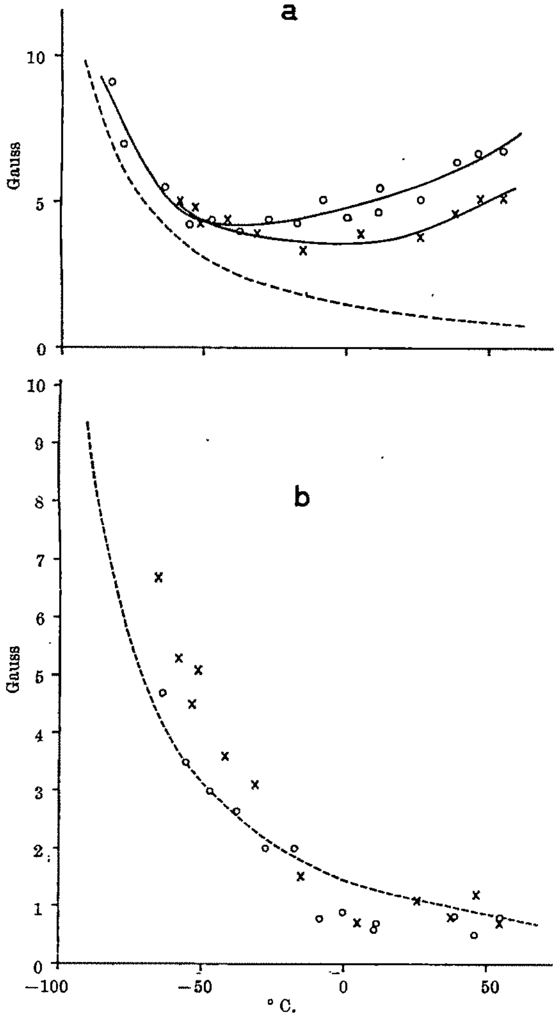

Fig. 1. Widths of hyperfine lines (measured between points of maximum and minimum slope) of 10^{-3} M and 10^{-4} M respectively acetone solutions of $[(C_2H_5)_2NCS_2]_2Cu$. Dashed curves are calculated from Kivelson's formulæ. a, Widths of $m_I = +\frac{1}{2}$ line; b, difference in widths between $m_I = -\frac{1}{2}$ and $m_I = +3/2$ lines. ×, 10^{-4} M; ○, 10^{-3} M

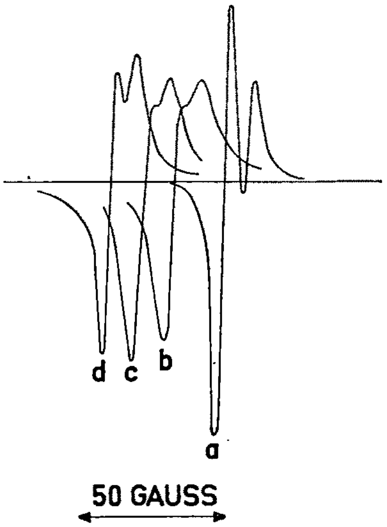

Fig. 2. $m_I = +3/2$ line of 10^{-3} M $[(C_2H_5)_2NCS_2]_2Cu$ in benzene–pyridine mixtures. The mole fraction of benzene is: a, 1·0; b, 0·9; c, 0·7; d, 0·0. Each line is an unresolved doublet due to the presence of two copper isotopes

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Electron Spin Resonance Spectra of γ-Irradiated Carbohydrates and α-Hydroxy Acids

The production of polymers[1] from a wide range of carbohydrates and α-hydroxy acids induced by γ-irradiation of their de-aerated aqueous solutions posed the problem of identifying the monomer radicals responsible initially for such polymerizations. Measurement of the rates of dimerization of simple hydropyranols[2] and the polymer yields from some twenty model carbohydrates[3] suggested that, with glucose, one of the primary reactions involved was a dimerization of the radical derived by abstraction of a hydrogen atom from the carbon of the hydroxymethyl group. The identification[4] of the radical obtained on γ-irradiation of polycrystalline glycollic acid, using electron spin resonance spectra, and the presence of the same radical in the liquid phase, inferred from the isolation of tartaric acid[5] from irradiated, de-aerated aqueous solutions of glycollic acid, suggested a close correlation between these systems. Moreover, recent work[6] on single crystals has enabled the proton coupling tensor elements for protons in certain environments to be determined accurately. With glycollic acid the peak separation (~61 Mc./sec.) of the polycrystalline sample corresponded closely with the average value (57 Mc./sec.) of the H(C) coupling which represents the isotropic part of interaction.

The polycrystalline carbohydrate and α-hydroxy acid samples were sealed off in evacuated glass phials (5 mm. × 20 cm.) and irradiated from a 180 c. cobalt-60 γ-radiation source for 15 hr. at room temperature. The samples could be kept at liquid-air temperature, until their spectra were recorded at ambient temperature on an X-band reflexion spectrometer[7] using small magnetic field modulation and phase-sensitive detection to present the first derivative of the absorption on a pen recorder. The magnetic field was swept comparatively slowly, taking up to 15 min. to record the spectra. The results shown in Table 1 were interpreted on the basis of the work of Whiffen *et al.*[6] and McConnell *et al.*[8] on irradiated single crystals. The spectra of 1-deoxy and 2-deoxy D-glucose could not be resolved.

Table 1

Compound	Type of spectrum and peak separation	Radical postulated
D-Glucose	doublet 42 Mc./sec.	$-\dot{C}HOH$ (carbon 6)
D-Mannose	doublet 35 Mc./sec.	$-\dot{C}HOH$ (carbon 6)
L-Rhamnose	doublet 14 Mc./sec.	$>\dot{C}OH$ (carbon 1, 2, 3 or 4)
D-Arabinose	doublet 8 Mc./sec.	$>\dot{C}OH$ (carbon 1, 2, 3 or 4)
D-Glucal	quadruplet 44 Mc./sec.	$-CH=\dot{C}-$ (carbon 1 or 2)
Mandelic acid	multiplet ~ 10 Mc./sec.	$C_6H_5\dot{C}(OH)COOH$
Hexahydromandelic acid	singlet 0	$C_6H_{11}\dot{C}(OH)COOH$

The spectrum obtained from D-arabinose is only consistent with hydrogen ejection from carbons 1–4 since ejection from C_5 would give a doublet with a peak separation of about 40–50 Mc./sec.. L-Rhamnose appears to give a similar radical since a radical formed at C_6 of the type $\rangle\mathrm{C{-}CH_2}$ would give a triplet (relative intensities 1 : 2 : 1) of total splitting 100 Mc./sec. while ejection of a hydrogen at C_5 would be expected to give rise to a quadruplet (1 : 3 : 3 : 1) of total splitting about 210 Mc./sec. (compare results frim 2-methyl malonic acid, Pooley, D., and Whiffen, D. H., unpublished results). The peak separation and anisotropic broadening observed with irradiated glucose and mannose are consistent with hydrogen ejection at C_6, resembling as they do the spectrum of polycrystalline glycollic acid. The quadruplet spectrum (relative intensities 1 : 3 : 3 : 1) of D-glucal can only arise because of hydrogen ejection at C_1 or C_2 with subsequent delocalization of free spin in the π-bond orbitals and interaction with the three protons—two on C_3 and one on C_1 or C_2. This result is consistent with the radical inferred from the identification of stable end-products from irradiated aqueous solutions in which hydroxylation and fission of the double bond were the predominant effects. The singlet spectrum of hexahydromandelic acid would be expected from a radical obtained by hydrogen ejection at the asymmetric carbon atom since any other radical would exhibit considerable α- or β-proton couplings. In the case of mandelic acid itself, the spectrum was also consistent with hydrogen ejection from the asymmetric carbon atom and subsequent delocalization of free spin and coupling to the aromatic protons, and here the total splitting of about 90 Mc./sec. was similar to that observed[9] in aromatic radical ions. Ejection of an aromatic proton was very unlikely.

Although the above results were obtained with polycrystalline solids and as such should be treated with reserve, in all cases they are in agreement with the radicals, which have been inferred from the isolation of stable end-products in irradiated, de-aerated aqueous solutions of these compounds.

We wish to thank the United Kingdom Atomic Energy Authority, Research Division, Harwell, for supporting these investigations and for a maintenance grant to one of us (H. D. S.), and Prof. M. Stacey, for his interest in this work.

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BIOPHYSICS

New Products from Wheat Starch, Flour and Gluten

Watson[1,2] has shown that graft polymers may be produced from certain substances of high molecular weight when they are masticated with monomers at ordinary temperatures in a machine in which high shearing forces are developed—the 'mechano-chemical reaction'. In the present work a laboratory machine of this type, invented by Watson[3], has been used to study the possibility of making, from different wheaten products, new substances of possible industrial importance.

In the first series of experiments, wheat starch or flour or gluten was masticated with a monomer, for example, styrene, methyl methacrylate, acrylonitrile or methacrylic acid, in the presence of glycerol. In all cases a product of hard, rubbery to rock-like, consistency was obtained, the type depending on the duration of mastication. In a typical example a mixture of 71 per cent starch, 16·5 per cent styrene and 12·5 per cent glycerol was masticated for 30 min., and from this powder a hard, dense product was obtained.

There is evidence from the present work that glycerol does not merely give a suitable consistency to the initial mixture for shearing but also it participates in the reaction. Thus, new products have been obtained by simply masticating starch and glycerol without the presence of a monomer. For example, 85 parts of starch and 15 parts of glycerol in the masticator for 20 min. gave a tough, rubbery product, which became harder as the time of mixing was increased. In the reaction with starch, glycerol may be replaced by various glycols, for example, ethylene glycol, or by glyceryl monoacetate. It cannot be replaced by mono-functional alcohols or by glyceryl diacetate. Similarly, wheat starch triacetate will not react with glycerol.

Compared with wheat starch, the products have a low solubility and high paste viscosity. The evidence suggests, therefore, that an essential step in the formation of these products from starch is the cross-linking of the starch chains by a bifunctional reagent. Furthermore, products are obtained by masticating wheat starch with either diethanolamine or formamide. The nitrogen contents of the products indicate that, if cross-linking has occurred, there is about one cross-link per six anhydroglucose units when diethanolamine is used and about one in three with formamide. By contrast, cross-linked starches prepared by conventional methods[4] usually have one cross-link per 50 or more anhydroglucose units.

Gluten (washed out of flour and freeze-dried) behaves differently from starch or flour. Masticated alone, it shows no obvious change; but with glycerol or glycerol and styrene it yields a hard product. On the other hand, unlike starch, it yields a similar product with glyceryl diacetate. By contrast, casein gives a hard product with glycerol but does not react with glyceryl diacetate.

The products from mastication of starch and flour with glycerol and monomers are practically insoluble in aqueous solutions and in the common organic solvents over a wide range of temperature. They have approximately the same vapour-pressure isotherm as the parent starch or flour. They can be hot-extruded to give fibres of a brittle character, with a breaking strength of 400–1,100 lb. per sq. in.

Full details of this work will be published elsewhere, including an account of further experiments to decide if, in some cases, the mechano-chemical effect is not predominant but activates a thermal reaction. Watson[5] has evidence that in certain cases this can occur.

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BIOCHEMISTRY

Paper Chromatography of Hexosamines and N-Acetylhexosamines

An improved method of paper chromatography has been used to aid the separation of galactosamine hydrochloride from other hexosamine hydrochlorides in acid hydrolysates of chondroitin sulphate and to investigate the isomerization of N-acetylgalactosamine in alkaline conditions.

Barium chondroitin sulphate was prepared from dried bovine nasal cartilage by extraction with 1 *N* sodium hydroxide at 70° for 30 min. followed by neutralization and precipitation with 'Cetavlon'. Paper chromatography of fractions from 'Dowex-50' of hydrochloric acid hydrolysates showed the presence of glucosamine, galactosamine and an unknown amino-sugar. This amino-sugar was separated by re-elution from 'Dowex-50' with 0·3 *N* hydrochloric acid and identified as talosamine. Its hydrochloride was differentiated from galactosamine and mannosamine hydrochlorides and its N-acetyl derivative[1] from N-acetylglucosamine, N-acetylgalactosamine and N-acetylmannosamine by paper chromatography (Table 1) in butanol/pyridine/water (6 : 4 : 3). In the solvent of Fischer and Nebel[2] the mobility of the N-acetyl derivative relative to N-acetylglucosamine was 1·09, the same as that of synthetic N-acetyltalosamine[3].

Table 1. Paper Chromatography of Hexosamine Hydrochlorides and N-Acetylhexosamines

	Hydrochlorides		N-acetyl derivatives	
	A (cm.)	*B* (cm.)	*A* (cm.)	*B* (cm.)
Galactosamine	7·2	9·0	13·7	8·1
Glucosamine	8·1	10·9	14·9	10·4
Talosamine	7·9	11·5	16·1	13·3
Mannosamine	8·6	12·3	14·9	11·5
Solvent front	31·6	38·6	31·1	37·6

Solvent: Descending butan-1-ol/pyridine/water (6 : 4 : 3). Time, 16 hr.; temperature, 21°. Whatman No. 1 paper either (*A*) untreated or (*B*) with preliminary treatment in 0·1 *M* barium chloride. Sprayed for hexosamines and N-acetylhexosamines. Distances moved from origin (cm.).

Ninhydrin oxidation of the amino-sugar was carried out on the base-line of paper chromatograms[4], which were then developed in butanol/ethanol/water (4 : 1 : 1) or in butanol/pyridine/water and sprayed with benzidine–trichloracetic acid reagent. The derived pentose was neither arabinose nor ribose, but coincided with the lyxose/xylose position. A similar chromatogram, after development in butanol/pyridine/water for 19 hr., was sprayed with 0·05 *M* borate buffer, *p*H 9·6, and subjected to ionophoresis at right angles, using a current of 0·4 m.amp./cm. width for 20 hr. Pentose markers had the following ionophoretic mobilities: xylose, + 13·3 cm.; arabinose, + 10·5 cm.; ribose, + 9·5 cm.; lyxose, + 7·5 cm. The derived pentose (+ 7·7 cm.) was clearly lyxose and the original unknown amino-sugar (not galactosamine) was talosamine.

The separation of the amino-sugars and of their N-acetyl derivatives was improved by preliminary treatment of the paper with 0·1 *M* barium chloride (Table 1), due partly to faster solvent movement and partly to variations in R_F. We find this procedure preferable to that of Fischer and Nebel[2] for investigation of mixtures of these compounds. Borate-treated papers[1] give low R_F values for N-acetylhexosamines and N-acetylmannosamine coincides with N-acetylgalactosamine.

The isomerization of N-acetylglucosamine under alkaline conditions is known[5]. With 2 mgm. N-acetylgalactosamine and 0·125 ml. 1 per cent (w/v) potassium carbonate at room temperature for 2 days there was about 5 per cent conversion to a product corresponding to the previously identified N-acetyltalosamine. No further conversion was apparent after 4 days treatment.

N-acetyltalosamine was found to be present in a sample of N-acetylgalactosamine prepared by the acetylation of galactosamine hydrochloride derived from sheep tracheal cartilage[6].

These results do not necessarily mean that epimerization of N-acetylgalactosamine residues was responsible for the appearance of talosamine, because a high proportion of terminal residues would require to be epimerized during the alkaline extraction in order to give the talosamine (1 per cent of the total hexosamine) detected. If talosamine does occur naturally in cartilage, it may be associated with protein since purified chondroitin sulphate[7] contained no amino-sugar other than galactosamine and a neutral polysaccharide fraction[8] contained no amino-sugar other than glucosamine, but proof of its occurrence requires to be shown without use of alkali.

We are indebted to Prof. R. Kuhn and Dr. R. Brossmer for a gift of mannosamine hydrochloride.

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Catalysis of Metalloporphyrin Formation: a Possible Enzyme Model for Hæm Iron Incorporation

RECENT investigations on the incorporation of metal ions into porphyrins solubilized in aqueous detergent solutions have revealed two factors having a marked influence on the reaction-rate. The first is related to the charge on the detergent and has been termed the electrostatic effect; the second is concerned with the presence of certain chelating agents, and has been termed the co-ordination effect.

The electrostatic effect is such that the rate of incorporation of cupric ions (Cu^{++}) into dimethyl protoporphyrin ester is 20,000 times faster in 2·5 per cent sodium dodecyl sulphate than in 5 per cent cetyl trimethylammonium bromide under otherwise comparable conditions. This effect can be attributed to the electrostatic attraction of the cupric ions to the anionic micelle–water interface, where presumably they are able to interact with the nitrogen atoms in the tetrapyrrole nucleus. A possible structure for the solubilized porphyrin in which the pyrrole nitrogens are exposed to the aqueous phase is shown in Fig. 1.

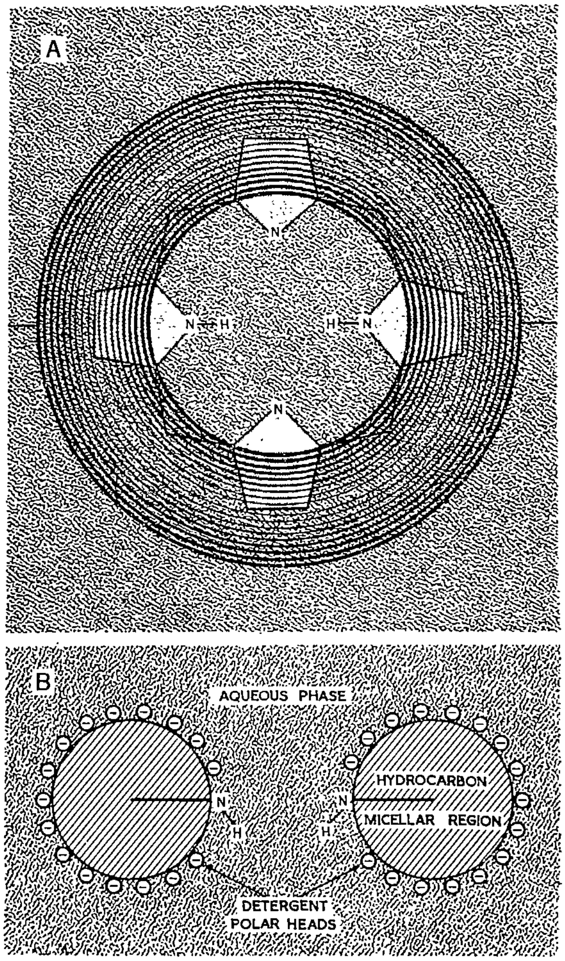

Fig. 1. A possible structure for the porphyrin – micelle complex with the porphyrin mainly immersed in the hydrocarbon region of the micelle and the nitrogen atoms in contact with the aqueous phase. *A*, plan; *B*, sectional view

The co-ordination factor is illustrated in Fig. 2, which shows the effect of increasing concentrations of 8-hydroxyquinoline (OX) on the initial rate of formation of copper protoporphyrin ester in a 2·5 per cent sodium dodecyl sulphate solution. It will be noted that the effect is maximal when the mole ratio of cupric ions to 8-hydroxyquinoline is 1 : 1, and that at higher concentrations of 8-hydroxyquinoline the rate is depressed. This suggests that the 1 : 1 $[Cu\ OX]^+$ complex is the active catalytic species. A similar effect has been observed in cationic and non-ionic detergent solutions. Because of the interplay of electrostatic and co-ordination factors in practice (one must, for example, compare the divalent cupric ion with the monovalent $[Cu\ OX]^+$ ion) it is difficult to assess the absolute catalytic effect of the co-ordination factor. However, it can be estimated to be 200–2,000-fold. The effect of other chelating and co-ordinating agents on the reaction-rate will be discussed in a further communication.

The temperature coefficient of the reaction ($Q_{10}\sim 2$) is unaffected by the presence of catalytically

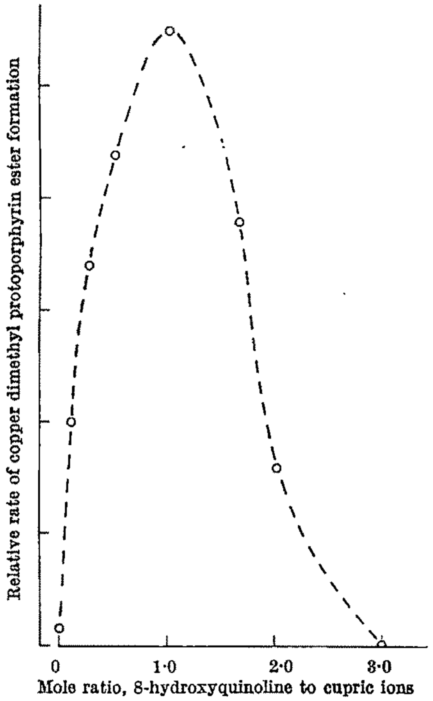

Fig. 2. Effect of varying the concentration of 8-hydroxyquinoline on the initial rate of formation of copper dimethyl protoporphyrin ester in 2·5 per cent sodium dodecyl sulphate at 40° C. The initial concentrations of cupric ions and dimethyl protoporphyrin ester were constant at $10^{-5} M$ and $10^{-6} M$ respectively. The pH was ~ 7·0

active $[Cu\ OX]^+$ species. This suggests that the co-ordination effect influences the probability of an effective collision (that is, the activation entropy) rather than the activation energy.

The maximum increase in rate yet observed from the combined influence of the electrostatic and co-ordination effects is 500,000-fold. This corresponds to the difference in initial reaction-rates between $[Cu\ OX]^+$ and dimethyl protoporphyrin ester in 2·5 per cent sodium dodecyl sulphate and cupric ions and dimethyl protoporphyrin ester in 5 per cent cetyl trimethylammonium bromide at comparable temperatures. Both effects have been observed to influence the rate of incorporation of ions other than cupric ions, for example, cobalt, nickel, ferrous iron and zinc.

The reaction studied in this system has features in common with an enzyme-catalysed reaction. Thus, the detergent solution, which consists of lipoidal micellar regions dispersed throughout an essentially aqueous phase, resembles the colloidal nature of the biological environment. The role of the detergent micelle in reacting with one of the substrates dimethyl protoporphyrin ester and simultaneously attracting the other substrate (cupric ions) to the same vicinity parallels the behaviour of an enzyme in bringing the reactants together—the porphyrin–micelle complex being analogous to an enzyme–substrate complex. The 8-hydroxyquinoline might be regarded as a co-factor which, by co-ordinating with one of the substrates (cupric ions) thereby facilitates the reaction. Both the electrostatic and co-ordination effects arise from changes in the entropy rather than the energy of activation, the electrostatic effect being associated with a concentration entropy term and the co-ordination effect with an orientation entropy term.

It is interesting to note that the large catalytic effect observed in this system has arisen solely from favourable changes in the entropy of activation. The importance of the entropy of activation in enzymic reactions has recently been pointed out[1] in connexion with the role of magnesium ions in enzyme syntheses involving adenosine triphosphate.

This hypothesis might have some relevance to the incorporation of iron in the biosynthetic formation of hæm. Preliminary experiments have indicated that the rate of incorporation of ferrous ions into porphyrins in this system is several hundred-fold greater than that observed in other aqueous systems[2–4], although still considerably less than the known enzymic rate[5–7]. However, the relative magnitude of the effects discussed here is such that the enzymic rate of incorporation might yet be equalled by a further chemical modification of the system.

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Gastro-Intestinal Absorption of Heparin and Synthetic Heparinoids

Heparin and synthetic heparinoids have to be administered parenterally to assure clinical efficacy[1]. Since oral administration of these drugs would be highly desirable, numerous compounds were tested for their ability to effect absorption from the gastro-intestinal tract. The criterion of absorption was the appearance of plasma lipæmia-clearing activity, which was first described by Hahn[2].

Several compounds ('adjuvants') were discovered to have this property, the best of these being certain salts of ethylenediaminetetraacetic acid (EDTA). Other active compounds will be the subject of future communications. The present communication describes the use of salts of EDTA to obtain absorption of heparin and synthetic heparinoids.

Rats weighing about 200 gm. were placed in individual cages and fed for 72 hr. a liquid diet consisting of 30 ml. of a 33 per cent casein hydrolysate and water *ad lib.* The drug was given by intubation of an aqueous solution at a volume of 10 ml./kgm.

Table 1. Rat and Dog Lipæmia-Clearing Activity 4 hr. after Oral Sulphopolyglucin plus EDTA

Animal	No. of animals	SPG (mgm./kgm.)	Sodium salt of EDTA (mgm./kgm.)	Lipæmia-clearing activity (C.U.*)
Rats	5	500	—	0·04 ± 0·03
	5	500	500	2·41 ± 0·74
	4	—	500	0·01 ± 0·01
Dogs	5	100	—	0·59 ± 0·41
	6	100	50	5·73 ± 1·04

* Mean ± standard deviation

Table 2. Lipæmia-clearing Activity and Clotting Time after Oral Heparin plus EDTA in Rats and Dogs

Rats			Dogs		
Heparin 50 mgm./kgm. Sodium salt of EDTA 500 mgm./kgm.			Heparin 100 mgm./kgm. Sodium salt of EDTA 50 mgm./kgm.		
Time (min.)	Clearing activity (C.U.*)	Clotting time (min.)	Time (hr.)	Clearing activity (C.U.*)	Clotting time (min.)
0	0·04 ± 0·03 (5)	7·0 ± 1·7 (16)	0	0·19 ± 0·18 (4)	11·3 ± 2·1 (8)
10	0·10 ± 0·09 (3)	11·0 ± 1·3 (3)	1	0·85 ± 0·70 (4)	>28 ± 21 (8)
30	1·46 ± 0·17 (3)	13·3 (2)	2	0·99 ± 0·66 (4)	>44 ± 22 (8)
60	0·31 ± 0·24 (3)	8·0 (1)	4	0·31 ± 0·45 (4)	18·1 ± 8·2 (8)

Figures in parentheses are number of animals. >, some animals had incoagulable blood (> 60 min.). *, Mean ± standard deviation.

At specified times, the rats were anæsthetized with pentobarbital sodium, and blood samples taken by cardiac puncture.

In dogs, after an overnight fast, the drugs were given orally in gelatin capsules. Blood samples were taken by venipuncture at specified intervals. Lipæmia-clearing activity of citrated plasma was determined[3] and expressed in clearing units (C.U.) calculated according to Grossman, except that incubation time was limited to 10 min., and the substrate was 'Ediol' (SchenLabs), diluted 1 to 50 with water. Whole-blood clotting time was determined by a three-tube Lee–White method[4] and indicated in minutes. When blood did not clot within 60 min., a value of 60 min. was used in calculating mean clotting time, but the '>' sign was added. All values for clearing activity and clotting time are given as mean ± standard deviation for groups of 5–10 animals or as specified.

The drugs used were heparin sodium U.S.P., potassium salt of sulphopolyglucin (SPG) and various salts of EDTA. SPG is a sulphated polysaccharide of 5 to 15 glucose units connected by α,1–4, and α,1–6 linkages, with 2–3 sulphate groups per glucose unit.

Typical experiments demonstrating the adjuvant property of sodium EDTA are presented in Table 1. Using lipæmia-clearing activity as an index of absorption, the results show, without question, the adjuvant properties of sodium EDTA. In dogs, where SPG alone produces measurable although somewhat erratic clearing activity, absorption of the heparinoid is increased about ten-fold by simultaneous administration of the sodium salt of EDTA. In rats, the effect of EDTA is even greater, since SPG alone is practically not absorbed.

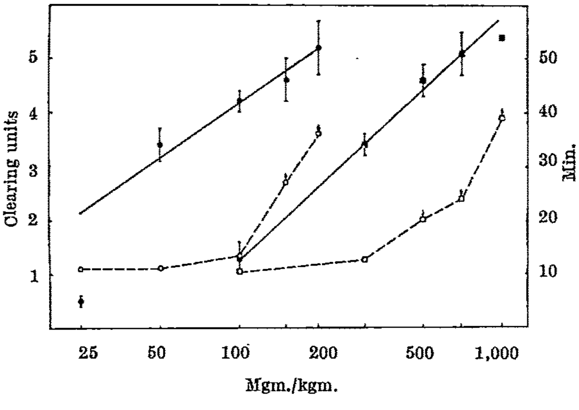

Fig. 1. Dose-response curves for lipæmia-clearing and anti-coagulant activities of SPG without and with EDTA in a ratio 4 : 1. Drugs given orally to groups of 5–10 dogs and blood taken 3 hr. later. Arrows indicate clotting times of more than 60 min. in some specimens. Limits in lipæmia-clearing curves are standard error. ● ○, EDTA; ■ □, no EDTA; —, clearing; – – –, clotting

Similar results with SPG have been found in every animal species studied (monkey, dog, cat, rabbit and rat), as well as in man[5]. The adjuvant property of EDTA has also been demonstrated for other synthetic heparinoids (polyethylene sulphonate, dextran sulphate and others).

The adjuvant property of the sodium salt of EDTA can also be demonstrated for heparin, using both clotting time and clearing activity as criteria of absorption (Table 2).

The effects of EDTA are again unequivocal. In rats, clearing activity reached a peak about 30 min. after administration of the drug combination, and then declined slowly to control-levels. A similar time course was obtained for the anti-coagulant effect, with doubling of the clotting time at about 30 min. after the drug and subsequent return to control-levels. Since it is known that heparin is not absorbed from the gastro-intestinal tract, control experiments without EDTA were not made.

In dogs, the typical heparin effects appeared slower than in the rats. The peak of clearing activity and anti-coagulant effects were reached about 2 hr. after drug combination and returned to control-levels after more than 4 hr.

Further experiments were carried out in dogs to establish dose-response curves for SPG, both with and without EDTA. The results in Fig. 1 show good correlation between dose and clearing as well as anti-coagulant activity. Statistical evaluation indicated that the efficacy of SPG was increased approximately five-fold when EDTA, in the ratio 1 : 4, was used as adjuvant.

Significant amounts of heparin and synthetic heparinoids can be absorbed from the gastro-intestinal tract when these drugs are given orally together with an alkali salt of EDTA. The mechanism of this adjuvant effect is not clear. There is evidence that the chelation of calcium and/or magnesium ions by EDTA may be involved for the following reasons: (1) Sodium, potassium and ammonium salts of EDTA are effective as adjuvants; (2) calcium and magnesium EDTA are not effective; (3) adding a calcium salt blocks the adjuvant action of alkali EDTA; (4) phosphate buffers are not effective; (5) the adjuvant has to be given orally; intravenous injection of the adjuvant does not influence the absorption of SPG from the gastro-intestinal tract.

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Changes of Hexose/Hexosamine Ratio and Degree of Branching in Glycoprotein from Rat Urine during the Development of Cancer

We have isolated, from the urine of patients with cancer, an abnormal glycoprotein (III)[1] from fraction II (richest in carbohydrate, but low in sialic acid) of the Anderson–MacLagan glycoprotein (I)[2], which was characterized by a decreased hexose/hexosamine ratio, increased oligosaccharide-branching due to hexosamine and L-fucose residue and increased molecular weight, as compared with the exactly corresponding glycoprotein of urine of non-cancerous origin[1] (IV). These changes were observed, however, on fractions III and IV prepared from urine of patients with different stages of cancer, and also from normal and non-cancerous subjects[1]. We wished, therefore, to study those changes under comparable conditions, such as experimental cancer or inflammation, so as to follow up changes during pathological processes. But technical difficulties were great, since the yield of III is only 2–3 per cent by weight of I, and we were limited by the small quantity of animal urine available. It was observed, however, that the characteristic changes can be recognized even at the stage of purity of substance II.

Urine of four groups of ten rats (Wistar strain, female) were collected under toluene. The rats were inoculated intraperitoneally with ascites hepatoma 136 (Sasaki Institute, Tokyo), and afterwards pooled urine was separated every four days and substance II glycoprotein fractions were prepared; substance I from the pooled rat urine was solubilized with water at *p*H 8·4 (insoluble part rejected) and precipitated at *p*H 4·2, and centrifuged. The supernatant was electrodialysed for 3 days to effect complete precipitation, and the supernatant was lyophilized and analysed without further purification. In two groups of ten rats, inflammation was set up by intraperitoneal injection of turpentine oil, and the urine of days 2–7 after injection were taken, similarly treated and analysed.

Table 1. CHANGES OF GLYCOPROTEIN FRACTION II IN INFLAMMATION AND DURING DEVELOPMENT OF CANCER

	Normal rats (before inoculation)	Inflammatory rats† (turpentine peritonitis)	Cancer-bearing rats* (ascites hepatoma 136, Sasaki)			
			Days after inoculation			
			1–4	5–8	9–12	13–death
Hexoses (ref. 3)	14·8	13·2	10·7	13·6	9·7	8·1
Pentoses (ref. 3)	5·3	3·1	3·0	4·0	3·5	3·2
Methyl pentoses (ref. 4)	3·4	3·2	3·0	3·2	3·2	3·4
Hexosamines (ref. 5)	8·1	8·1	8·4	10·9	8·5	9·1
Carbohydrate terminals (ref. 6)‡	5·6	5·5	4·6	6·5	8·1	8·1
Ratio of hexose to hexosamine	1·8	1·6	1·3	1·2	1·1	0·9
Ratio of total carbohydrate§ to terminal carbohydrate	17·7	19·9	18·1	20·6	32·6	38·4

For methods of determination, see the corresponding references.
* Mean values of four groups of ten rats.
† Mean values of two groups of ten rats.
‡ Expressed as percentage of the amount of formic acid evolved on periodate oxidation for 12 hr. at 5° C.
§ Excluding sialic acid.

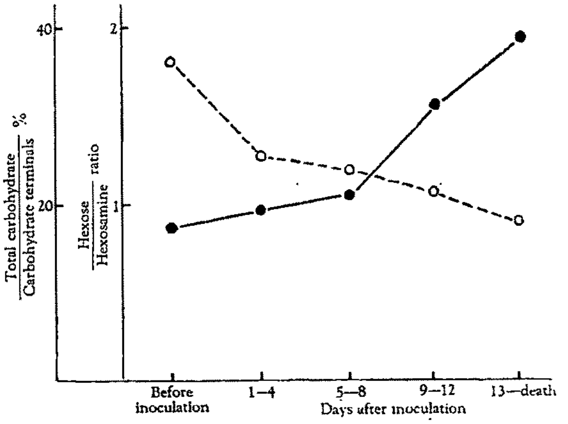

Fig. 1. Changes of degree of branching (full line) and ratio of hexose to hexosamine (dotted line) of the glycoprotein II from rats during development of cancer

As is shown in Table 1 and Fig. 1, the non-amino- to amino-sugar ratio decreased, and the ratio of the total carbohydrate to end-group carbohydrate increased with the development of cancer, but these ratios remained unchanged in the case of inflammation.

In view of the significant role of oligosaccharide branching and the hexose/hexosamine ratio in the determination of the biological specificity of mucosubstances[7], it is considered that the lost hexoses and increased branching observed in the abnormal glycoprotein must reflect lost specificity of the normal molecule, and leads to the appearance of the structure responsible for pathological specificity.

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Fractionation of Compound Lipids by Dialysation in an Organic Solvent against an Organic Solvent

Compound lipids in general are not dialysable in aqueous solution or emulsion because they form micelles. Their dialysability in organic solvents, however, has not been examined in detail; but it seems to be largely dependent on the state of the organo-micelle[1]. It was therefore thought that by dialysation of individual compound lipids in organic solvents against successive changes of solvents of different polarity, the so-called 'high-molecular lipids'[5] or 'mucolipids'[2,3] would remain undialysable if they are really of high molecular weight.

We have examined the dialysability of various compound lipids in a number of organic solvents, and the following findings are of special interest: (1) Chol-

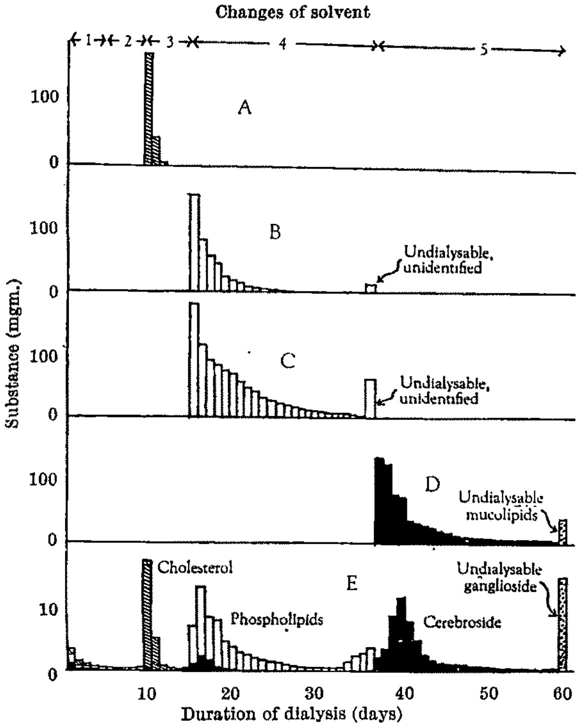

Fig. 1. Dialysability of cholesterol (*A*) in chloroform; I (*B*); mixture of II and III (*C*) in chloroform–methanol (2 : 1 v/v); IV (*D*) in pyridine and of a mixture of these with V (*E*). Dialysis was carried out by using a Visking tube, 1 cm. × 20 cm. against a 100-ml. aliquot of various solvents. Solvent 1 : benzene/petroleum ether (1 : 1, v/v). Solvent 2 : benzene/chloroform (1 : 1, v/v). Solvent 3 : chloroform. Solvent 4 : chloroform/methanol (2 : 1, v/v). Solvent 5 : pyridine. Temperature, 5 ± 1. The abscissa represents the duration of dialysis in days and the ordinate is the amount of substance dialysed in mgm. In experiment (*E*), 20–50 mgm. each of the mixed material dissolved in 80 ml. of benzene petroleum ether (1 : 1) placed in a 1 cm. × 40 cm. Visking tube were dialysed in 100 ml. of solvent

esterol, phosphatidyl-cholin (I), -ethanol amine (II) and -serine (III), and cerebroside (IV) can be dialysed in different polarity of the media used through Visking 24 Å. membrane (see Fig. 1). A trace of undialysable portion was recognized in substances I–IV, even in chromatographically pure substances I–III (ref. 4), but the undialysable remainders of I–III have not been identified as yet. Ganglioside (V) prepared from strandin[5] fraction cannot be dialysed in any of the media.

(2) Substance IV, prepared from protagon[6], crystallized thrice from ethanol, twice from glacial acetic acid, can be separated into a dialysable fraction (VI) and an undialysable fraction (VII) by dialysation for two weeks in pyridine (outside liquid changed every day). Substance VII was separated into water-soluble supernatant (VIII) and gelatinous precipitate (IX) when dialysed against water (150 mgm. of VIII and 230 mgm. of IX from 6·5 gm. of IV).

The fast dialysable (within 2–3 days) fraction of (V) was identified as almost pure cerebroside by analysis ; N, 1·70; hexose, 18; P, 0·01; SO_4, 0·2 per cent; $[\alpha]_D^{25}$ in pyridine, 4·5°. The hydrolysate gave only galactose (a typical infra-red spectrum). The slowly dialysable (7–14 days) fraction of (V) contains mainly galactose and also a small amount of galactosamine, but no glucose.

From substance VIII was isolated by chromatography on cellulose[7], two main peaks eluated with methanol/chloroform/water (7 : 3 : 0·5 by vol.). They were typical of mucolipids but different from ganglioside[8] or 'brain mucolipid'[3]; they were characterized by strong dextro-rotation, ($[\alpha]_D^{18}$ in pyridine = +68° and +84° respectively), but both contain glucose, galactose, galactosamine and a trace of sialic acid. Substance IX has not yet been purified, but is believed to be a glycolipid linked with polypeptide (the hydrolysate gave such amino-acids as glutamic acid, aspartic acid, serine, glycine, leucine and valine); N, 3·5 per cent; hexose, 25; P, 0·8; sialic acid, 5·5 per cent. It is noticeable that only the undialysable fractions (VII and VIII) contain a considerable amount of glucose.

(3) Fractional dialysation of artificial mixtures of cholesterol, I, II, III, IV and V has been tried. Although up to the present simultaneous dialysation (co-diffusion) of a small amount of IV with the majority of II and III has been observed, almost successful separation of individual compound lipids has been accomplished, as is shown in Fig. 1*E*. Substance V, which is thought to be the only high-molecular lipid among these mixtures, has been recovered as the undialysable remainder.

This type of fractionation may be of considerable importance, since it does not include any drastic treatment and can resolve the individual lipids in their native state.

Full characterization of the individual fractions will appear in detail elsewhere.

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Fractions of Trypsinized Casein giving Different Patterns of Bacterial Growth

THE rate of growth of a strain of *Streptococcus equisimilis* in a medium comprising acid-hydrolysed casein, glucose and yeast extract has been shown in these Laboratories to be markedly increased by the addition of a small amount of a tryptic digest of casein. While investigating this effect, which had been shown not to be due to the ability of the enzymic digest to supply free amino-acids, it was found to be possible by chromatography on paper in *n*-butanol/acetic acid/water (25 : 6 : 25 by vol.) to fractionate the digest in such a way as to separate compounds possessing growth-stimulating activity of two distinct types. Thus constituents of the digest possessing approximately the same R_F as that of glycine resulted, after a very short lag, in a sudden increase in the rate of growth of the organisms which, however, was of short duration (Fig. 1*a*). On the other hand, the effect of constituents of the digest with R_F values similar to that of valine reached a maximum only after a prolonged lag and differed again from the previous effect in that the high rate of growth was sustained (Fig. 1*b*).

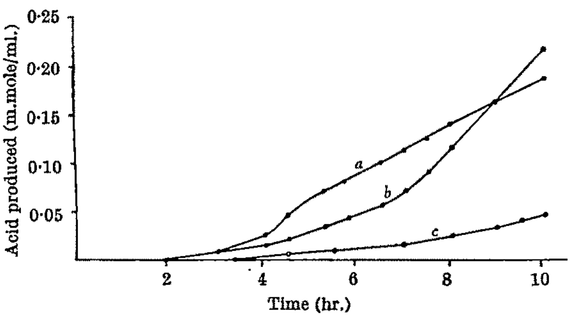

Fig. 1. Growth patterns of a strain of *Streptococcus equisimilis* showing effect of fractions of trypsinized casein separated chromatographically. For convenience, growth is expressed as m.mole/ml. of lactic acid produced. *a*, Basal medium + glycine fraction ; *b*, basal medium + valine fraction ; *c*, basal medium

It seemed almost certain from the protein nature of the starting material that the active substances in the casein digest were peptides, and indeed, an active peptide with the same R_F value as that of valine has now been isolated chromatographically from a tryptic digest of casein. The effect of this peptide was of the predicted type, as rapid growth-stimulation occurred only after a long lag period. Fractions obtained by filtration of casein digests through 'Sephadex *G*-25' (ref. 1) were further resolved by a two-dimensional technique involving the successive use of paper chromatography and high-voltage electrophoresis[2]. By these means, the presence has been confirmed of a great multiplicity of peptides in both the glycine and valine regions of chromatograms (Fig. 2).

It has been known for some time that certain natural or synthetic peptides exert a stimulatory effect on micro-organisms, particularly the *Lactobacilli*[3], while more recently, Kihara and Snell[4] have advanced the attractive theory that this stimulatory effect resides in the ability of peptides in some instances to overcome blocks in the metabolism of individual amino-acids.

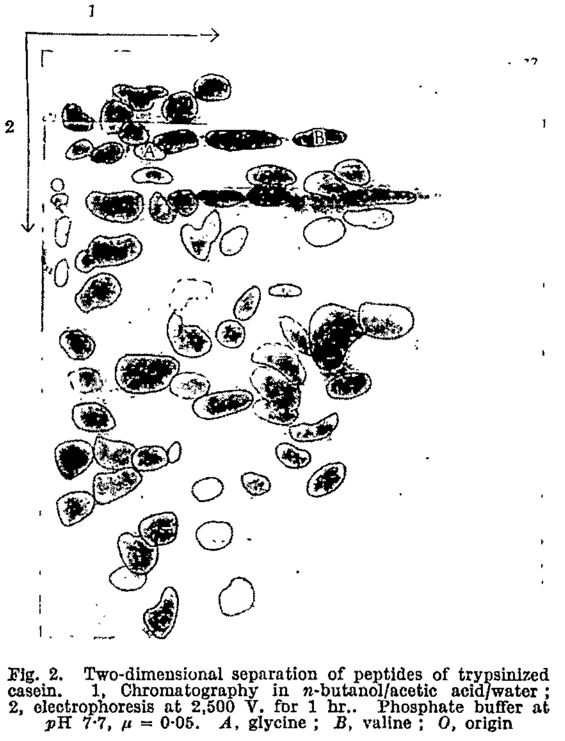

Fig. 2. Two-dimensional separation of peptides of trypsinized casein. 1, Chromatography in *n*-butanol/acetic acid/water ; 2, electrophoresis at 2,500 V. for 1 hr.. Phosphate buffer at *p*H 7·7, μ = 0·05. *A*, glycine ; *B*, valine ; *O*, origin

In the present work the diverse effects shown by the two peptide fractions might be interpreted as indicating the changing requirements of the bacterium for specific peptides at different stages of growth. On the other hand, it is possible that the present results relate to current theory as follows : Pattern 1*a* may represent the effect of a group of peptides which help the organism to overcome metabolic blocks but which, through being present in submiminal amounts, fail to show sustained activity. Pattern 1*b* may represent the effect of a group of peptides which are not of themselves active but which give rise to active substances as a result of structural modifications arising through the action of enzymes produced by the bacterium. The long lag may then be regarded as the period required for the synthesis or action of the appropriate enzyme system. Alternatively, the lag may represent the interval necessary for the micro-organism to elaborate the system for transporting the peptides across the cell wall. These and other related problems are at present under investigation.

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Biosynthesis of Choline *in vitro* by Rat Liver

In conformity with the observation that only one of the three methyl groups of choline, after oxidation to betaine, can be transferred to homocysteine[1], it was shown from *in vivo* studies[2] that only the conversion of dimethylethanolamine to choline is a transmethylation, the influence of folic acid being confined to steps leading to the formation of monomethylethanolamine and dimethylethanolamine from ethanolamine. The present *in vitro* experiments were carried out to ascertain, more directly, the origin of the methyl groups of choline, and their relationship to folic acid.

Since homogenation resulted in complete loss of ability to form choline[3], several rat liver slices were incubated, separately, with ethanolamine, monomethylethanolamine and dimethylethanolamine in presence of (3-^{14}C) serine or (Me-^{14}C) methionine. The involvement of folic acid in one or more of the methylations in choline biosynthesis from ethanolamine, can be inferred from the significantly lower incorporation of activity into phosphatidyl choline by liver slices of folic acid-deficient rats as compared with those of the folic acid-supplemented controls, except when dimethylethanolamine and (Me-^{14}C) methionine were incubated together (Table 1). This lack of effect in the latter case would suggest that the methylation of dimethylethanolamine is predominantly a transmethylation. On the other hand, the appreciable amounts of activity incorporated from the methyl group of methionine into phosphatidyl choline in presence of ethanolamine and monomethylethanolamine, even in folic acid deficiency (Table 1).

Table 1. Folic Acid Deficiency and Incorporation of (Me-^{14}C)-Methionine and (3-^{14}C)Serine into Phosphatidyl Choline

Additions	(Me-^{14}C)methionine		(3-^{14}C)serine	
	+ Folic acid	− Folic acid	+ Folic acid	− Folic acid
	Total counts/min./vessel in phosphatidyl choline *			
Ethanolamine	1,452	502	1,043	268
Monomethylethanolamine	1,133	392	616	115
Dimethylethanolamine	1,918	1,486	906	172

* Average values for five independent incubations.

Each incubation vessel contained 500 mgm. of normal or folic acid deficient (ref. 4) rat liver slices in 5 ml. Krebs–Ringer phosphate buffer *p*H 7·4, containing 3×10^{-4} *M* (Me-^{14}C)methionine or (3-^{14}C)-serine and $3 \cdot 8 \times 10^{-4}$ *M* either ethanolamine, mono- or dimethyl-ethanolamine. Incubations were performed in air for 2 hr. at 37° C. The reaction was stopped by addition of 2 ml. of a 3 : 1 mixture of ethyl alcohol–ethyl ether. Extraction of phospholipids, hydrolysis and separation of choline were according to Pilgeram *et al.*, ref. 3.

would indicate that transmethylation is also involved in providing methyl groups for dimethylethanolamine synthesis. The methyl groups of choline, therefore, seem to be derived by transmethylation as well as folic acid-dependent reactions, the latter being predominant with the first two steps and the former with the last one. These observations are in keeping with recent findings of Venkataraman and Greenberg[5], who have defined an active soluble enzyme system in liver, capable of synthesizing all the choline methyl groups from formaldehyde, tetrahydropteroylglutamic acid serving as a co-factor, and of Bremer and Greenberg[6], who have shown that rat liver microsomes could synthesize monomethylethanolamine and dimethylethanolamine, as well as choline, by transmethylation.

There is a graded decrease in the activity of phosphatidyl choline formed in presence of ethanolamine and monomethylethanolamine, possibly because, on an equimolar and random basis, for every ethanolamine and monomethylethanolamine molecule, there will be 3 and 2 methyl groups respectively entering. The rate-limiting nature of earlier methylations[6] would explain the higher incorporation with dimethylethanolamine (Table 1).

Experiments in Table 2 serve to show that the observed folic acid-dependent incorporation of the β-C of serine into phosphatidyl choline, synthesized from dimethylethanolamine (Table 1) probably occurs through prior conversion to the methyl moiety of methionine[8]. The β-C of serine is incorporated more extensively into methionine than into choline of the acid-soluble fraction. The incorporation reaches a maximum in 0·5 hr., while it increases steadily for 2 hr. in phosphatidyl choline. Arnstein and Neuberger[9] have also indicated from *in vivo* work that methionine synthesis precedes choline synthesis, the hydroxymethyl group of serine being an important precursor of the methyl group of methionine.

Table 2. Relative Rates for Incorporation of (3-^{14}C)Serine into Acid-soluble Methionine, Choline and Phosphatidyl Choline

Incubation time (hr.)	Acid-soluble fraction				Phosphatidyl choline	
	Methionine		Choline			
	A	*B*	*A*	*B*	*A*	*B*
	Total counts/min./vessel*					
0	489	450	234	228	0	0
½	18,237	5,408	1,736	2,440	381	478
1	13,469	15,016	913	1,902	927	1,202
2	10,263	14,720	551	1,608	1,228	1,287
3	8,998	10,124	378	750	1,278	1,306

* Average values for five different incubations.

Each reaction vessel contained 400 mgm. rat liver slices in 5 ml. of Krebs–Ringer phosphate buffer, *p*H 7·4, containing 3×10^{-4} *M* (3-^{14}C)serine and $3 \cdot 8 \times 10^{-4}$ *M* each of ethanolamine and homocysteine in experiments *A* and dimethylethanolamine and homocysteine in experiments *B*. Incubations were performed in air at 37° C. The reactions were stopped at different time intervals by adding trichloracetic acid (5 per cent). Choline, methionine and serine were added, each at 1-mgm. level, as carrier and the amino-acids separated essentially according to the method of Moore and Stein (ref. 7). Choline was eluted finally by 1·5 *N* hydrochloric acid. Phosphatidyl choline was isolated as explained in legend for Table 1.

This investigation is being extended with the sub-fractions of the cell.

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Reaction of Digitalis Compounds with Antimony Pentachloride

Antimony pentachloride has been used as an agent for the detection of steroids in paper chromatography by a number of investigators[1–4]. It has been found that antimony pentachloride is a useful agent for detecting certain digitalis compounds (digoxin, digitoxin and digitoxigenin) on paper chromatograms.

Crystalline digitoxin and digitoxigenin (obtained from Nutritional Biochemicals Corp., Cleveland, Ohio) in chloroform and crystalline digoxin (obtained from Burroughs Wellcome and Co., Inc., Tuckahoe, New York) in a 1 : 1 solution of 95 per cent ethanol and chloroform were placed on a 0·8 cm.^{2} area on Whatman No. 7 paper in varying amounts. Antimony pentachloride in chloroform (20 gm. in 100 ml.) was sprayed on the paper. An immediate reaction occurred. All three compounds became yellow in colour and the digoxin and digitoxin quickly took on an intense purple colour. The digitoxigenin slowly took on a green colour at the 50- and 25-μgm. level, but remained yellow with lesser amounts.

Table 1. Reaction of Digitalis Compounds with Antimony Pentachloride

Compound	Colour before heating	Minimum amount detectable (μgm.)	Colour after heating	Minimum amount detectable (μgm.)
Digoxin	Purple	1·0	Black	0·5
Digitoxin	Purple	1·0	Black	0·5
Digitoxigenin	Green or yellow	1·0	Darker green or yellow	0·5

After heating the sprayed paper at 84° C. for 5–10 min. the digoxin and digitoxin became intensely black, while the digitoxigenin turned a darker shade of green or yellow.

Table 1 shows the changes in colour before and after heating and the least amount detectable. After heating the compounds were much more easily visible at the 1·0–5·0 μgm. range than before heating.

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RADIOBIOLOGY

A General Method for the Quantitation of Immune Cytolysis

THE cytolytic effect of immune serum has been the subject of study by a number of investigators. It has been shown[1] that cells other than erythrocytes undergo lytic changes in the presence of antibody and complement. It has been further shown[2], using cells labelled with phosphorus-32, that the lysis of such cells is accompanied by a release of intracellular proteins, similar to the release of hæmoglobin from lysed erythrocytes. It appeared that the immune lysis of tissue cells is quite analogous to the immune lysis of erythrocytes. On this basis, a test was developed using radioactive labelled cells which provides a means of quantitating immune lysis for cells derived from a variety of tissues.

Mouse Ehrlich ascites tumour cells grown in *CF* No. 1 female mice and the corresponding immune rabbit serum were used for the demonstration of this method. The tumour cells were collected from the peritoneal cavity of the mouse, washed 3–5 times by centrifugation and decantation with a diluent composed of veronal buffered saline, *p*H 7·4 (ref. 3), containing 1 per cent bovine albumin (Armour Laboratory, Kankakee, Illinois). The washed tumour cells were labelled with 50 μc. of chromium-51 ('Ra-chromate', Abbott Laboratory, North Chicago) per ml. packed tumour cells. After an incubation period of 1 hr. at room temperature, the labelled cells were washed five times with diluent. An uptake of 60–70 per cent of the radioactivity was obtained. The cell concentration was then adjusted to 1 per cent on the basis of either packed cell volume or cell count. It was found that a 1 per cent suspension corresponding to 4 × 10^6 cells/ml. was optimal for these tests. It was also found that only those cells were suitable which had been collected and labelled on the same day the test was performed.

The total volume of the cytolytic test was 3·0 ml., consisting of 1·0 ml. serum dilution, 1·0 ml. complement dilution (lyophilized guinea pig serum, Sharp and Dohme, Philadelphia) and 1·0 ml. of the chromium-51-labelled cell suspension. (A protocol for a typical antibody cytolytic titration is given in Table 1.) The test was incubated for 1 hr. at 37° C. and centrifuged. Then the clear supernatant fluids were gently removed by aspiration. Finally, 1·0-ml. aliquots of the supernatant liquids were counted for radioactivity. The values obtained (antibody or complement titration) were corrected for non-specific loss of radioactivity from the cells (tube 7*c* of Table 1) and then the percentage of specific lysis was calculated by dividing the corrected counts by the number of counts corresponding to 100 per cent lysis.

In order to obtain a 100 per cent value for cell lysis, it was necessary, because insoluble as well as soluble components of the cell had been labelled, to determine the amount of radioactivity associated with the soluble components only. Ultrasonic vibration was considered to be the most suitable method for cell disruption; therefore, an aliquot of the labelled cell suspension was sonated for 15 min. (magnetostriction oscillator, model *S*-102*A*, Raytheon Mfg. Co., Waltham, Mass.) and the lysed cell suspension centrifuged for 1 hr. at 16,000*g*. The clear supernatant fluid was diluted 1 : 3, and a 1·0-ml. aliquot used for counting. The counts were corrected for non-specific loss of radioactivity and were used as the value for 100 per cent lysis. This value agreed well with the number of counts found when excess complement and antibody were used to obtain maximum lysis. The values for 100 per cent lysis varied between 71 and 76 per cent of the corrected total radioactivity present.

All further treatment of the data was the same as that described by Kabat and Mayer[4] for the determination of hæmolysin titre in terms of 50 per cent hæmolytic units. The values obtained for percentage lysis (Y) of different amounts of serum or complement were converted to $Y/(1-Y)$ values and the amount of serum versus the corresponding $Y/(1-Y)$ values was plotted on logarithmic paper. The amount of serum required for 50 per cent lysis was estimated from the graph (where $Y/(1-Y) = 1{\cdot}0$). Repeated titrations of the same serum, carried out on different days, were found to agree within 5 per cent.

Table 1. TYPICAL PROTOCOL FOR THE QUANTITATION OF ANTIBODY CYTOLYTIC ACTIVITY

Tube No.	Ml. serum*	Ml. diluent (alb.-V.B.)	Ml. complement (four 50 per cent units)	Ml. 1 per cent cells (^{51}Cr labelled)	Counts/min./1·0 ml. aliquot supernatant	Corrected counts (−7*c*)	Per cent lysis (Y)	$\frac{Y}{1-Y}$
1	1·0	—	1·0	1·0	10,749	8,657	77	3·35
2	0·8	0·2	1·0	1·0	9,735	7,643	68	2·13
3	0·6	0·4	1·0	1·0	6,835	4,743	42	0·724
4	0·5	0·5	1·0	1·0	4,418	2,326	21	0·266
5	0·4	0·6	1·0	1·0	2,982	890	8	0·087
6	0·3	0·7	1·0	1·0	2,424	332	3	0·031
7*c*	—	1·0	1·0	1·0	2,092	—	—	—
†8*c*	—	2·0	—	1·0	17,117	15,025	—	—
‡9*c*	—	2·0	—	1·0	13,282	11,190	—	—

* Initial dilution of serum 1 : 480. † Not centrifuged, total number of counts. ‡ Supernatant from sonated cells, value for 100 per cent lysis.

Antiserum titrations were carried out in the presence of four 50 per cent units of complement. The complement titrations were carried out using optimally sensitized cells[3]. In this particular system, it was found that complement had from twenty-five to thirty 50 per cent cytolytic units per ml. All the components of this system were checked for anti-complementary activity in the sheep cell hæmolytic system and were not found to be anticomplementary in the concentrations used.

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Protective Effect of orally administered S,β-Aminoethylisothiuronium.Br.HBr against X-radiation Death in Mice

S,β-Aminoethylisothiuronium.Br.HBr (AET) is of known value in mice, and given parenterally in an appropriate dose will convert a $LD_{100/30}$ dose of whole-body X-radiation to essentially a $LD_{0/30}$ (ref. 1). The oral administration of AET prior to X-radiation is said to be effective (ref. 2 and Doherty, D. G., personal communication), but no definite results have yet been published. Oral cysteamine has been reported to be effective in both mice and rats with a longer duration of action than intraperitoneal injection[3], but this has been disputed[4].

The outstanding advantage of the oral use of any chemical is, of course, ease of administration. The purpose of this communication is to present data on the oral administration of AET to mice, its toxicity, its value and its duration of action.

Young adult female mice, Walter Reed–Bagg strain, weighing 22–26 gm. were used in this work. The AET used was dissolved in a phosphate buffer of *p*H 7·2. The *p*H was then adjusted to 7–7·5 by the addition of the necessary amount of concentrated sodium hydroxide. The final concentration of AET was always about 100 mgm./c.c. based on the weight of the di-bromide salt. All solutions were prepared just before using.

Experimental animals were given 1,500 mgm./kgm. AET by gastric catheter (volume about 0·3 c.c.) while controls were given the same amount of the buffer solution alone.

At various times after AET administration exposure to 770 r. of whole-body X-radiation was carried out. Each exposure group consisted of experimental and control animals, totalling 20–30 mice. After exposure each group was housed in the same large cage, with standard laboratory food and water always available. Mortality and body-weight of the survivors were determined daily for 30 days.

The source of X-ray was a 250 Maxitron, operating at 250 kVp., 30 m.amp. Inherent filtration was equal to 3·2 mm. beryllium and half-value layer of 1·0 mm. aluminium and 1·0 mm. copper was added. The dose was measured in air in the appropriate mid-position of the animals with a Victoreen chamber. The rate of X-radiation was about 30 r./min. and the target source distance was 70 cm.

Toxicity analysis revealed that doses of AET of 1,200 mgm./kgm. or less were non-lethal to these mice. At 1,500 mgm./kgm. there was a 20 per cent mortality in the next 24 hr. Such a result was also noted in the treated X-radiated animals ; however, all such deaths were discarded from the experimental groups reported. At doses of 1,800 mgm./kgm. or greater there was 100 per cent mortality in 48 hr.

Fig. 1 presents the results of this experiment. A total of seven groups of control mice showed a remarkably similar 30-day mortality. For purposes of statistical comparison the control groups were averaged together.

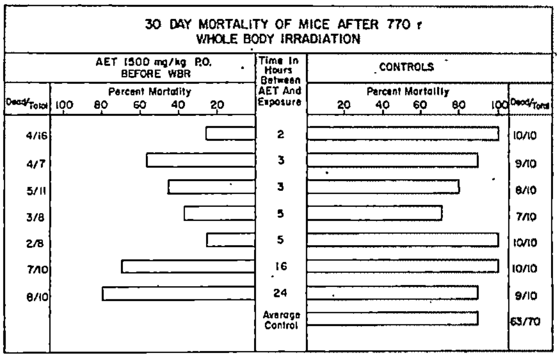

Fig. 1

AET by mouth exerts a significant protective effect at 2, 3 and 5 hr. after ingestion, but this is lost at 16 and 24 hr. Chi-square analysis of the 2 hr., 3 hr. (combined) and 5 hr. (combined) gives a $P > 0·001$ when compared with the control $LD_{90/30}$ in a total of 70 mice. The protection achieved is not so dramatic as when AET is given parenterally.

The loss of body-weight following X-radiation was similar in all groups for the first week. Thereafter, the controls continued to lose weight and eventually died, while the AET-treated mice began to regain weight and by 30 days had generally exceeded their pre-X-radiation body-weight.

AET is as effective at 5 hr. as at 2 hr. after ingestion. Duration of the protective action is unknown, but it is not apparent at 16 hr. Nor is the earliest time for protection following oral administration known from this experiment, except that it exerts its effect at least within 2 hr. Other reports indicate that protective action is apparent within a few minutes of ingestion for AET[3] and cysteamine[4].

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PHYSIOLOGY

Repetitive Hyperpolarizing Response of the Nerve Fibre of Crayfish

The electrical properties of the myelinated nerve fibre in potassium-rich solution were observed by Stämpfli[1], Mueller[2] and Tasaki[3]. Those of the unmyelinated nerve fibre were observed by Segal[4], Tasaki[3] and Moore[5]. When the membrane potential is depolarized by potassium, the potential difference across the membrane produced by the inward current pulse is larger than that produced by the outward one and exhibits a kind of discontinuous increase at a certain current intensity (the current-potential relation is non-linear). The time-course of the potential difference produced by the inward current has two steps. This phenomenon is called 'anodal threshold phenomenon' by Segal[4] and 'hyperpolarizing response' by Tasaki[3]. Tasaki reported that the hyperpolarizing response in the giant axon of the squid was slightly different from that in the myelinated nerve fibre.

In the present work similar experiments were made on the single motor axon (fast closer) in the claw of the crayfish (*Procambarus clarkii*), which was smaller than the giant axon of squid and was 30–50μ in diameter. Our results accorded approximately with those obtained in the nerve fibre of other animals. Responses obtained in the crayfish giant axon sometimes resembled the hyperpolarizing response obtained in the node of Ranvier of the myelinated nerve fibre and sometimes resembled that in the squid giant axon. Besides, they often appeared in the form of repetitive responses which were not observed in the other animals.

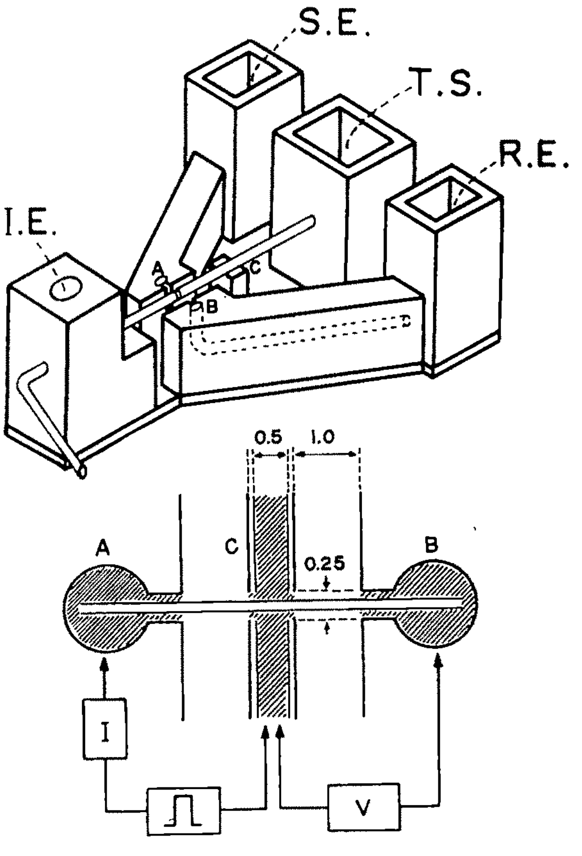

Fig. 1. Arrangement used for recording the membrane potential variation in the crayfish unmyelinated nerve fibre. The portions of the axon in pools *A* and *B* were narcotized with 0·2 per cent cocaine solution. Each pool was insulated with mineral oil

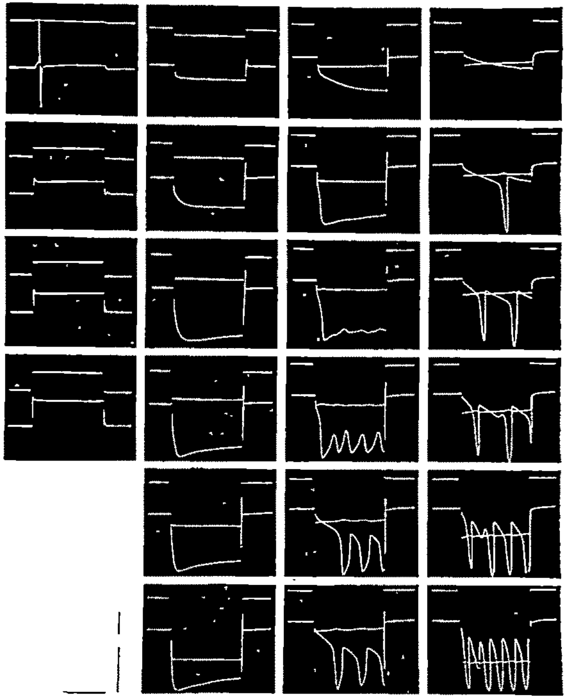

Fig. 2. Simultaneous recording of the membrane current (upper trace) and the membrane potential (lower trace) in a crayfish nerve fibre treated with potassium chloride. The concentration of potassium was increased in the order from left to right. The calibrating bar for current (upper) is 10^{-7} amp., for voltage (lower) is 50 mV. and for time is 30 msec.

A single nerve fibre was mounted in the chamber with three pools as shown in Fig. 1. The stimulators were connected with *A*-pool and *C*-pool and the potential difference between *C*-pool and *B*-pool was recorded. *C*-pool was irrigated with the test solution. The amount of potassium ions in the medium was altered by mixing isotonic potassium chloride solution with van Harreveld solution. Sodium was replaced with potassium, while chloride, calcium and magnesium were kept normal. The portions of the axon in *A*-pool and *B*-pool were narcotized with a 0·2 per cent cocaine solution. Each pool was insulated with mineral oil.

Fig. 2 shows the potential which was produced across the membrane depolarized by potassium when a square current pulse of 50 msec. was applied. In Fig. 2, the upper trace of each photograph shows the applied current and the lower trace shows the potential produced. The concentration of sodium ion was decreased and that of potassium was increased in the order from left to right. The repetitive hyperpolarizing response was observed in the potassium-rich media (the third and fourth columns of Fig. 2). Such response could be produced no matter whether the solution contained a small quantity of sodium or not. The response resembled the ordinary action potential in several respects and had the all-or-none character with a clear threshold, displaying a critical hyperpolarization. As the concentration of potassium was increased, this response became sharper and larger. The amplitude and the duration of the response in isotonic potassium chloride solution were about 80 mV. and about 5 msec. respectively. As the stimulus current was increased, the latency became shorter. The frequency of the repetitive response became higher on increasing the inward current. The repetitive response could last for more than 10 sec., and, when it ceased, the membrane potential reached the potential level lower than the peak of the response by about 20 mV.

The membrane in the potassium-rich media shows the same voltage-current characteristics as the semiconductor. The repetitive response may be produced by a negative resistance property of the membrane as known from semiconductor theory. In other words, the factor which causes the repetitive response may be explained by the spontaneous change between the two stable states of the potassium permeability, one of which is the increased state and the other of which is the decreased state[1,3,4,5].

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Transmission of Swelling Characteristics by Mitochondria of Regenerating Liver

As an outcome of previous work on the fine structural changes of cell particles during induced hepatic carcinogenesis[1-3] and rapid liver growth[3], it has been found that the mitochondria isolated from regenerating rat liver transmit their swelling characteristics to the hepatic mitochondria of the host rats following intraperitoneal injection.

Male Sprague–Dawley rats (average 280 gm.), fed 'Purina chow', were used. The animals were killed 12 hr. after partial hepatectomy, where two-thirds of the liver was removed under light ether anæsthesia. The mitochondrial fractions isolated as described[2] from two animals were pooled, suspended in 3 ml. 0·25 *M* sucrose, and injected intraperitoneally to rats. The controls were injected with mitochondria from intact, non-hepatectomized rats. For the preparation of the mitochondrial membrane (crista) and intra-cristal protein fractions, the mitochondria from two partially hepatectomized rats were pooled, and suspended in 5·5 ml. of distilled water that contained, per litre, 1 ml. of a phosphate buffer (411 gm. dipotassium hydrogen phosphate + 163 gm. potassium dihydrogen phosphate per litre). The suspension was incubated for 40 min., then centrifuged for 12 min. at 25,000*g*. The sedimented membrane fraction was resuspended in 3 ml. of 0·9 per cent sodium chloride solution, while a concentrated sodium chloride solution was added to the decanted supernatant to bring the final concentration of sodium chloride to 0·9 per cent. Both fractions were injected intraperitoneally to separate animals. The swelling of the liver mitochondria of the injected animals was measured at the time-intervals shown in Fig. 1, by the optical density method as described previously[2]. All experiments were carried out in triplicate.

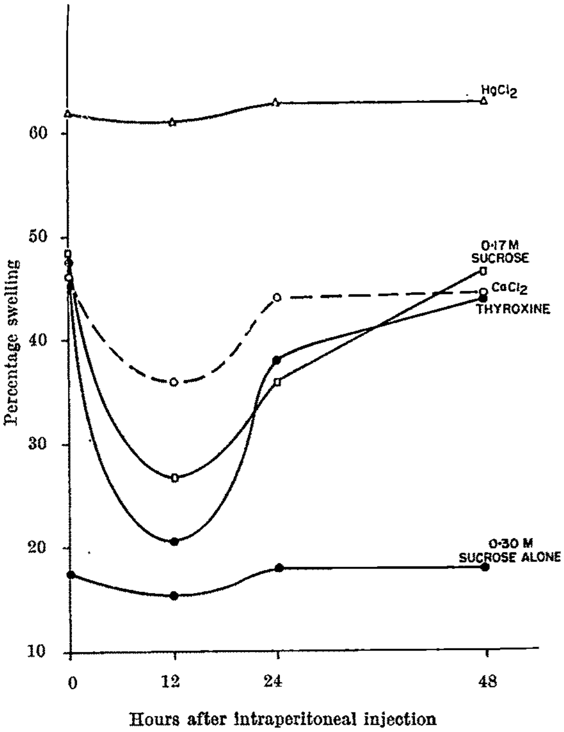

Fig. 1. Effect of intraperitoneally injected liver mitochondria obtained from partially hepatectomized rats on the hepatic mitochondrial swelling of the host rats. The test systems for mitochondrial swelling were 1×10^{-5} *M* thyroxine or mercuric chloride, or 5×10^{-3} *M* calcium chloride in 0·30 *M*, *p*H 7·4 sucrose, or 0·17 *M*, *p*H 7·4 sucrose alone. Each point on the curves corresponds to the swelling at 40 min.

In contrast to the results shown in Fig. 1, the mitochondria isolated from the control rats, which were injected with liver mitochondria from intact, non-hepatectomized animals, exhibited no, or only very slight, change in swelling. In the experiments with the isolated membrane and intra-cristal protein fractions corresponding alterations in mitochondrial swelling were observed in the thyroxine and calcium chloride test systems when the rats were injected with the membrane fraction. However, unlike whole mitochondria, injection of this fraction did not produce changes in the swelling induced by 0·17 *M* sucrose. The intra-cristal protein fraction did not produce noticeable changes in swelling with either of the test systems used.

That rapid tissue growth is associated with low levels of mitochondrial swelling has been shown previously with rat hepatoma[2-4], and with rat liver following partial hepatectomy or intraperitoneal injection of 20-methylcholanthrene[3]. In view of these results, the transmissible decrease of mitochondrial swelling, produced by mitochondria of regenerating liver, found by us correlates with the stimulation of hepatic mitotic rate by injected whole homogenates of rapidly growing tissues[5,6] or isolated microsomes and mitochondria from rapidly dividing cells[7]. Since tissue growth is associated with enhanced rate of protein synthesis which is in turn dependent on the rate of production of adenosine triphosphate, it is not unexpected that growth affects the mitochondrial membrane site of intense oxidative phosphorylation and thus, the characteristics of swelling. Hence, further evidence is provided in support of the correlation between rapid tissue growth and low values of mitochondrial swelling[2,3]. A more detailed study of the reported phenomenon is in progress.

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Incorporation of Ethanol-I-^{14}C into Fatty Acids in the Normal and Alloxan Diabetic Rat

Recent work in this laboratory has shown that, in the mouse, ethanol-1-^{14}C is incorporated *in vivo* into phospholipids, cholesterol, and fatty acids at approximately the same rate and to the same degree as acetate-1-^{14}C (ref. 1). It is well known that in diabetic animals a metabolic block exists in the synthesis of fatty acids from acetate[2]. It was of interest to determine whether ethanol can be used to any greater degree as a source of building blocks for fatty acid synthesis in diabetic animals, or whether a similar metabolic block occurs.

Long-Evans rats of 250–350 gm. maintained on Wayne laboratory chow were used. Alloxan diabetes in rats was produced by the subcutaneous injection of 150 mgm. alloxan per kgm. in citric acid buffer according to Klebanoff and Greenbaum[3]. Rats were considered diabetic when urine sugar was greater than 250 mgm. per cent two weeks after injection of alloxan. Acetate-1-^{14}C and ethanol-1-^{14}C were obtained from Nuclear-Chicago. Solutions of radioactive acetate and ethanol were measured by means of a Packard Tri-Carb scintillation counter.

The incubation medium consisted of 2 or 4 μc. of ethanol-1-^{14}C or acetate-1-^{14}C diluted with 170 μM of the non-radioactive compound in 4 ml. 0·1 M phosphate buffer, *p*H 7·4. Approximately 500 mgm. of liver slices cut by means of a Stadie-Riggs tissue slicer were inserted into 25-ml. Erlenmeyer flasks containing the incubation medium, the flasks were gassed with pure oxygen and incubated for 2 hr. at 37°. At the end of the incubation time the medium was decanted on filter paper and the total lipids in the slices were isolated by the method of Baruch and Chaikoff[4]. The chloroform solutions of lipids were plated on copper planchets and the radioactivity was determined in a Nuclear-Chicago gas-flow counter using the end window.

In agreement with my *in vivo* work in mice, rat liver slices incorporate ethanol and acetate into liver lipids at approximately equal rates and to the same degree (Table 1). In diabetic animals ethanol and acetate incorporation was severely and equally depressed. In the diabetic rats, the rate of ethanol incorporation into liver lipids was 22 per cent of normal, while acetate incorporation was 17 per cent of normal. This small difference in incorporation is not considered significant. The fact that the liver slices from the diabetic animal were able to utilize ethanol and acetate to approximately the same extent in their slow rate of lipid synthesis indicates that the diabetic animal is able to convert ethanol to acetate at an adequate rate and that the metabolic block must occur beyond this point. Recent work elsewhere[5] has suggested that ethanol and acetate do not participate equally in fat metabolism. We infer from these experiments, however, that ethanol and acetate are equivalent as a source of liver lipid synthesis both *in vivo* and *in vitro*.

Table 1. INCORPORATION OF ETHANOL-1-^{14}C AND SODIUM ACETATE-1-^{14}C INTO LIVER LIPIDS IN NORMAL AND DIABETIC RATS

	Ethanol-1-^{14}C c.p.m./gm. liver slice	Acetate-1-^{14}C c.p.m./gm. liver slice
Normal (average of 12 animals)	1,111 ± 90	1,033 ± 14
Alloxan diabetic (average of 6 animals)	251 ± 49	176 ± 61

The incubation flasks contained 170 μM (2 μc.) of the radioactive compound in 4 ml. 0·1 M phosphate buffer *p*H 7·5. Incubation time was 2 hr.

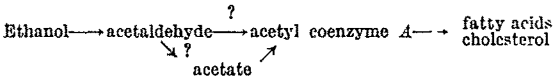
When normal liver slices are presented with either ethanol or acetate as a source of lipid building blocks, acetate is the preferred substrate. Isotopic competition studies show that equimolar amounts of non-radioactive acetate inhibited the incorporation of radioactive ethanol into lipids 75 per cent (Table 2). On the other hand, non-radioactive ethanol did not depress entrance of acetate-1-^{14}C into the lipid-building metabolic sequence more than about 10 per cent. Table 2 also shows that in alloxan diabetic animals a parallel relationship exists.

Table 2. INCORPORATION OF ETHANOL-1-^{14}C AND SODIUM ACETATE-1-^{14}C INTO LIVER LIPIDS ALONE AND IN THE PRESENCE OF EQUIMOLAR AMOUNTS OF NON-RADIOACTIVE ACETATE AND ETHANOL RESPECTIVELY

	Ethanol-1-^{14}C c.p.m./gm. liver slices	Ethanol-1-^{14}C + acetate c.p.m./gm. liver slices	Acetate-1-^{14}C c.p.m./gm. liver slices	Acetate-1-^{14}C + ethanol c.p.m./gm. liver slices
Normal (average of 3 animals)	2,294 ± 480	710 ± 142	1,947 ± 318	1.752 ± 216
Alloxan diabetic (average of 3 animals)	378 ± 16	80 ± 25	227 ± 106	201 ± 21

Conditions are as described for Table 1 except that the medium contained 4 μc. of the radioactive compound and the incubation time was 3 hr.

Whether ethanol and its oxidation product, acetaldehyde, enter the acetate pool as acetate *per se* or directly as an activated 2-carbon fragment has not yet been definitely settled. These alternatives are expressed in the following metabolic sequence :



The enzyme aldehyde dehydrogenase, which probably oxidizes the bulk of the acetaldehyde, yields acetate as the product[6]. My results suggest that ethanol follows the metabolic sequence : acetaldehyde → acetate → activated acetate, since ethanol incorporation into lipids is depressed by additional acetate, while non-radioactive ethanol dilutes the radioactive acetate incorporation not more than 10 per cent. If acetaldehyde were directly converted to acetyl coenzyme *A*, a reciprocal dilution effect between the two radioactive precursors should be observed. It is not likely that the presence of acetate is inhibiting the oxidation of acetaldehyde since this reaction is known to be irreversible. Furthermore, I have shown that acetate does not slow the rate of ethanol oxidation[7]. My results best fit the theory that acetaldehyde from ethanol is converted to free acetate before entering the metabolic pool.

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Effect of Splenectomy on the Resistance of Rats to Histamine Stress

The spleen has been suggested by many investigators as a site of production of hormones or hormone-like substances; interrelationships have been postulated with the pituitary, adrenal, gonad and thyroid glands. The present communication on the relationship of histamine tolerance to splenectomy provides additional evidence for a splenic humoral contribution.

Two hundred and ten female Wistar rats averaging 75 gm. were matched by weight and divided into three groups (*A*, *B* and *C*) representing respectively splenectomized rats, sham-operated animals and normal controls. The splenectomies were completed under open-ether anæsthesia, as described by Farris and Griffith[1]. The splenic pedicle was ligated and cut as close to the hilus as possible. The incisions were closed in two layers, surgical gut being used for the abdominal wall, and silk for the skin surfaces. Sham-operated females were subjected to the same operative procedures except for the surgical removal of the spleen. After four weeks, 60 animals in each group were selected and equal numbers tested for histamine resistance. The histamine challenge was at four dose-levels, 450, 600, 750, 900 mgm./kgm. of body-weight. Identical procedures were utilized for male animals. Finney's method of probit analysis was used to calculate the LD_{50} values and dose-response lines[2].

Table 1 presents the results. Comparisons between the LD_{50} values for groups *A* and *B* indicate that splenectomy induced 5·1 per cent increase in the tolerance of female and 14·9 per cent increase in the tolerance of male rats to histamine stress. In the males, the increase approached statistical significance ($P = 0·13$). In no instance did any of the differences between the sham and unoperated controls attain or approach statistical significance. It is of interest that the increase in tolerance observed in the male was greater than the comparable increase in female rats. This finding suggests the possible existence of a sex difference in splenic function, a suggestion supported by previous reports of sex differences in response to histamine in parathyroidectomized[3] and thymectomized[4] animals.

Table 1. Effects of Splenectomy on the LD_{50} Values of Male and Female Rats

	Group *A*: splenectomized rats	Group *B*: sham-operated rats	Group *C*: normal control rats
I. *Male*			
LD_{50} (mgm./kgm. ± *S.E.*)	754·3 ± 68·2	656·6 ± 63·4	686·6 ± 56·7
Percentage difference between LD_{50} values	*A* : *B* + 14·9 per cent *A* : *C* + 9·9 per cent	*B* : *C* − 4·4 per cent	
P values	*A* : *B*, 0·13 *A* : *C*, 0·30	*B* : *C*, 0·64	
II. *Female*			
LD_{50} (mgm./kgm. ± *S.E.*)	703·8 ± 49·9	669·6 ± 54·1	661·1 ± 55·7
Percentage difference between LD_{50} values	*A* : *B* + 5·1 per cent *A* : *C* + 6·5 per cent	*B* : *C* + 1·3 per cent	
P values	*A* : *B*, 0·48 *A* : *C*, 0·38	*B* : *C*, 0·88	

A : *B*, results of comparisons between results for group *A* and group *B*, *A* : *C*, group *A* and group *C*, and *B* : *C*, group *B* and group *C*, respectively.

That splenectomy tends to increase tolerance to histamine stress is in accord with the protective action against epinephrine-induced pulmonary œdema[5] and toxic doses of 2-thio-5-methyl-uracil[6] induced by splenectomy.

Although the investigation does not offer conclusive evidence concerning a splenic endocrine function, the results provide inferential support for such a hypothesis.

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An Effect of Chlorpromazine on Rat Mitochondrial Membranes

The phenothiazines used in therapy appear to affect membranes in many biological systems. Thus, Greig *et al.*[1], among others, have shown that promethazine, a phenothiazine type of antihistaminic drug, prevents the usual deterioration of red blood cells during storage. This presumably is a membrane effect. The frog gastrocnemius muscle swells when placed in distilled water. This water imbibition can be partially prevented by chlorpromazine[2]. A few *in vivo* experiments may be cited as well. Thus, the metrazol-enhanced passage of dyes into the brain is antagonized by chlorpromazine[3] ; the absorption of sugar from the intestine[4] and drugs from subcutaneous depots[5] are both retarded by chlorpromazine. Close inspection of the entire literature dealing with the actions of chlorpromazine on all types of biological systems revealed that most of the test systems employed contained semipermeable membranes of one type or another (cellular or sub-cellular). For this reason the possibility should be considered that these structures are the primary loci of attack by the drug, while the numerous other reported effects may be secondary.

Prominent among the membranes thought to be affected by chlorpromazine are those of the mitochondrion[6–8]. Roizin *et al.*[8] have presented preliminary evidence that pretreatment of rats with this drug caused changes in the size and structure of brain mitochondria. Bernsohn *et al.*[9] believed that the inhibitory effect of chlorpromazine on the enzyme hexokinase present in rat brain homogenate was an indirect one, since they were unable to demonstrate inhibition of a purified yeast hexokinase with this drug. However, no experiments with the non-particulate mammalian enzyme were performed. Although it is true that in a few isolated instances chlorpromazine does inhibit non-particulate enzyme preparations directly, this does not negate the possibility that the pharmacologically important action of this drug is concerned with changes in biological membranes. Finally, it must be considered that chlorpromazine membrane effects, such as slowing or preventing substrate uptake, or changing enzyme leakage from mitochondria, for example, could mistakenly be interpreted as an inhibition of a particular enzyme under observation.

Since a majority of the papers scrutinized employed either isolated mitochondria or tissue preparations containing mitochondria, an investigation of the effect of chlorpromazine on the permeability of these organelles to water or water-soluble ions was undertaken. It is known that the optical density of a suspension of mitochondria in an aqueous medium is a function of their volume[10]. Swelling results in a lowering of optical density which can easily be followed spectrophotometrically at the arbitrary wave-length of 520 mμ. Such swelling can be induced in both brain and liver mitochondria from Wistar rats by a variety of means, including osmotic dilution, phosphate ions plus osmotic dilution, thyroxin and many detergents, including 'Triton *X*-100'. The effect of chlorpromazine on these different types of mitochondrial swelling is shown in Table 1. Under the conditions of these experiments, chlorpromazine considerably inhibits all the types of swelling, many at low concentrations. Occasionally, effects were noted at concentrations as low as 10^{-6} *M* for brain using 'Triton' as a swelling agent, or 10^{-7} *M* for liver with thyroxin as the swelling agent. The concentrations of chlorpromazine are smaller than those generally necessary to produce changes in enzyme activities or in the level of oxidative phosphorylation.

Table 1. INHIBITION OF MITOCHONDRIAL SWELLING BY CHLORPROMAZINE

Mitochondrial source	No. of exp.	Swelling agent	Molar concentration of chlorpromazine inhibiting mitochondrial swelling 30 per cent
Brain	6	Phosphate + water	5×10^{-4}
	15	'Triton *X*-100' + sucrose	10^{-5}
Liver	6	Water	2×10^{-4}
	6	Phosphate + water	5×10^{-5}
	20	Thyroxin + sucrose	$1–2 \times 10^{-6}$
	6	None	5×10^{-6}*

* Prevention of endogenous swelling when mitochondria were suspended in 0·3 *M* sucrose.

Mitochondria from 50–100 mgm. of rat brain or 12–20 mgm. of rat liver suspended in 0·1–0·2 ml. of 0·25 *M* sucrose were added to 0·25–0·30 *M* sucrose, or water or 0·25 *M* phosphate buffer at *p*H 7·4. Thyroxin when added to liver mitochondria was present at a final concentration of 10^{-5} *M* ; 0·44 ml. of a solution of 0·125 per cent (v/v) 'Triton *X*-100' was usually added to brain mitochondria when needed. The final volume in all cuvettes was 3·0 ml. Mitochondrial swelling caused a fall in optical density which was measured by means of a Beckman Model *DU* spectrophotometer at 520 mμ. The change in optical density was generally followed over a 10-min. period.

These experiments therefore give some support for the idea that chlorpromazine primarily causes a change in membrane permeability.

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Effects of Chlorpromazine and Lysergic Acid Diethylamide on the Rate of Habituation of the Arousal Response

THE responsiveness of an animal to sensory stimulation is dependent to a large extent on past experience as well as the precise conditions to which the animal is subjected. A continually repeated auditory stimulus presented in a stable environment gradually produces indifference whereas the association of a reward or a punishment brings about heightened attentiveness and increases the arousal value of the auditory stimulus[1]. Systemic administration of certain drugs may produce similar effects but for a wider range of stimuli. Blocking of sensory-induced electrocortical arousal and behavioural indifference characterize some of the central effects of chlorpromazine in cats[2]. On the other hand, *d*-lysergic acid diethylamide (LSD 25), administered in small doses, produces increased responsiveness to most sensory stimuli[3]. Chlorpromazine has also been shown to block conditioned avoidance behaviour and markedly decrease the ability of the animal to learn simple positive conditioned responses[4].

It may be that the effect of chlorpromazine on arousal responses and conditioned avoidance behaviour is due not to an all-or-nothing blocking of the responses but to an increased rate of negative learning or habituation. Conversely, LSD 25 may interfere selectively with this latter process, thereby inducing increased responsiveness. The effects of these two drugs have therefore been compared on the rate of habituation of an arousal response evoked by an auditory stimulus, both in terms of the behaviour of the animal and the electrical activity of the cerebral cortex.

Experiments were carried out on five chronic cat preparations carrying permanently implanted cortical recording electrodes. For recording purposes the animals were housed in a double-walled, semi-sound-proofed cubicle fitted with a one-way observation window. Thus the behaviour of the animal could be correlated concomitantly with the electrocortical activity. An auditory stimulus of 1,000 c./sec., obtained from an audio-oscillator, was fed into a loudspeaker fitted in a side wall of the box.

The rate of habituation of the arousal response was found to be dependent on a number of factors such as the intensity, the duration and the rate of presentation of the stimulus. Thus the experimental procedure was standardized and the auditory stimulus was presented at intervals of 5–10 min., depending on whether or not the required depth of sleep was indicated in the electrocorticogram. The duration of the stimulus was kept constant at 8 sec., while the animal was always allowed a period of acclimatization in the cubicle before each experiment was started. Each animal was habituated to the auditory stimulus at a known intensity following an injection of normal saline. When habituation was complete a very loud, brief noise was suddenly presented in an attempt to dishabituate the animal. A period of 2–3 weeks then elapsed before the same procedure was repeated after the injection of the appropriate drug. The experiments were conducted therefore in a control, drug, control sequence in order that some assessment of the amount of retention of habituation from experiment to experiment could be made. The results obtained are illustrated graphically in Fig. 1 where the number of trials required to produce habituation of the electrocortical arousal response has been plotted against the intensity of the 1,000 c./sec. tone, expressed in decibels above an arbitrarily chosen reference-level (0·1 mV. into a 15-ohm impedance).

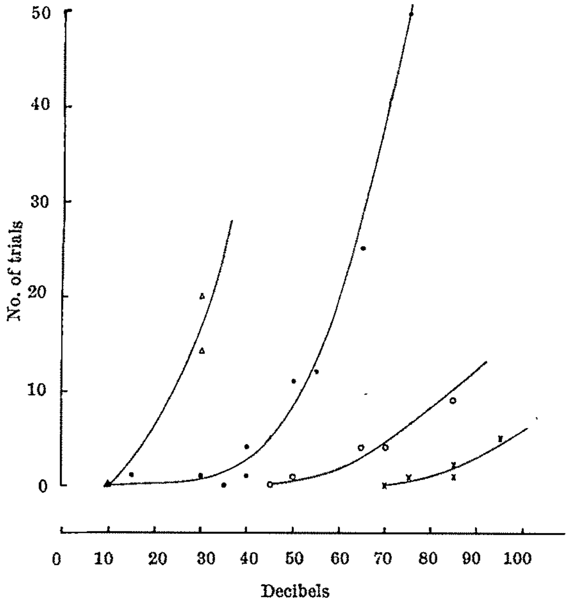

Fig. 1. Effect of chlorpromazine and *d*-lysergic acid diethylamide on the rate of habituation of the electrocortical arousal response evoked by a 1,000 c./sec. pure tone at different levels of intensity. △, 15 μgm./kgm. LSD 25 ; ●, control ; ○, 5 mgm./kgm. chlorpromazine ; ×, 10 mgm./kgm. chlorpromazine. Both drugs injected intraperitoneally

The pattern of habituation of the electrocortical arousal response was similar to that reported by Sharpless and Jasper[6]. Continued presentation of the auditory stimulus at a constant intensity brought about a progressive decrease in the period of electrocortical desynchronization until the electrical pattern of low-amplitude fast activity characteristic of the wakeful state, failed to appear. Behavioural arousal, which was indicated in the initial trials by ear movements, opening of the eyes and usually some head or body movements, or in the later stages by slight eye movements only, disappeared three to four trials before habituation of the electrocortical response.

The intraperitoneal injection of 5 mgm./kgm. or 10 mgm./kgm. of chlorpromazine was followed by an increase in the intensity of stimulation necessary to elicit the arousal response. There was also a marked increase in the rate of habituation. Where previously 45–50 presentations of the auditory stimulus had been required to produce habituation of the electrocortical arousal response, 3–4 or, with the higher dose of the drug, 1–2 trials were now sufficient to produce a similar effect using the same intensity of stimulation (Fig. 1 at 70 db.). Moreover, the period of desynchronization in the electrical record evoked by the tone before habituation took place was much shorter than in the control experiments. Behavioural arousal became habituated even more rapidly than the electrocortical response and when present in the initial trials was not very marked, being indicated by slight ear and eye movements or occasionally, at the higher intensities of stimulation, by the lifting of the head.

In contrast, following the administration of LSD 25 in doses of 10–15 μgm./kgm., intraperitoneally, the animals became increasingly responsive to all modalities of stimulation, so much so that periods of sleep activity in the electrocorticogram were very difficult to obtain. Presentation of the auditory stimulus was followed by abrupt arousal and long-lasting periods of alerting, both electrocortically and behaviourally. Thus, due either to the lack of the appropriate level of sleep activity in the electrical record or to the failure of the response to become habituated during the period when the drug was acting, it was possible to assess with some degree of accuracy the rate of habituation in only two out of a total of ten experiments with this drug (Fig. 1). These experiments demonstrated that even at low intensities of stimulation a greater number of trials was necessary before habituation occurred, indicating that the injection of LSD 25 produced a decrease in the rate of habituation.

In conclusion, it would appear that the changes in responsiveness of animals to sensory stimulation following systemic administration of LSD 25 or chlorpromazine could be brought about by the ability of these drugs to interfere selectively and antipodally with processes concerned in habituation or negative learning. The fact that there is also an increase in the intensity of auditory stimulation necessary to evoke an arousal response following chlorpromazine suggests that the effect of this drug on sensory-induced arousal is not produced solely by the increased rate of negative learning. There appears to be a decrease in the significance or meaning attached to the novel tone by the animal after this drug is injected.

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Biological Activity of Some Humic Fractions isolated by means of Partition Chromatography

Humus has often been said to possess effects on plant growth over and above the value to plants of the plant nutrients it may release during growth.

Attempts have been made by Forsyth[1] and by Trojanowski[2] to extract humus and separate the constituents. Forsyth separated the aqueous extract of humus (fulvic acids), and Trojanowski isolated, by means of chromatography, the compounds of a group of hymatomelanic acids (ulmic acids), which have so far been little investigated. Hymatomelanic acids have now been isolated from various peats and composts using Trojanowski's method.

The crude material was freed from bituminous matter with a mixture of benzene and ethanol. Calcium and iron ions were removed by 0·2 *N* hydrochloric acid. The material was then extracted with ethanol, and the hymatomelanic acids precipitated from the ethanol extract by calcium acetate at *p*H 6·8. The precipitate was washed with 0·5 *N* hydrochloric acid and water, and then dissolved in acetone, the insoluble residue being removed by centrifuging. The acetone solution was transferred to a column of Whatman cellulose powder (30 gm. cellulose in a tube of diameter 3·5 cm.), saturated with acetone/water (20 : 1 vol.), and developed with *n*-butanol saturated with water + acetone (1 + 1 vol.). Under ultra-violet light five fluorescent bands were detected. The bands were eluted successively with acetone and water, and the eluates re-chromatographed on Whatman paper No. 1 in order to check their homogeneity. The paper chromatogram was developed with water/saturated *n*-butanol/acetone/ 0·5 *N* hydrochloric acid (12 : 12 : 1·5 vol.). The R_F values of the spots were 0·53, 0·73, 0·95 (for fractions from low peat), and 0·45, 0·48, 0·53, 0·69, 0·95 (for fractions from compost).

The activity of the fractions was determined by the *Avena* split coleoptile test of Thimann and Schneider[3]. Controls used were : (1) re-distilled water + sodium chloride in the same quantity as used for the preparation of the solutions of hymatomelanic acid fractions, (2) indole-3-acetic acid solution (0·0025 gm./1,000 ml.), (3) solutions prepared from the ash of hymatomelanic acid fractions.

A stimulating influence on growth was found in hymatomelanic acid fractions from low peat with R_F value 0·53 and 0·95 (concentrations of 0·01 gm./100 ml.) and in fractions obtained from compost with R_F values 0·45 (concentration 0·01 gm./100 ml.), R_F 0·53 (concentrations 0·03 gm./100 ml.) and R_F 0·95 (concentration 0·09 gm./100 ml.). The stimulating influence of these fractions is similar qualitatively to that of indole-3-acetic acid. No free indole compounds were found in the fractions examined. Under these conditions unpurified complexes of hymatomelanic acids did not show any physiological activity. The observed effects could not have been caused by the mineral constituents of the humic preparations.

A possible explanation for the findings seems to be that curvatures of this type are due to inherent differences in the response to humic substances of adjacent layers of tissue.

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HÆMATOLOGY

Age and Molecular Weight of Dextrans, their Coating Effects, and their Interaction with Serum Albumin

Dextrans coat blood platelets and red cells[1] and interact with human serum albumin[2]. Recently two new problems have presented themselves. The first is that the interaction of the same sample of dextran with serum albumin appeared to be less after the dextran had been stored at 4° C. for about four years. and that the interaction of 'Synkol' (a Russian dextran of unknown age) was still less. The second is that, while the coating effects of dextrans of varying molecular weight scarcely varies, the interaction of the higher molecular weight dextrans with serum albumin has not been measured.

Effect of age of dextran on coating. Through the kindness of Dr. Norman Wheeler of the Abbott Co., we have been able to obtain two specimens of a dextran with a molecular weight similar to that of 285*W*3*A* of the Commercial Solvents Co. These two samples of dextran, made by the same process, had been stored for 6 and 40 months respectively, and will be referred to as 'new' and 'old' Abbott dextran. Their viscosity relative to that of saline buffered at *p*H 8 and their coating effect on human platelets and red cells are compared in Table 1 with that produced by 'old' dextran 285*W*3*A*. The viscosities of the new and the old Abbott dextrans are virtually identical, and are about 0·97 times that of old dextran 285*W*3*A*. The coating effects of the new and the old Abbott dextrans, determined in a vertical electrophoresis cell and expressed as U/U_0 (see ref. 1), are virtually identical, and almost the same as those of 285*W*3*A*. The coating effects do not seem to depend

Table 1

	η/η_1	Platelets U/U_0 (per cent)	Red cells U/U_0 (per cent)
Abbott new	1·22	1·31 ± 2·7	1·09 ± 1·9
Abbott old	1·23	1·32 ± 3·1	1·09 ± 1·7
285*W*3*A*	1·27	1·30 ± 2·4	1·09 ± 1·5

Table 2

	Mol. wt. × 10^{-3} (mean)	K_1	K_2	K_3	K_4	$-A$	$+D$	α_1	α_2	α_3	α_4
Abbott 'new'	86	44	42	45	42	4	4	0·0018	0·0013	0·0014	0·0017
Abbott 'old'	89	44	42	46	42	4	5	0·0018	0·0013	0·0014	0·0017
C.S.C. No. 6	194	59	26	34	50	8	8	0·0018	0·0008	0·0011	0·0015
C.S.C. No. 7	225	59	32	40	52	7	8	0·0018	0·0010	0·0012	0·0016
C.S.C. No. 8	412	59	33	41	51	8	8	0·0018	0·0010	0·0012	0·0016

on the time during which these dextrans have been stored, at least up to 40 months.

Effect of age of dextran on interaction with serum albumin. The interaction with serum albumin of new and old Abbott dextrans was compared by the micro-Tiselius method already described[2]. The highest concentration of serum albumin (Cohn's Fraction V, kindly supplied to us by the American Red Cross) was 2 per cent, and the highest concentration of dextran 1·5 per cent; the *p*H of the systems was 8·55, using Michaelis buffer. (The interaction of dextran 285*W*3*A* with serum albumin is the same between *p*H 6·6 and 9·6 in Michaelis buffered systems, and is the same at *p*H 8·6 in Michaelis and Dole buffered systems.)

The first two rows of Table 2 show the results obtained for new and old Abbott dextrans. Here K_1, K_2, K_3 and K_4 are the constants (slopes) of the four straight lines, passing through the origin, for albumin, dextran, dextran in the serum albumin–dextran mixture, and albumin in the serum albumin–dextran mixture, while α_1, α_2, α_3 and α_4 are the specific refractive increments for the same four lines. The interaction of these two Abbott dextrans with human serum albumin are substantially the same, although one had been stored for 6 months and the other for 40 months. The values $-A$ and $+D$ may indicate the extent of transfer of albumin to dextran when interaction produces a new species, but, except for saying that the formation of the new species always results in a transfer of albumin to dextran, it is not safe to be more definite.

Effect of molecular weight on interaction with serum albumin. Experiments on coating of human platelets and red cells have not shown any dependence on the molecular weight of Commercial Solvents Co. dextrans of mol. wt. 77,100–412,000 [1]. Samples of these dextrans, referred to as Nos. 6, 7 and 8, have been kept for 4 years at 4° C. in the solid state; they can each be made up as a 6 per cent solution in 1 per cent sodium chloride, dialysed against large volumes of Michaelis buffer at *p*H 8·55, and allowed to interact with 2 per cent serum albumin. This interaction is measured as already described[2].

The results are shown in rows 3, 4 and 5 of Table 2, K_1, etc., α_1, etc., and $-A$ and $+D$ having the same meaning as described for rows 1 and 2. The values suggest that there is a more extensive interaction when the molecular weight of the dextran is high, but the computations are difficult in the best of circumstances, and their reliability is lessened by the solutions of the higher dextrans being slightly opalescent; this makes the spikes slightly fuzzy, and reduces the accuracy with which the areas under them can be measured.

The coating of human red cells and platelets thus seems to be the same for dextrans of molecular weight 86,000–412,000. Low molecular weight dextrans (mol. wt. 86,000) interact to the same extent with human serum albumin whether they have been stored for 6 months or 40 months. Dextrans of high molecular weight appear to interact with human serum albumin to a greater extent than do those of low molecular weight, but the differences are small, and may be due to technical difficulties.

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PATHOLOGY

Leukæmogenesis by Urethan in New-born Swiss Mice

AFTER earlier evidence that urethan induces or enhances the formation of pulmonary adenomas in rodents[1,2], more recent reports suggest a multipotential carcinogenic activity of this chemical. Salaman and Roe[3] and Graffi *et al.*[4] first demonstrated that urethan initiates skin carcinogenesis although it does not induce skin tumours when administered alone. Tannenbaum and Silverstone[5], by long-term application of urethan to the skin of inbred mice, observed not only lung adenomas but also tumours of the mammary gland, interscapular fat, and lacrimal glands. Kawamoto *et al.*[6] reported the augmenting influence of urethan on leukæmogenesis induced in low-leukæmic strains of mice by X-rays, œstrogenic hormone or methylcholanthrene. Berenblum and Trainin[7] showed that urethan treatment following X-irradiation results in a striking increase of leukæmogenesis in *C57BL* mice.

So far as the induction of leukæmia is concerned, the enhancing property of urethan is considered by these investigators as a 'co-leukæmogenic' effect, since it is generally accepted that urethan by itself is not leukæmogenic. The influence of urethan on leukæmogenesis, however, has not yet been studied in new-born mice, although there is good evidence that new-born animals are particularly susceptible to viral or chemical leukæmogenic agents[8,9].

In the present work new-born Swiss mice, less than 24 hr. old, were injected subcutaneously with a single dose of 0·05 ml. of a 4 per cent solution of urethan in distilled water. The Swiss mice were originally obtained from the Arsal Co., Rome. The incidence of spontaneous leukæmia in these animals, as controlled on several hundreds of old mice bred at random in this laboratory, is less than 1 per cent.

The results of the present experiment, which refer to a 6-months period of observation, were as follows: thirteen out of the 60 male and female mice given urethan at birth (an incidence of 21·6 per cent), developed malignant lymphomas between 73 and 157 days of age. The average age for onset of the disease was 107 days. All the animals showed striking enlargement of the thymus, which filled almost completely the chest cavity. In eight mice there was also marked involvement of superficial and deep lymph nodes as well as spleen. In addition, multiple lung adenomas were observed in all cases. Histologically, the lymphomatous lesions were of the lymphoblastic–lymphocytic type. Infiltrations of heart, lungs, liver, kidneys, gonads and bone marrow were found microscopically. In the peripheral blood there was in some cases a remarkable lymphocytosis with many immature cells of the lymphoid series, although the total number of leukocytes did not show abnormalities. In a single case, however, the leukocyte count at the final stage of the disease was about 300,000 per mm.^{3}.

From the results reported here, it would appear that urethan, *per se*, has the property of inducing leukæmia when a single dose is injected into new-born animals. The occurrence of leukæmia following the administration of an agent, such as urethan, which has been considered hitherto free from leukæmogenic activity, is probably to be regarded as dependent on factors inherent to the particular stage of development of the animals at birth[9,10].

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Influence of a Liver Fraction on the Phagocytic Activity of the Reticulo-Endothelial System in Mice

The bacterial pyrogens (lipopolysaccharides) are reported to have a marked influence on the resistance of experimental animals to bacterial challenge[1]. The reaction follows a particular pattern : during the first few hours after injection there is a drop in the resistance to infection, followed by an increase which can be quite considerable. Halpern *et al.*[2] have shown that the injection of lipopolysaccharides in mice is followed by increased phagocytic activity of the reticulo-endothelial system. On the other hand, when increased phagocytic activity is achieved by some other procedure, namely, immunization with *BCG*, the lethal effect of bacterial endotoxins is greatly enhanced[3]. The explanation for this may be found in the fact that a stimulated reticulo-endothelial system will have a greater ability to phagocytize the endotoxins without the concordant ability to neutralize their toxic effect on the organism. After finding that a particular leucocytosis-promoting liver fraction had the properties of stimulating the resistance against bacterial challenge in mice, as well as in human beings, we found it of great interest to investigate whether this fraction would have some influence on the phagocytic activity in mice.

Table 1.- Effect of Liver Fractions on the Phagocytic Activity of the Reticulo-endothelial System

	No. of animals	Phagocytic index *K*	*W*/*WLS* *	Corrected phagocytic index
Experiment *A* :		Liver fraction I	0·2 mgm./mouse	6 hr. before testing
Treated	5	0·025	16·9	4·9
Controls	5	0·025	16·35	4·7
Experiment *B* :		Liver fraction I	0·2 mgm./mouse	24 hr. before testing
Treated	5	0·030	14·1	4·3
Controls	5	0·020	14·8	4·0
Experiment *C* :		Liver fraction I	0·4 mgm./mouse	24 hr. before testing
Treated	5	0·027	14·1	4·2
Controls	5	0·020	14·8	4·0
Experiment *D* :		Liver fraction I	0·4 mgm./mouse	48 hr. before testing
Treated	5	0·021	13·7	3·7
Controls	3	0·027	15·3	4·5
Experiment *E* :		Liver fraction II	0·4 mgm./mouse	24 hr. before testing
Treated	5	0·022	13·0	4·5
Controls	3	0·026	13·7	4·0

All animals tested with 16 mgm. carbon/100 gm. mice intravenously.
All liver fractions were given intraperitoneally.
* Ratio, body-weight to weight of liver and spleen.

Table 2. Effect of Liver Fractions (L.F.) on the Experimental Infection by *Salmonella Danysz* in Mice

Infection *	Substance	Dose intraperitoneal route	Time of challenge (hr.)	Mean survival time (days)
1,000 germs of *Salmonella Danysz* (intravenously)	Control	—	6	4·4
	L.F. I	0·20 ml./20 gm.	6	5·0
700,000 germs of *Salmonella Danysz* (intraperitoneally)	Control	—	6	3·55
	L.F. I	0·20 ml./20 gm.	6	4·1
	L.F. I	2 × 0·20 ml./20 gm.	first injection, after 6 hr. ; second injection, after 48 hr.	4·1

* 10 mice/group.

The liver fractions used are designated fractions I and II and were administered in doses of 0·2 mgm. dry substance per animal. The substance was dissolved in twice-distilled, pyrogen-free water and injected intraperitoneally. The control mice received saline injections. The phagocytic function of the reticulo-endothelial system was measured by the kinetics of blood clearance of 16 mgm./100 gm. mice of colloidal carbon (C11/1431*a*) according to the method described previously (ref. 3 and unpublished work by Dedichen, J., Laland, P., and Laland S.). The test was carried out 6, 24 and 48 hr. after the administration of the liver fraction. The results are shown in Tables 1 and 2. Stimulation of phagocytic activity could not be established by the method used.

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Abnormalities in the Fine Structure of the Neuromuscular Junction in Patients with Myasthenia Gravis

In a recent communication, Bickerstaff *et al.*[1] were unable to demonstrate differences between the synaptic regions of myasthenic and non-myasthenic patients when muscle biopsies were examined by electron microscopy.

Although a detailed study of the fine structure changes in several biopsied cases of myasthenia gravis is being prepared for future publication, we would like to report at this time that definite alterations in both the presynaptic and postsynaptic regions of the neuromuscular junctions do occur in patients with myasthenia gravis.

Muscle biopsies were obtained from intercostal muscles and peripheral muscles of myasthenic patients without recourse to methylene blue staining or electrical stimulation and recording. Normal human endplates for examination were obtained from children and adults subjected to thoracotomy for repair of congenital or acquired cardiac defects.

Pieces of muscle obtained at the time of primary muscle incision were placed in buffered (pH 7·35) 4 per cent formol-sucrose fixative[2] for 15–20 min., washed and then put buffered (pH 7·35) into 1 per cent osmium tetroxide for 15 min. The blocks of muscle were rapidly dehydrated in graded ethanol and embedded in mixtures of 30 per cent methyl methacrylate and 70 per cent butyl methacrylate or 'Epon 812' (Shell Chemicals, New York). Thick sections were examined by phase microscopy and the blocks were trimmed to contain selected endplate areas. Silver sections were studied in an RCA *EMU 3D* electron microscope.

Two kinds of abnormalities were observed in the myasthenic neuromuscular junctions : (*a*) focal areas of decreased electron density in the normally electron-dense portion of the sarcolemmal membrane within the secondary synaptic clefts ; (*b*) extensive disorganization of endplate structure characterized by shrunken axon filaments, decreased numbers of secondary synaptic clefts, widening and clubbing of secondary synaptic clefts, and decreased numbers of mitochondria in the sole plate area.

Figs. 1 and 2 illustrate the appearance of a normal human neuromuscular junction. The axon terminal filament (*A*) and overlying Schwann cell (*S*) are separated by the primary synaptic cleft (*PSC*) from the elaborately infolded sarcolemma which forms the secondary synaptic clefts (*SSC*). A mitochondrion in the axon filament is present at *M*. Fig. 2 shows a drawing illustrating the relationships of these structures and the finely granular 'basement membrane' (*BM*) within the synaptic clefts. Figs. 3 and 4 illustrate disorganization of the secondary synaptic clefts and the moth-eaten appearance of the sarcolemma in the secondary synaptic clefts of a myasthenic neuromuscular junction. Figs. 5 and 6 illustrate at greater magnification the clubbing of the secondary synaptic clefts and the focal loss of material in the sarcolemma within the clefts.

In many of the endplates examined, abnormalities in the layered 'basement membrane' within the synaptic clefts were observed. Fig. 3 shows a single layer of 'basement membrane' in most areas whereas Fig. 5 shows loss of layered 'basement membrane' in most areas.

Pore-like defects in the axolemma described by Bickerstaff *et al.*[1] were seen in normal neuromuscular junctions as reported by others[3].

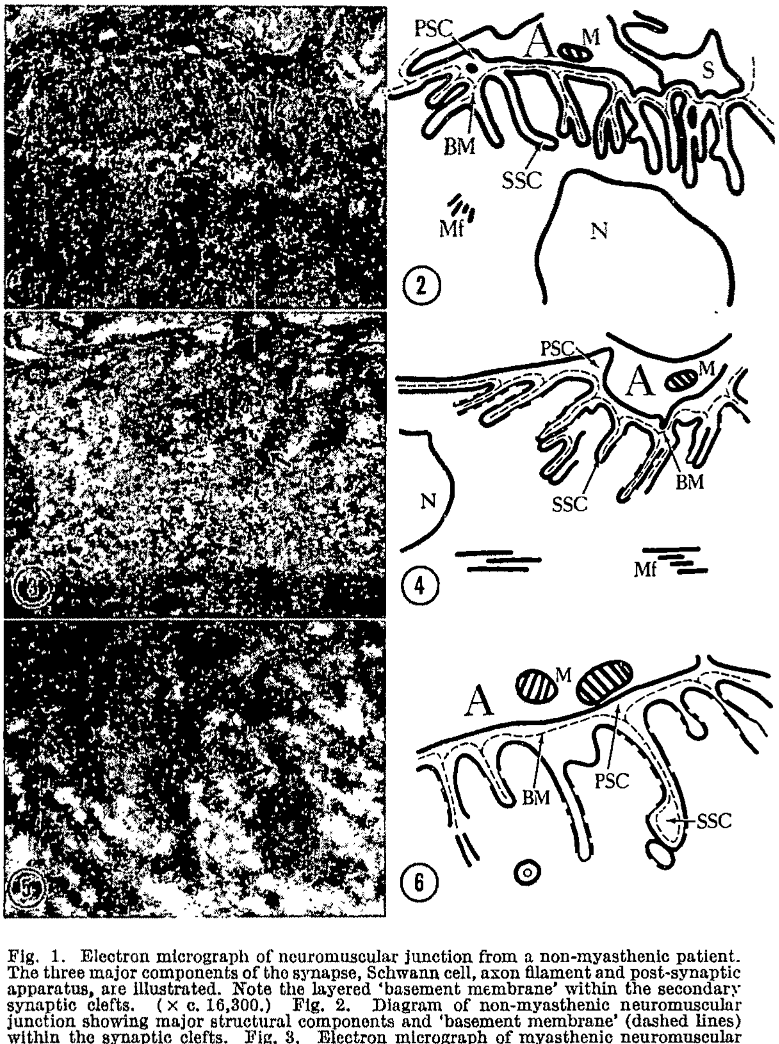

Fig. 1. Electron micrograph of neuromuscular junction from a non-myasthenic patient. The three major components of the synapse, Schwann cell, axon filament and post-synaptic apparatus, are illustrated. Note the layered 'basement membrane' within the secondary synaptic clefts. (× c. 16,300.) Fig. 2. Diagram of non-myasthenic neuromuscular junction showing major structural components and 'basement membrane' (dashed lines) within the synaptic clefts. Fig. 3. Electron micrograph of myasthenic neuromuscular junction showing disorganization of the synaptic clefts and focal areas of thinning and disruption of the sarcolemma within the secondary synaptic clefts (marked with arrow). (× *c.* 16,000). Fig. 4. Diagram of myasthenic neuromuscular junction prepared from Fig. 3 showing the changes illustrated in Fig. 3. Fig. 5. Electron micrograph of myasthenic neuromuscular junction showing swelling and clubbing of secondary synaptic clefts, moth-eaten appearance of the sarcolemma within the secondary clefts and loss of layering of the 'basement membrane' within the secondary clefts. (× *c.* 24,000.) Fig. 6. Diagram of myasthenic neuromuscular junction prepared from Fig. 5 showing the changes illustrated in Fig. 5

A, Axon filament ; *BM*, 'basement membrane' ; *M*, mitochondria ; *Mf*, muscle filaments ; *N*, nucleus ; *PSC*, primary synaptic cleft ; *S*, Schwann cell ; *SSC*, secondary synaptic cleft

Although it is premature to attempt to correlate the abnormalities observed in the fine structure of myasthenic neuromuscular junctions with the physiological abnormality in these patients, our investigations indicate that definite morphological alterations occur which may be related to the functional disability.

We wish to thank Dr. Kermit Osserman for making these biopsies available for examination.

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BIOLOGY

Variation in the Secondary Sex-Ratio of the Grey Seal *Halichoerus grypus* (Fab.) during the Breeding Season

Hewer[1] has suggested that the secondary sex-ratio (that is, the sex-ratio at birth) of the grey seal is probably close to equality. Since this publication, considerably more data have been collected on the grey seal colony on the Farne Islands, Northumberland, by members of the Natural History Society of Northumberland, Durham and Newcastle upon Tyne. Of 1,433 calves which were tagged, sexed and aged between 1952 and 1959 inclusive, 731 were males and the remaining 702 females; a result which does not differ significantly from a 1 : 1 sex-ratio and which confirms Hewer's findings.

Despite the very nearly equal sex-ratio obtained, distinct differences were found when the sex-ratio was examined in relation to the time of the breeding season. Coulson and Hickling[2] have shown that there is little annual variation in the breeding season of the Farne Island colony of grey seals, and it is therefore reasonable to group data collected over a number of years into the same fortnightly periods. As is shown in Table 1, there is a distinct tendency for the sex-ratio to change from a preponderance of males at the beginning of the breeding season (in late October) to a markedly higher proportion of females at the end of the breeding season ($\chi^2 = 8\cdot56$, 3 d.f., $P < 0\cdot05 > 0\cdot01$). This result could be produced by the male calves being born, on average, earlier than the female calves. This, in fact, does occur but the difference is small, the average difference being less than 3 days.

Table 1. Sex-Ratio of Grey Seal Calves on the Farne Islands according to their Date of Birth

Year	Date of birth							
	Oct. 14–27		Oct. 28–Nov. 10		Nov. 11–24		After Nov. 24	
	♂	♀	♂	♀	♂	♀	♂	♀
1952	8	4	16	18	12	8	8	8
1953	12	8	11	16	5	10	3	8
1955	5	8	43	42	20	25	9	14
1956	7	6	43	47	47	47	24	32
1957	36	22	114	96	67	60	13	18
1958	1	1	57	38	45	38	11	10
1959	2	2	69	61	34	41	9	14
Total	71	51	353	318	230	229	77	104
Sex-ratio	100 : 71·8		100 : 90·1		100 : 99·6		100 : 135·1	

While seasonal differences in the sex-ratio of domestic and laboratory mammals have been recorded by Parkes[3], this type of difference in the sex-ratio has not been recorded before in the Phocidae.

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Dissociation of Isohæmagglutinin Formation and Tolerance of Skin Grafts in Chicks

The administration of homologous whole blood into the embryo or new-born animal can produce either tolerance of skin grafts[1] or lead to the suppression of the isohæmagglutinin formation[2]. The causal relationship between these two types of immunological areactivity, however, remains obscure. It could be due to the fact that an identical antigenic stimulus is needed for agglutinin formation and transplantation immunity. There is evidence that red blood cells and some other tissue cells have some antigens in common. After the transplantation of tumours in mice and rats[3] and after the transplantation of skin in mice, rabbits and chicks[4], agglutinin formation was revealed even in those instances where the participation of red blood cells transferred in the graft could be ruled out. On the other hand, at least in mammals, red blood cells alone were not able to produce immunity against skin grafts[5].

In the present experiment, we investigated the formation of isohæmagglutinins in 13 chicks tolerating skin grafts after previous immunization with blood of the donor of the graft. Tolerance was produced in six individuals by mutual application of the skin graft immediately after hatching, in seven individuals by a similar skin transplantation with simultaneous administration of 0·5 ml. of heparinized blood of the donor. At the age of 6–12 weeks the tolerant chicks with the feathered graft, which had taken, were immunized with six 1-ml. doses of fresh citrated blood of the donor, administered intravenously. Plasma specimens for estimating the agglutinin titres were withdrawn in three-day intervals for two weeks after immunization was started.

In 10 from a total of 13 chicks tolerating the skin graft on the sixth to tenth day after the beginning of immunization, the formation of isohæmagglutinins against red blood cells of the donor of the graft and blood was detected (Table 1). The antibody response was relatively uniform, the maximum titres being 1/8–1/16. The grafts remained even after immunization in most chicks tolerating the graft for at least six weeks without any signs of destruction. Only in two animals they disintegrated between the fourth and sixth week after immunization. The immune response following the immunization by blood of the donor thus did not influence the fate of the tolerated homografts. Agglutinins developed in all animals where the tolerance was produced by the graft alone.

Table 1

Tolerance induced on the first day after hatching by :	No. of animals	Maximal titres of agglutinins after immunization	No. of animals with feathered grafts more than 6 weeks after immunization
Skin grafting	6	16, 16, 16, 16, 8, 8	4
Skin grafting and whole blood	7	16, 16, 8, 8, 0, 0, 0	7

Two grafts were destroyed between the fourth and sixth weeks after immunization. Three tolerant animals did not produce agglutinins even after repeated immunization.

but only in 4 out of 7 chicks where the tolerance was produced not only by the graft but also by the administration of blood on the first day after hatching. The remaining three tolerant animals did not develop any agglutinins, even after repeated immunization. Serological evidence was provided that the erythrocytes of the donors differed as to their antigens from those of their tolerant partners. These three animals not only tolerated grafts but also the red blood cells which were administered to them immediately after hatching.

From the results it appears that the cells of the skin graft alone are not capable of producing tolerance for red blood cell antigens. This is the reciprocal picture of the former finding that erythrocytes alone, administered during the adaptive period, are unable to produce tolerance of the skin graft in chicks[6]. If we can assume that this dissociation is caused by the antigen difference of red blood cells and tissue cells, then it can be also concluded from our results that some erythrocyte antigens are absent in tissue cells.

It cannot be ruled out, however, that the dissociation of both types of tolerance could be caused by factors other than antigen differences between erythrocytes and the cells of the skin graft. It is, for example, possible that the transplantation alone on the first day after hatching produces in the chicks some type of a local immunological tolerance. Finally, the temporarily tolerated skin graft could survive further also on grounds other than the persistence of the state of specific immunological activity of the recipient.

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Inhibition of the Development of a Marine Sessile Community

BETWEEN October 1958 and September 1959 two panels of 'Tuffnol', each 2 ft. square, were suspended on either side of a raft at Port Royal, Jamaica. The purpose of these panels was to collect data on the settlement of an ascidian, *Ascidia nigra*. Each panel was examined at 2-monthly intervals, the two panels being examined on alternate months. When a panel had been examined it was cleaned before replacement on the raft. In this way a fresh settlement surface was provided each month. In the course of the two months in which a panel was exposed a dense growth of sessile animals always developed on it, usually dominated by *Balanus amphitrite* and *Didemnum maculosum*, with smaller numbers of other ascidians (*A. nigra*, *Diplosoma macdonaldi*, *Symplegma viride*, *Polyclinum constellatum*), serpulid worms, lamellibranchs, bryozoans and the alga *Enteromorpha sp.*

On October 1, 1959, it was necessary to find an alternative place to hang the panels, and they were moved to another raft close which already had hanging from it six panels that had been in the water continuously for 26 months. These latter panels were dominated by a community of sponges of several species, sea anemones (*Aiptasia tagetes*) and ophiurans (*Ophiothrix angulata*) with an associated fauna of crustaceans, polychæte worms, and molluscs. There were very few ascidians in these communities.

The panel which was raised and examined on November 1, 1959, had a fairly normal community on it ; but it should be noted that this would have developed from larvæ settling before the panels were shifted. The panel examined on December 1 had been in the new position for the whole of the 2 months of exposure, and had a completely different appearance from anything hitherto seen. Considerable portions of the panel had not been colonized at all, and elsewhere there were only small numbers of barnacles and serpulids, a few sponge colonies, a few colonies of the ascidian *Botryllus planus* and 16 specimens of *A. nigra*. On January 4, when the other panel was again examined after 2-months exposure at the new site, the effect was more marked. Barnacles and serpulids were almost entirely absent from this panel, there were no *A. nigra*, only one small colony of *D. maculosum*, and along one margin of the panel there were several oysters (*Isognomon alatus*). Otherwise small thin sponge colonies and a few filaments of algæ were the only noticeable colonisers.

The reduction in colonization was not due to a reduction in available larvæ or to any physical change in the environment such as temperature or salinity changes in the water. This is amply demonstrated by the fact that on a similar raft, moored 20 ft. from this one, normal colonization occurred. This other raft had eight clean panels attached to it on October 1, and by December 1 they were all covered by a *Balanus-Didemnum* community with all the usual associated animals. Only four of these panels were on the same level in the water as the test panels on the other raft : these had an average of 28 *A. nigra* on each.

Four of the above eight panels were removed on December 1 and replaced by clean panels. By January 4 the latter had developed a normal *Balanus-Didemnum* community, and the two panels on the level of the test panels had 48 and 51 *A. nigra* respectively. Observations could not be continued into 1960.

It is clear from the foregoing observations that development of a normal sessile community was inhibited on the two panels inserted in among the mature sponge-anemone-ophiuran community, and it is also clear that this was a purely local effect not observed 20 ft. away. In every respect other than a small difference in panel size and the presence of the sponge community the two environments seemed identical. Each raft was the same size and in the same depth of water and the arrangement of the panels on them was the same in each case. The inhibition was not due to any ageing effect of the panels as the same panels developed normal communities at a later stage when removed from the sponge community. The only possible explanation is that the mature sponge-anemone-ophiuran community actively inhibited the development of a primary community on the clean panels. It is not possible at present to say what form this inhibition takes: it could either prevent larval settlement or prevent survival and growth of young sessile stages. The absence of large barnacle settlements suggests the former possibility as dead shells would probably persist if metamorphosis had occurred. Nor is it possible to say what is its cause, but it is probably an attribute of the sponges rather than other members of the mature community. The high filtration-rate of a sponge community could deplete the surrounding water of food for the young primary colonizers, or the secretion of mucus or chemical toxin might kill the young stages.

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The Deepest Recorded Fishes

THE number of fishes from the hadal zone (in depths between about 6,000 and 11,000 m. in the deep-sea trenches) is very limited. The *Princesse-Alice* collected *Bassogigas profundissimus* (Roule) at 6,035 m. in the east Atlantic Ocean[1], the *Galathea* collected a species of Liparidae (Kermadec Trench, 6,660–6,770 m.) and *Bassogigas* sp. (the Sunda Trench, 7,160 m.)[2], and the *Vitjaz* collected *Careproctus* (*Pseudoliparis*) *amblystomopsis* Andriashev and another species of that genus from the Kurile-Kamtchatka Trench (7,210–7,230 m.) and from the Japan Trench (6,156 and 7,587 m.)[3–5]. Thus, the last-named depth is the hitherto greatest known depth from which any fish has been brought up.

During the truly remarkable dive of the U.S. Navy bathyscaphe, the *Trieste*, in the Mariana Trench in January 1960, the vessel remained on the bottom for 20 min. at a (corrected) depth of 35,800 ft. (10,912 m.) The only living creatures observed by the two men on board, Engineer Jacques Piccard and Lieut. Don Walsh, were a "shrimp" and an animal which Piccard[6] described as "a true fish", being "apparently of the sole family, about a foot long and half as wide. . . . [It] moved away from us, swimming half in the bottom ooze, and disappeared . . ." (p. 235). It was "tentatively described as *Chascanopsetta lugubris*" (p. 230). Walsh[7] mentioned it as a "flat-fish" which was "white and about a foot long".

There are several reasons for considering these statements as being somewhat dubious. First, flatfishes are commonly known as a primarily littoral order with a number of bathyal, but no single truly abyssal species (Chabanaud's record[8] of *Bathysolea profundicola* (Vaillant) having been captured between 2,000 and 4,000 m. depth is erroneous, the species being known only from between 250 and 1,290 m.). The only two species which penetrate beyond 1,500 m. are *Citharichthys dinoceros* Goode and Bean (201–1,746 m.) and *Poecilopsetta beani* (Goode) (203–1,639 m.), both from the West Indies. The deepest recorded flatfish from the *Galathea* Expedition was from 750 m. depth[9], and no flatfish was collected in or close to any of the trenches investigated. *Chascanopsetta lugubris* Alcock was previously recorded only between depths of 220 and 977 m.

Secondly, it seems very probable that certain groups of the trench fauna have a distinct lower limit. Decapod crustaceans and bryozoans are certainly not able to penetrate into the hadal zone and other important marine groups such as cirripeds, asterids, echinids, tunicates, and fishes have not been recorded at depths exceeding some 7,000–7,500 m.[10]. It is true that the number of trawlings deeper than 7,500 m. is limited to (at least) 22, but the majority of these catches have constantly yielded representatives of the really significant, hadal animals, such as actinians, polychaetes, isopods, amphipods, prosobranchs, lamellibranchs, and holothurians. Negative records are naturally of less value, but should be taken into account also.

Thirdly, it seems to appear from most of the observations made from bathyscaphes that even zoologists have great difficulties in recognizing the creatures seen through the porthole. In the case in point the animal was observed "swimming half in the bottom ooze". If it was not a fish, its remarkable size would indicate that it was probably some kind of holothurian, for example perhaps related to the bathypelagic, cushion-shaped *Galatheathuria aspera* (Theel). Hansen and Madsen[11] found the gut of this extraordinary animal to be partly filled with remains of pelagic forms and with various bottom foraminiferans and sponge spicules. The animal is almost 1 ft. long and oval in outline.

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A New Intermediate Host of Schistosome Trematodes from New South Wales

RECENTLY schistosome larvæ have been discovered in the snail *Siphonaria denticulata* Quoy and Gaimard. Cercariæ from infected snails cause a typical schistosome dermatitis in man.

Schistosome dermatitis occurs along parts of the coast of New South Wales. Infections usually occur in sandy estuarine lakes where the snail *Velacumantus australis* (= *Pyrazus australis*) harbours the schistosome, *Austrobilharzia terrigalensis* Johnston[1]. However, cases of schistosome dermatitis have occasionally been reported from marine rock pools where no *Velacumantus* snails occur. An examination of a number of different species of snails from such places showed that *Siphonaria denticulata* harboured schistosome cercariæ. A typical schistosome dermatitis followed exposure to these cercariæ.

An examination of 100 *Siphonaria* from part of a rock platform at Cape Banks, Sydney, showed an infection-rate of 35 per cent. At low tide this area is often crowded with the gull *Larus novae-hollandiae.*

Twenty-five budgerigars (*Melopsittacus undulatus*) were exposed to naturally emerged cercariæ, or cercariæ from crushed snails. Immature schistosome worms were recovered from the liver or lungs of five birds. The worms appear to belong to the sub-family Bilharziellinae.

It is interesting to note that the host snail, *Siphonaria,* belongs to a pulmonate family (the Siphonariidae). Members of this family bear a superficial resemblance to limpets, but may be distinguished from them by a groove which runs down inside the right-hand side of the shell. Members of the Siphonariidae are normally found in the upper littoral zone of rocky shores. Their distribution is almost world wide, but they are especially common around the shores of the Pacific and Indian Oceans[2].

Because the Siphonariidae have such a wide geographical distribution, and their normal habitat is often the resting place of birds of the seashore, one might expect them to be the hosts of larval schistosomes in many other places.

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Transmission of Gall-Diseases of Cacao, Mango, and Pigeon Pea

'CUSHION-GALL' disease of cacao is widely distributed throughout South America[1] and has been reported also from Ghana[2] and Nigeria and Ceylon[3]. The disease has been found in most old cacao plantings in British Guiana, in the 'green-point' form, and was recorded and described by A. W. Bartlett[4] as long ago as 1905, although his report was only rediscovered in 1959. The presence of the 'flowery' type has yet to be confirmed.

Hutchins[2] has transmitted the disease successfully in Costa Rica by tissue implantation, while workers in Ghana (Tinsley, T. W., personal communication, November 1959) have transmitted a similar disease using the single cotyledon technique.

In the present work the 'green-point' type gall of cacao was transmitted by using the half-bean technique: galls were produced three weeks to a month after inoculation. Washings from the surface of galls, or extracts of macerated gall tissue transmitted at a rate of 1/7. The germination-rate of half-beans treated with gall inoculum was markedly lower than that of controls treated with distilled water.

Galls similar to the 'green-point' form of cacao have been found on mango and pigeon pea. Distilled water washings, and extracts of gall tissue of these species were inoculated into cacao half-beans. Galls were visible in the axils of the cotyledons about one month after inoculation.

The transmission-rates were:

	Washings		Tissue extract	
Mango	13/35	16/25	15/35	17/25
Pigeon pea	7/25		9/25	

The galls produced by inoculation from mango material were indistinguishable from those produced by inoculations from cacao 'green-point' material. While most of the galls produced on plants inoculated with pigeon pea gall were similar to cacao-gall, in some, the axillary bud enlarged very considerably and became chlorotic, with no proliferation of other buds. The germination-rate of the treated plants was comparable with those of controls with distilled water.

Transmission of the mango gall into a two-month cacao seedling was also obtained by inoculation of the axillary bud with tissue extract.

In the absence of any knowledge of vectors of 'cushion-gall' of cacao the significance of these results is difficult to assess. However, the ease with which the diseases of mango and pigeon pea can be transmitted to cacao under laboratory conditions would suggest that the cultivation of these two species in close proximity to cacao should be avoided.

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Puccinia polysora on *Tripsacum laxum* and *Zea mays*

THERE would appear to be the impression that, in the Carribbean, the same strain of *Puccinia polysora* attacks both *Tripsacum laxum* (Guatemala grass) and *Zea mays*. In Trinidad during 1953–54 an attempt to cross-inoculate uredospores from the two species proved completely unsuccessful. Successful inoculation of the same species was a matter of routine. It was not possible to detect any morphological differences between the strains, but they are certainly quite distinct. *T. laxum* is at present free from attack by *P. polysora* in Eastern Nigeria.

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MICROBIOLOGY

Slime-wall Formation in the *Salmonellae*

THE formation of a 'slime wall', which is usually a property of *Salmonella paratyphi B* but not of *S. paratyphi B* var. *java,* has long been used as a criterion of differentiation between these two organisms[1–4]. According to Birch-Hirschfeld[5], the slime-wall consists of a nitrogen-free polysaccharide containing more than 40 per cent of glucose. It is formed on solid media, optimally at temperatures of about 20–25° C. and not at 37° C. In this laboratory it is regularly demonstrated by incubating platings on solid media, initially at 37° C. overnight, and afterwards at 21° C. The slime-wall is usually evident after 24 hr. at 21° C. In the search for a reliable medium on which to demonstrate its formation, it was found that the concentration of phosphate exerted a powerful influence on the production of the polysaccharide. The basic solid medium used in these experiments contained 2 per cent of 'Bacto' dehydrated nutrient broth (Difco Laboratories), 0·85 per cent sodium chloride and 1·2 per cent New Zealand powdered agar ; it is referred to hereafter as Difco agar. Sörensen buffer, *p*H 7·0, was mainly used as the phosphate source. It was added to Difco agar in concentrations of *M*/5 doubling in dilution to *M*/160 ('phosphate agar'). The favourable effect on slime-wall production was strongest at *M*/5 and diminished progressively with the fall in phosphate concentration, little effect being observed beyond *M*/20, which was adopted as the lowest phosphate concentration for routine use. In strains of *S. paratyphi B* that were slime-wall positive on Difco agar, the amount of slime produced was greatly increased in the presence of phosphate. Strains that formed slime in small amounts or irregularly on nutrient agar produced it in abundance when phosphate was added. Some cultures that were consistently negative on nutrient agar secreted slime on *M*/5, *M*/10 and *M*/20 phosphate agar, others on *M*/5 and *M*/10, and yet others on *M*/5 phosphate agar only.

I was able to classify *S. paratyphi B* by these reactions. Strains that produced slime freely on Difco agar corresponded to classical *S. paratyphi B* and did not ferment D-tartrate. Strains forming slime sparsely or not at all on Difco agar, but abundantly on the same medium reinforced with *M*/20 Sörensen buffer, were mostly isolated from cases of food poisoning, but occasionally from cases of enteric fever. These were usually D-tartrate positive. Strains that formed slime only on *M*/5 phosphate agar were D-tartrate positive and came almost entirely from cases of food poisoning, although one strain reacting in this way has caused enteric fever. The typing phages of Felix and Callow[6,7] divided these strains into groups of types characteristic of each of the three varieties described. Exceptions to these reactions, in addition to the one already quoted, were found, however ; for example, one phage type, associated only with food poisoning, was slime-wall negative and D-tartrate negative. From this stock I have isolated both slime-wall positive and D-tartrate positive clones. It has been possible in a number of other strains to isolate slime-wall negative mutants from slime-wall positive stocks, and vice versa (see ref. 2). Within slime-wall positive lines, mutants were found that varied in the amount of slime they produced. These variations were often apparent only on phosphate agar.

An exploration of organisms of every group of the Kauffmann–White scheme of *Salmonella* serotypes revealed that, while very few indeed synthesized slime on Difco agar, the great majority produced it on phosphate agar. The most abundant production of the polysaccharide was on media containing *M*/5 phosphate, and some strains produced it only on such media. Others were able to form it when the phosphate concentration was reduced to *M*/10, and yet others on *M*/20 phosphate agar. The slime wall is uniform in appearance throughout all the *Salmonellae* tested, usually taking the form of a raised marginal ring, clearly demarcated and opaque by transmitted light and rather 'silky' when viewed by oblique illumination. Obvious slime production occurs only when the *p*H is 6·5 or higher. Between *p*H 7·0 and *p*H 8·0 it is formed freely, *p*H 7·0 being used as a routine in my experiments. Below *p*H 6·5 the colonial morphology is 'rough'.

Buffers other than Sörensen's were tried at *p*H 7·0 ; but few stimulated better slime formation than unbuffered Difco agar. Sodium glycerophosphate was very active, however, and a concentration of *M*/5, adjusted to *p*H 7·0 with sodium dihydrogen phosphate or hydrochloric acid, produced colonies that were completely mucoid after 24 hr. at 21° C. The metallic ions in the phosphates were not essential for slime-wall synthesis, for glycerophosphoric acid adjusted to *p*H 7·0 with ammonium hydroxide was equally active.

The presence of a fermentable carbohydrate, in the form of 0·5 per cent glucose, increased the amount of slime formed but was not essential for its synthesis in strains that produced it freely on Difco agar. Some strains forming slime only on phosphate agar, however, required the addition of glucose for its production. (The basic Difco medium employed in these experiments contained no detectable reducing sugar.)

The slime is not formed under anaerobic conditions.

It is not yet apparent whether the phosphate operates purely as a non-toxic buffer that does not inhibit slime-wall synthesis, or whether it participates in the synthesis. Some of the other buffers tested were clearly inhibitory in the higher concentrations used.

Whether or not the polysaccharide of which the slime is formed is identical in all *Salmonellae*, it is evident that the system that synthesizes it is similar throughout. This system is now under closer examination. Work is in progress on the conditions governing slime formation, the chemical and immunological nature of the polysaccharides, and on the possible effects of phosphate on the elaboration of other antigens. The results of these investigations will be published at a later date.

Note added in proof. An exploration of other members of the Enterobacteriaceae, for example, *Escherichia coli* and *Arizona*, has shown that the phenomenon of slime-wall formation in the presence of relatively high phosphate concentrations is widely distributed in this family.

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CYTOLOGY

Chromosomes of the Wild Mushroom

VERY little work has been done on the cytology of the wild mushroom *Agaricus campestris* Fr. var. *tetrasporus*. Most investigations have been confined to cultivated mushrooms (reviewed by Evans[1,2]). The cytology of the wild form is also of considerable interest since such information may contribute to our understanding of why it has not been possible to cross the wild with the cultivated form in attempts at introducing commercially desirable characters, such as an improved flavour.

Colson[3] examined both varieties of mushroom and found that the haploid chromosome number, seen as bivalents at meiosis, was 9 in both varieties. In a variety grown on a commercial scale in Great Britain, Evans[1] found a higher haploid chromosome number, $n = 12$, which he attributed to either: (*a*) different strains of the fungus having different chromosome numbers or (*b*) differences in chromosome number being due to limitations of technique. Previous observations were made on sectioned material whereas Evans's observations were made by means of a squash technique which facilitated study of entire cells.

Since the only previously recorded chromosome number of the wild mushroom was by means of a section technique, the work reported here was carried out by means of a squash technique in order to ensure that entire cells were being examined.

Fruit-bodies of the wild mushroom were collected from permanent pasture land at Aberystwyth, at the stage when the veil had just ruptured revealing part of the hymenium. Gills, at this stage, were fixed in Evans's mixture[1] of chloroform, ethanol and glacial acetic acid (7 : 5 : 2) immediately, in the field. Squash preparations were obtained with acetic orcein after sufficient hydrolysis of tissue with hydrochloric acid (to remove basophilly of the cytoplasm) for the study of meiosis in the basidium. Twelve bivalents were observed at the first division of meiosis. The clearest count obtained was at diakinesis (Fig. 1). It was more difficult to separate the bivalents after they had become orientated around the metaphase plate. Another difficulty in counting bivalents was non-synchronization of separation of chromosomes of the bivalents, at late metaphase–early anaphase.

Thus the chromosome number of both wild and cultivated mushroom varieties, as determined by a squash technique, is $n = 12$, which supports the suggestion of Evans[1] that "previous counts may have erred on the low side" due to the inadequacy of the sectioning method for chromosome counting.

It appears that the failure to hybridize the wild and cultivated varieties[4] does not simply depend on a difference in chromosome number. It is possible that an incompatibility system[5] and a difference in breeding system mechanism are involved. Thus research on the breeding mechanism, so far, indicates that the cultivated (2-spored) variety is a homothallic heterokaryon[2,6], whereas the wild variety is a heterothallic heterokaryon like most other 4-spored hymenomycetes[4].

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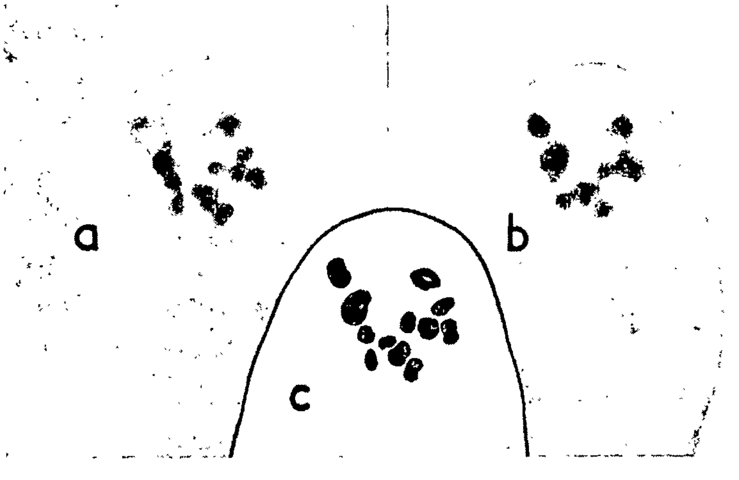

Fig. 1. Twelve bivalents at diakinesis. *a* and *b*, Photomicrographs at two foci; *c*, drawing of the group of bivalents as seen under the microscope. (× 6,000)

GENETICS

Glutamic Dehydrogenase in Single-Gene Mutants of *Neurospora* Deficient in Amination

FINCHAM[1,2] described some amination-deficient (am^-) mutants of *Neurospora crassa* which lacked the glutamic dehydrogenase and, therefore, could grow only very slowly in minimal medium in the absence of exogenous L-glutamic acid. Normal growth of the am^- mutants occurred in media containing glutamate, aspartate, alanine, ornithine or arginine, but not glycine, serine, threonine, histidine, lysine or citrulline[2]. The effectiveness of various amino-acids in supporting the growth of the am^- mutants was later correlated[3] to the efficiency of enzymatic transamination between these amino-acids and α-ketoglutarate in cell-free extracts. The growth of am^- mutants on exogenous sources of amino-acids is thus without doubt due to their conversion first to glutamic acid by transamination.

The very slow growth of the am^- mutants on minimal medium, however, has never been explained. It could perhaps be due to the formation of traces of glutamic acid by transamination between α-ketoglutarate and some other amino-acids initially synthesized by the cells. So far as we are aware, however, the only major path of entry of ammonia in *Neurospora* is the reductive amination of α-ketoglutarate. It is therefore difficult to visualize how the am^- cells produce other amino-acids in the absence of glutamic dehydrogenase. Earlier, Sanwal[4] had demonstrated the presence of two different glutamic dehydrogenases, one specific for diphosphopyridine nucleotide (DPN) and another for triphosphopyridine nucleotide (TPN) in a related fungus, *Fusarium*. In following up this work with wild-type strains of *Neurospora crassa*, Sanwal and Lata[5] succeeded in separating and purifying the TPN- and DPN-specific glutamic dehydrogenases. The glutamic dehydrogenase of *Neurospora* was reported to be specific for TPN[2] but later found[6] to be non-specific with regard to its coenzyme requirement.

The discovery of two different glutamic dehydrogenases[5] raised some interesting questions pertaining to the one-gene one-enzyme hypothesis. If single-gene *am*$^-$ mutants had lost the ability to grow in the absence of some exogenous source of L-glutamate, did it mean that one gene mutation had affected two different glutamic dehydrogenases? To test this point we used two *am*$^-$ mutants, 32213 *R*1*a* and 47305 *A* (both single-gene mutants) obtained through the courtesy of Dr. M. B. Mitchell of the California Institute of Technology, Pasadena. The wild-type strain, R^+, was kindly supplied by Dr. P. K. Issac of the Department of Botany, University of Manitoba. The strains were grown in shake cultures from a conidial inoculum[5] in the minimal medium *N* of Vogel[7] supplemented with 0·1 per cent L-glutamate and the specific activity of the DPN-specific and TPN-specific glutamic dehydrogenases was determined[5] in cell-free extracts of 24-hr. and 48-hr. mycelial growth. Table 1 summarizes the results of this investigation.

Table 1. SPECIFIC ACTIVITY OF DPN- AND TPN-SPECIFIC GLUTAMIC DEHYDROGENASES OF WILD-TYPE STRAIN R^+, *am*$^-$ *32213 R*1*a* AND *47305 A* OF *Neurospora*

Strain	Hr. of growth	DPN-enzyme	TPN-enzyme
R^+	24	90	320
	48	87	101
*32213 R*1*a*	24	32	none
	48	63	none
47305 A	24	61	none
	48	83	none

It will be seen from Table 1 that the wild-type strain produces both enzymes, but the *am*$^-$ mutants produce only the DPN-specific glutamic dehydrogenase. It is clear, therefore, that the mutation of one gene has led to the loss of only one enzyme, the TPN-specific glutamic dehydrogenase. The slow growth of the *am*$^-$ mutants on minimal medium is now understandable on the basis of the presence of the DPN-specific enzyme, but it is not easy to understand why the mutants cannot grow as well as the wild-type strain in the minimal medium. We have demonstrated[5] that the affinity of the TPN-specific enzyme for α-ketoglutarate is higher than the DPN-specific enzyme, but this fact alone is not sufficient to explain the slow growth of the mutants unless it is argued that the DPN-specific enzyme is primarily a degradative enzyme and the TPN-specific enzyme is biosynthetic[4,5]. We shall publish data elsewhere in support of this argument.

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* Post-Doctoral Fellow of the National Research Council of Canada (1960–61).

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SOIL SCIENCE

Interpretation of the Upper Plastic Limit of Clays

THE upper plastic limit, or liquid limit, of clays is regarded as that water content at which sufficient free water is present to allow clay particles to slip past one another under a certain applied force. It can also be interpreted as the distance between particles or between structural units of particles at which forces of interaction between the clay particles become sufficiently weak to allow easy movement of particles or units relative to each other. This interpretation allows an easier explanation of the influence on the liquid limit of such factors as exchangeable ion, salt concentration and *p*H of the clay paste. For this interpretation it is convenient to define an 'interaction volume' for a unit mass of clay particles as that volume within which individual particles or units of particles interfere with the movement of adjacent particles or units. The interaction volume will decrease as the applied force increases.

In addition to the influence of exchangeable ions on the liquid limit, for which published results have been summarized[1], it is necessary to know the influence of electrolyte concentration since this controls interparticle forces and particle arrangement in many clay pastes. Liquid limit values were measured according to a standard soil mechanics procedure of determining the number of blows required to close a groove in the clay paste samples using the Casagrande liquid limit device. Three or four water contents were used for which the number of blows fell between 15 and 40, and the water content at the 25-blow point (the liquid limit) was read from a plot of water content versus the logarithm of the number of blows. Water content was expressed on oven-dry weight of soil corrected for the weight of salt. Values for liquid limit are influenced by the procedure and by the drying and preparation history of the sample. These factors were kept constant, and only relative values of liquid limit are discussed.

The dominant interparticle force in pastes of montmorillonite with monovalent exchangeable cations is a repulsion arising from osmotic activity of the diffuse ion-layer around the particles[2]. Fig. 1 shows the measured liquid limit of sodium montmorillonite at different concentrations of sodium chloride. An increasing salt concentration, which results in a decrease in interparticle repulsion, decreases the liquid limit. The repulsion between particles can be considered to determine the interaction volume, keeping the particles in a fixed configuration with respect to each other and preventing free movement. As the repulsion is decreased, the particles become free to move at lower water contents or lower interparticle distances, and the liquid limit decreases.

When the repulsion is decreased by substituting a divalent exchangeable ion such as calcium, the liquid limit is also decreased. However, for calcium montmorillonite, increasing salt concentration does not decrease swelling by the predicted amount, and in Fig. 1 it did not decrease the liquid limit appreciably. Repulsion between individual particles resulting in large interparticle distances is not the dominant force. The clay paste consists of units of particles separated by 9 Å. within the unit. Larger separations occur between these units[3]. This structure or particle arrangement, which changes little with increasing salt concentration, determines the interaction volume or the water content at which interaction between units is low enough to allow easy movement.

In kaolinite pastes interparticle forces also control the liquid limit through their influence on particle arrangement. The dominant influence of particle arrangement is shown in Fig. 2 by the values with distilled water. The untreated clay has a *p*H of 4 and the particles are flocculated in an edge-to-face arrangement which results in a high interaction volume[4]. The neutral clays with a *p*H of 6·5 are still flocculated but the forces of attraction are lower. Fewer edge-to-face perpendicular contacts result in a lower interaction volume. In the dispersed sodium-saturated clay at *p*H 10 the interparticle force-resisting movement is the repulsion between particles which keeps them in more parallel arrangement. This is low for the kaolinite and the effective interaction volume is small. With increasing salt concentration the structure of all samples changes to face-to-face particle arrangement[4] with lower interaction volume than the edge-to-face but higher than the parallel arrangement. If the minimum in liquid limit for the dispersed clay at 0·01 *N* sodium chloride is real, it may result from decreased repulsion due to increasing salt concentration which initially decreases interaction volume more rapidly than the flocculated particle arrangement increases it.

For other clays the variation of liquid limit with electrolyte concentration may show a different pattern depending upon the interaction volume resulting from the interparticle forces. Limited measurements with a clay from the St. Lawrence Lowlands, which contains illite and chlorite, show the liquid limit passing through a shallow maximum at intermediate salt concentrations. This could result from the formation of a flocculated structure which becomes more compact as repulsion decreases due to further increases in salt concentration.

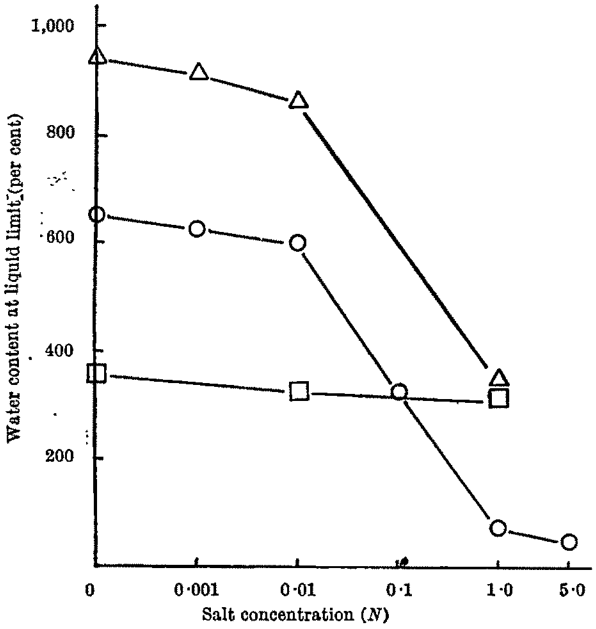

Fig. 1. Influence of salt concentration on liquid limit of montmorillonite clay with different exchangeable cations. △, sodium montmorillonite + sodium chloride; ○, natural bentonite + sodium chloride; □, calcium montmorillonite + calcium chloride

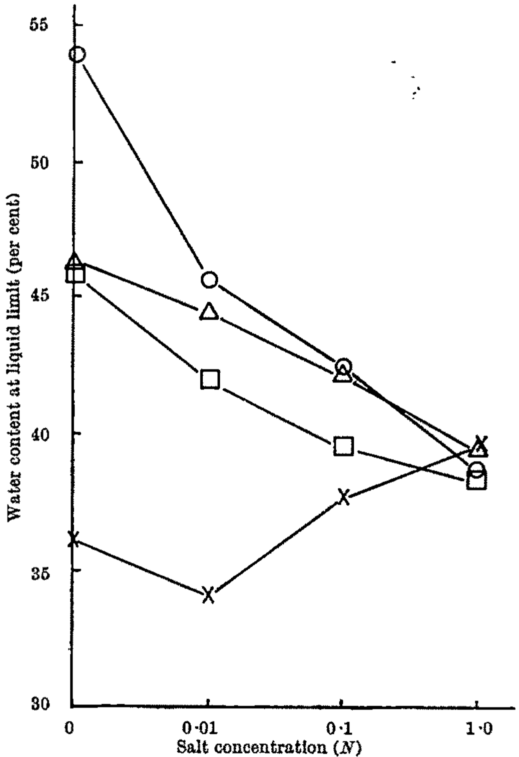

Fig. 2. Influence of salt concentration on liquid limit of kaolinite clay at different *p*H and with different exchangeable cations. ○, natural kaolinite (*p*H 4) + calcium chloride; △, sodium kaolinite (*p*H 6·5) + sodium chloride; □ calcium kaolinite (*p*H 6) + calcium chloride; ×, sodium kaolinite (*p*H 10) + sodium chloride

This interpretation of liquid limit controlled by interparticle forces explains why the liquid limit decreases with increasing valence of exchangeable ion for high-swelling clays, but increases for low-swelling clays where interaction volume is determined largely by particle arrangement. Here polyvalent ions lead to a more flocculated structure with a higher interaction volume.

At very high electrolyte concentration and in the presence of dissolving salt, clay pastes may become very stiff or show an increase in liquid limit. This effect has been termed ionotropy[5]. A definite structure or particle arrangement results from the salt diffusing into the clay paste. The intensification of *d* (001) spacings in X-ray diffraction patterns of salted clay pastes[6] indicates that the clay particles are in parallel orientation.

I wish to acknowledge the grant-in-aid of research from the National Research Council, Canada, under which this work was done, and the assistance of Mr. R. K. Chang, who made most of the measurements.

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Quebec.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, April 17

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. P. Alexander: "Science and Epistemological Atomism".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Education of Engineers—All's Well?" opened by Sir Hamish D. MacLaren, K.B.E.

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Annual General Meeting.

Institute of Metal Finishing (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. J. Edwards: "Plating of Zinc-Based Die-Castings".

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 7 p.m.—Discussion on "Co-ordinating the Needs of Hydraulic Machinery and Civil Engineering".

Monday, April 17—Tuesday, April 18

Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals (at the Royal Commonwealth Society, Craven Street, London, W.C.2)—Discussion on "The Appraisal of Powders for Pressing and Sintering".

Tuesday, April 18

Institute of Fuel (at the Royal United Service Institution, Whitehall, London, S.W.1), at 5.30 p.m.—Prof. Hoyt C. Hottel (Massachusetts Institute of Technology: "Radiative Transfer in Combustion Systems"* (Twenty-ninth Melchett Lecture).

Wednesday, April 19

British Association for Commercial and Industrial Education (at Senate House, University of London, W.C.1), at 10.40 a.m.—Conference on "New Routes to Further Education".

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Annual General Meeting and Presidential Address.

Bone and Tooth Society (at the Institute of Orthopædics, 234 Great Portland Street, London, W.1), at 5.15 p.m.—Dr. Franklin C. McLean (University of Chicago): "The Parathyroids and Vitamin D in Calcium Homeostasis".

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Dr. E. F. Drion: "The Intercorrelation between the Nutrients Consumed by a Group of Families in the Netherlands"; Mr. J. A. Heady: "Diets of Bank Clerks".

Institution of Electrical Engineers, Supply Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. A. N. Arman, Dr. F. J. Miranda and Mr. G. R. Bishop: "Progress in Oil-Filled Cables and Their Accessories".

British Institution of Radio Engineers, Medical Electronics Group (at University College Medical School, University Street, Gower Street, London, W.C.1), at 6 p.m.—Dr. C. N. Smyth: "Instrumentation for Obstetrics".

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Undergraduate Project Work in Full-time and Sandwich Courses in Mechanical Engineering".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. K. L. Blaxter: "The Nutritional Physiology of Livestock Under Natural Conditions" (Fernhurst Lecture).

Society of Instrument Technology, Data Processing Section (at 26 Portland Place, London, W.1), at 6.15 p.m.—Annual General Meeting. 7 p.m.—Mr. T. H. Flowers: "Electronic Telephone Exchanges".

Wednesday, April 19—Thursday, April 20

Institute of Rural Life at Home and Overseas (at the Colonial Office, Sanctuary Buildings, Great Smith Street, London, S.W.1), at 2.30 p.m. on Wednesday and 10.30 a.m. on Thursday—Eleventh Annual Conference on "The Land in Trust—at Home and Overseas".

Thursday, April 20

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. P. Benjamin and Mr. C. Weaver: "The Adhesion of Evaporated Metal Films on Glass"; Mr. C. J. S. Chapman, Mr. J. M. Gregory, Mr. R. W. Hill and Mr. M. W. Johns: "Nuclear Alinement of Cobalt and the Decay of Cobalt-55".

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Annual General Meeting followed by Scientific Papers.

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. R. Melville: "Bracken Regeneration from Sporelings"; Dr. John Ramsbottom: "A Letter from the Swedish Consul announcing the Arrival of the Linnean Collections"; Prof. L. J. Audus: "Recent Studies on Geotropism".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. K. Stein: "On Holomorphic Maps in the Space of Several Complex Variables".

Institution of Electrical Engineers, Utilization Section (joint meeting with the London Graduate and Student Section, at Savoy Place, London, W.C.2), at 6 p.m.—Mr. P. J. H. Rata: "Aluminium in Electrical Engineering"; Mr. A. D. Swanston and Mr. B. W. Towell: "Diesel Railway Locomotives"; Mr. T. E. S. Rickard: "Automatic Programming for Hot Reversing Mills".

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Mr. C. H. C. Brown: "Some Laboratory Investigations in connexion with the Development of Cationic Bitumen Emulsions".

Thursday, April 20—Friday, April 21

British Kinematograph Society, and the Television Society (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2)—Convention on "Television and Film Techniques".

Thursday, April 20—Thursday, May 4

Engineering, Marine, Welding and Nuclear Energy Exhibition (at Olympia, London).*

Friday, April 21

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Annual General Meeting. 7 p.m.—Prof. F. Bergel, F.R.S.: "Newer Developments in Carcino-Chemotherapy".

Institution of Electronics (in the Large Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 7 p.m.—Mr. T. D. Towers: "Modern Transistors and Their Applications".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer in Mathematics—The Principal, Borough Road College, Isleworth, Middlesex (April 21).

Assistant Lecturer in Physical Chemistry—The Registrar, The University, Hull (April 26).

Assistant Lecturers (2) (one with qualifications in the field of pure mathematics and the other with qualifications in the field of applied mathematics) in the Department of Mathematics—The Registrar, The University, Manchester 13 (April 27).

Assistant Lecturer in Statistics; a Lecturer or Assistant Lecturer in Applied Mathematics; and a Lecturer in Pure Mathematics—The Registrar, The University, Sheffield (April 29).

Assistant Lecturer or Lecturer in Pure Mathematics—The Registrar, University College, Singleton Park, Swansea (April 29).

Demonstrator in the Department of Geography—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (April 29).

Senior Lecturer in Inorganic Chemistry (Durban); and a Lecturer in Inorganic Chemistry (Pietermaritzburg), University of Natal, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, April 30).

Assistant Chief Geologist (with an appropriate university degree (preferably with honours), together with wide geological experience in the investigation of sedimentary basins and particularly in exploration and development of oilfields) in the Bureau of Mineral Resources, Department of National Development, Commonwealth of Australia, to be responsible for the Bureau's sedimentary programme of geological investigation of the sedimentary basins of Australia; prepare advice on problems related to the search for oil; and to assist the Chief Geologist in technical and administrative matters—Official Secretary (Ref. 61/2083), Australia House, Strand, London, W.C.2 (May 1).

Assistant Director (with post-doctoral research experience, preferably in the application of the electron microscope and related physical methods in biology or medicine) of Research in the Electron Microscope Section of the Cavendish Laboratory—The Secretary of the Appointments Committee of the Faculty of Physics and Chemistry, Chemical Laboratory, The University, Lensfield Road, Cambridge (May 1).

Lecturer or Assistant Lecturer (honours graduate in zoology or physiology) in the Department of Zoology—The Registrar, The University, Leeds 2 (May 1).

Lecturer (with an honours degree in physiology or an allied subject, or equivalent training) in the Department of Physiology—The Secretary, The University, Edinburgh (May 1).

Lecturer or Assistant Lecturer in the Department of Organic Chemistry—The Registrar, The University, Leeds 2 (May 5).

Research Demonstrator (with experience or interest in stratigraphy, structure and/or stratigraphical palæontology) in the Department of Geology—The Registrar, University College of South Wales and Monmouthshire, Cardiff (May 5).

Assistant Lecturer (preferably with interests in ecology, particularly its physiological aspects) in Botany—The Registrar (Room 22, O.R.B.), The University, Reading (May 6).

Demonstrators (2) in the School of Agriculture—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (May 6).

Postdoctoral Fellow in the Department of Chemistry, to carry out research into the reactions of halogen atoms with organic molecules, in collaboration with Dr. J. M. Tedder—The Registrar, The University, Sheffield 10 (May 6).

BURN RESEARCH FELLOW (graduate, less than 30 years old, with two to three years research experience) IN PHARMACOLOGY at Oxford—Secretary to the Trustees, Department of Pharmacology, University of Oxford (May 15).

DEMONSTRATOR IN BOTANY—The Registrar, The University of Adelaide, Adelaide, South Australia (May 15).

READER IN PHYSIOLOGY at the Royal Free Hospital School of Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 18).

ASSISTANT LECTURERS IN APPLIED OR PURE MATHEMATICS at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, May 19).

LECTURER (with a good honours degree in zoology, and preferably some experience of teaching and research) IN ZOOLOGY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, May 30).

CHAIR OF GEOGRAPHY in the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, May 31).

LECTURER or ASSISTANT LECTURER (preferably candidate interested in genetics and ecology) IN ZOOLOGY—The Registrar, Pius XII College, Roma, Basutoland, Southern Africa (May 31).

CHAIR OF PHYSIOLOGY in Monash University, Clayton, Victoria, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, June 10).

ASSISTANT LECTURER IN INORGANIC CHEMISTRY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

PHYSICISTS AND CHEMISTS (with an honours degree or equivalent in physics or chemistry with at least three years relevant experience, or Associates of the Royal Institute of Chemistry) as Reactor Operating Staff at Dounreay Experimental Reactor Establishment, Thurso, Caithness, Scotland, to take control of a shift team responsible for the safe and efficient operation of the high flux Materials Testing Reactor, its ancillary plant and the experimental test rigs under irradiation in it—The Personnel Manager, D.E.R.E., U.K.A.E.A., Dounreay, Thurso, Caithness, Scotland, quoting Ref. 214/34.

PLANT PATHOLOGIST (male, aged between 28 and 45, with an honours degree in botany having specialized in mycology, together with at least two years postgraduate experience, and preferably experience in plant diseases in the tropics, especially diseases of cocoa) in Sierra Leone, to advise the Director of Agriculture on the application of phytosanitary measures and to undertake investigations into the control of plant diseases—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD/63/15/019.

RESEARCH ASSISTANTS (graduates) IN THE DEPARTMENTS OF BOTANY AND ZOOLOGY, CHEMISTRY, MATHEMATICS, METALLURGY (GEOLOGY), AND PHYSICS—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

RESEARCH ASSISTANTS (with a degree in chemistry or chemical engineering, or with equivalent qualifications such as Grad.R.I.C.) IN THE DEPARTMENT OF CHEMISTRY, for experimental work on the thermodynamic properties of gases and of liquid mixtures—The Bursar, The University, Reading.

SENIOR LECTURER (well qualified academically in mathematics, and preferably with industrial and teaching experience) IN MATHEMATICS—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 8/G.

SENIOR TECHNICIAN or TECHNICIAN IN THE CLINICAL ISOTOPE LABORATORY—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

The Reason for Studying Plant Diseases. By Prof. N. F. Robertson. (An Inaugural Lecture delivered in the University of Hull, 10 February, 1960.) Pp. i+14. (Hull: The University, 1960.) 2*s*. 6*d*. [191

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 20: The Rearing of Chickens. Pp. 15. (Belfast: Ministry of Agriculture, 1960.) [191

Ordnance Survey. Map of Ilfracombe and District. (Sheet 856.) Scale: 1 : 25,000 (about 2½ inches to One Mile). (Chessington: Ordnance Survey, 1961.) Paper 6*s*. 6*d*. net. [191

Office of the Minister for Science. Scale of Enterprise in Farming: a Report by the Natural Resources (Technical) Committee. Pp. vi+110. (London: H.M. Stationery Office, 1961.) 6*s*. net. [191

Flora of Tropical East Africa. Edited by Dr. C. E. Hubbard and E. Milne-Redhead. Aizoaceae. By C. Jeffrey. (London: Crown Agents for Oversea Governments and Administrations, 1961.) 4*s*. [191

Society for Freedom in Science. Occasional Pamphlet No. 20: The Two Aspects of Science. By Sir George Thomson. Pp. 5. (Oxford: Assistant Secretary, Society for Freedom in Science, Department of Zoology, University Museum, 1960.) 1*s*. 3*d*. [191

Laboratory Animals Centre, Carshalton. Collected Papers, Vol. 9, 1960: Report of a Symposium on the Provision of Animals for Cancer Research, held at the Royal Society of Medicine, 23rd May, 1960. (Carshalton, Surrey: Laboratory Animals Centre, M.R.C. Laboratories, 1960.) 12*s*. 6*d*. [191

Johnson, Matthey and Co., Ltd. Publication No. 1302: Electrical Contacts. Pp. 30. (London: Johnson, Matthey and Co., Ltd., 1960.) [191

Manchester Public Libraries. The 108th Annual Report of the Libraries Committee, 1959–1960. Pp. 36+2 plates. (Manchester: Manchester Public Libraries, 1960.) [191

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Other Countries

Smithsonian Miscellaneous Collections. Vol. 140, No. 5: A Revision of the Ordovician Bryozoan Genera *Batostoma*, *Anaphragma*, and *Amplexopora*. By Richard S. Boardman. Pp. i+28+7 plates. (Publication 4426.) (Washington, D.C.: Smithsonian Institution, 1960.) [191

The Carlsberg Foundation's Oceanographical Expedition Round the World 1928–30 and previous "Dana" Expeditions. "Dana" Report No. 54: Further Studies on the Non-Nucleated Erythrocytes of *Maurolicus mülleri*, and Comparisons with the Blood Cells of Related Fishes. By Vagn Kr. Hansen and Karl Georg Wingstrand. Pp. 15+3 plates. (Copenhagen: Andr. Fred. Høst & Son, 1960.) 5 Danish kr. [191

Australian Atomic Energy Commission. Eighth Annual Report for the year ended 30th June, 1960. Pp. 63. (Coogee, N.S.W.: Australian Atomic Energy Commission, 1960.) [191

United States Department of the Interior: Fish and Wildlife Service. Fishery Bulletin No. 178: Natural History of the Sandbar Shark *Eulamia milberti*. By Stewart Springer. Pp. iv+1–38. 35 cents. Fishery Bulletin No. 180: Fecundity of Red Salmon at Brooks and Karluk Lakes, Alaska. By Wilbur L. Hartman and Charles Y. Conkle. Pp. iv+53–60. 15 cents. (Washington, D.C.: Government Printing Office, 1960.) [191

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A TRIUMPH FOR SCIENCE

IN *Nature* of October 12, 1957, p. 719, we were glad and proud to congratulate the scientists and technologists of the U.S.S.R. on the successful launching of the first artificial Earth satellite (*Sputnik I*). Less than a month afterwards (*Nature*, 180, 931; 1957), we again had occasion to congratulate the U.S.S.R., when the second satellite (*Sputnik II*) was launched, this time containing a dog. Though both these satellites eventually disintegrated in space, enough evidence had been obtained, especially from *Sputnik II*, to indicate that it was possible for living things (even mammals) to withstand the strain of being launched into orbit. Since then, Russian, American and other scientists have made astounding progress in space research using rockets, satellites, etc. Two more recent achievements, perhaps most important of all, were the placing into orbit of satellites containing dogs and recovering them with the dogs alive. This was done by scientists in the U.S.S.R. on August 16, 1960, and again on March 9 of this year.

The significance of these great achievements, all made so recently as during the past four years, needs no stressing now; but one cannot refrain from expressing concern that though each achievement has deservedly received world-wide acclaim, the science and technology behind each are, by and large, known only to the chosen few.

Now again, on April 12, a still further point in scientific advancement was reached, for on that day scientists and technologists in the U.S.S.R. reaped a rich reward for concentrated and ingenious research by successfully launching another space-vehicle (*Vostok*) into space. This satellite carried, among other things, a man (Major Yuri Alekseyevich Gagarin), travelled in orbit around the Earth in 89 min. 6 sec., and a little later was brought back safely to Earth, with Major Gagarin unharmed—all in about 108 min. Moreover, the Russians claim that every part of the space-vehicle was recovered intact and can be used again. (See also p. 307 of this issue.)

The entire world, scientists and non-scientists alike, has learned of this great feat with awe and admiration, and we hasten to praise and congratulate the scientists and technologists who were responsible. Moreover, Major Gagarin is to be commended on his courage and devotion to duty.

It seems timely, therefore, once again to emphasize what we consider to be the true significance of the magnificent results obtained in space research by Russian, American and, indeed, other men of science. We may ask: Are these great feats being worked for, paid heavily for and finally achieved amidst world applause mainly for political prestige or military advantage, or for the advancement of science in its truest sense, that is, the enhancement of man and his culture ? There is certainly no gainsaying the scientific results, for they are there for all to see; and the scientists and technologists concerned (most of them, apart from Kapitza, Sedov and Blagonravov, for example, unknown outside the U.S.S.R.) are, it is assumed, being awarded their just honours and dues. But the time has long since passed when the scientist either need not or does not concern himself with the consequence of his work; so the most important question now is: How are these magnificent scientific achievements to be utilized in the near and distant future ? Quite rightly, we think, the scientist himself should not be the sole arbiter in this problem. But it is for everyone to see that the right people make the right decisions for the sake of world progress and peace. We are often being told that it is the people of a nation and the peoples of the world who decide policy and make final decisions, and that their so-called leaders, especially in the political arena, are not really their leaders but actually their servants. If this is the case, then the world community must see that scientific results are used wisely; but the community can do so only if it has a true understanding and appreciation of the significance and message of science.

But it is very doubtful if the layman really appreciates what is going on in the world of science to-day, despite the greater attempts at improving education both through specialized agencies such as universities and technical colleges and through mass media. Our formal academies of teaching and learning, such as schools and universities, cannot, by themselves, prepare the ground for the most desirable growth, development, appreciation and application of the new knowledge. Neither will the plausible projects of increasing the number of universities and technical colleges solve the problem; they can only scratch the surface.

There are other means of education, equally important, albeit mainly mass education—television, sound broadcasting, the cinema, books and, above all, the newspaper Press; but, alas, all too often these soon become reduced to arenas for political propaganda.

Ignorance provides the most fertile ground for undesirable propaganda of all sorts, especially politics. So to what extent does the Press attempt to give science its fair due, and thus lay the foundation for creating the type of mind that can assess wisely the value of any form of scientific achievement, whether it be in space research or antibiotics ? It is very significant, alarmingly so, in fact, that the Press seems to have developed a poor sense of values, and preys on the weakness and foibles of human nature rather than attempts to stimulate the interest of its readers in things of greater moment and of inestimably more value to man and his cultural development. It is a sad reflexion that it takes a man travelling in orbit around the Earth to give science one of its all too few banner headlines in the newspapers. The same could be said of other aspects of cultural development which aims at international collaboration and peace. It is relevant here to refer to a recent American survey which has shown that a certain film actress was mentioned more often than anyone else in the world in the daily newspapers of 1960.

Another comparison may not come amiss. A photograph of a section of a crowd in Moscow, taken just after the *Vostok* news was released, seemed reminiscent of a photograph of a crowd outside the entrance to a London hospital. But there was a difference, in that the former wore, with understandable justification, jubilant and happy expressions on their faces, whereas the latter seemed taut, anxious and expectant, for they were a crowd of Press photographers anxious to take photographs of visitors to another film actress lying ill in the hospital. The sense of values of both Press and public on the two sides of the Iron Curtain seems to be vastly different.

Many of the comments on the recent news from the U.S.S.R., at any rate in the British Press, betrays a deplorable lack among some writers of a true appreciation of the technique, aims and objectives of science. Moreover, a sense of proportion seems to be sacrificed to sensationalism. For example, one feature article is devoted entirely to urging Britain to award some form of honour to Major Gagarin. It is fairly certain that to bestow on Major Gagarin an honorary George Cross, in recognition of his courage and bravery, would meet with popular acclaim; but, alas, the article in question directs all its proposals to British scientific societies, including a suggestion that the Royal Society of London should consider conferring the great scientific distinction of foreign membership on the Major. We have no doubt that it can be left to the Royal Society to maintain its sense of proportion, and while, perhaps, officially congratulating the Academy of Sciences of the U.S.S.R., continue to confine its distinguished foreign membership to scientists who have personally made outstanding contributions to fundamental scientific knowledge.

Another writer refers to scientists as being the "most optimistic and dangerous men in the world". Men of science can ill afford to ignore such absurd suggestions and comments about themselves, in spite of the fact that most ill-informed statements concerning science appear in the so-called 'popular' newspapers. The fact that science is so misrepresented should give cause for grave concern, for it is such newspapers which enjoy the largest circulation and are therefore read by the majority of people.

The official announcement of Russia's recent great scientific victory reads (in part) as follows:

"We, *Soviet people building Communism* [italics ours] have had the honour of being the first to enter into outer space. The victories in the exploration of the cosmos are regarded by us as not only the achievement of our people but also of entire mankind.

"We gladly place them at the service of all peoples in the name of progress, happiness and the well-being of all human beings on earth. We do not place our achievements and discoveries at the service of war but at the service of peace and security of peoples.

"On this solemn day, we address once again the peoples and governments of all countries an appeal for peace. May all people, irrespective of race and nation, colour, religious beliefs and social condition, devote all their efforts to safe-guarding stable peace throughout the world".

This is a most noble statement. We, as scientists, subscribe wholeheartedly to it and to the jubilation of Soviet Russia; indeed, we feel we have a share in it. Science cannot recognize national frontiers. There is no such thing as Communist science, or American science or British science; there is just *Science*. Moreover, present-day science is built up on science of the past (Sir Isaac Newton contributed largely to this latest Russian victory, three hundred years ago); and present-day science is the basis for the science of the future. Science, like other cultures, and disciplines, has been, is and will be in a state of evolution, and the pace of such evolution depends mainly on a host of genera and species of scientists, none of whom can afford to ignore the rest. While, therefore, reading with satisfaction the official Russian announcement, we think it is a pity that the words printed in italics were included. This great achievement is not a victory for Communism, any more than the great achievements in space research in America are a victory for democracy—in the political sense. All such achievements are the victories of science over Nature.

Conversely, it is equally disturbing to read that the news was received in the United States with "little surprise but deep disappointment", and that in Cape Canaveral a man working in the American space programme commented that the recent news from Russia "just makes me sick".

So, as a corollary, it is essential that we all realize that if the scientists and technologists do not get the sympathetic understanding and practical support they deserve, then those in political authority, coupled with their advisers, must accept the immediate blame; though the ultimate blame must rest on all those responsible for our education and for the mass dissemination and interpretation of knowledge.

One views with no little concern the utilization of science for political propaganda. The layman is not prepared for such news, and the majority of the public is in no position to evaluate the results. One otherwise quite intelligent person, on hearing the news, just commented: "I am now really afraid". It seems that the general public throughout the world is accepting as a fact that space research is now a race between Communists and the rest—mainly between the U.S.S.R. and the U.S.A. This cannot be a good omen for world peace, neither is it to the advantage of science in its dispassionate search for truth. The President of the United States has said that he is tired of being second in the space race with the U.S.S.R. Perhaps now, instead of making further efforts to become first, greater effort can be made by all the great Powers to arrange for a global pooling of scientific knowledge, thus forming the basis of H. G. Wells's ideal of a *World Encyclopædia*; but while there is so much political bad faith and open schism between countries, coupled with the hitherto tardy recognition that science is useful in peace as wel as in war, there seems to be little hope of such international collaboration. Moreover, it is futile to blame any one country for such refusal to collaborate, in spite of the fact that collaboration must be based on an all-or-none policy.

Despite the fact that the U.S.S.R. has been the first in some of the initial stages of space research (for there are undoubtedly many more stages to follow) it must be recognized that American contributions (though comparatively less spectacular and hence not having such 'hot' news or political propaganda value) to this now fashionable aspect of scientific research are by no means, therefore, of secondary importance. In fact, during these initial years, even if all data and results could be duly weighed and evaluated, it would be difficult to say which of these great Powers has contributed most, so far, to our knowledge of terrestrial and extra-terrestrial science; and since no one authority is in possession of all the facts it is virtually impossible to say which, from the scientific point of view, has done most towards the establishment and development of space research. Yet, in the long (perhaps very long) run, it will be the scientific assessment which really counts.

The kind of scientific competition, both inter- and intra-national, that we are experiencing to-day cannot be a healthy one. Claims for priority sometimes become quite nauseating. It is all bound to lead to misrepresentation, exaggeration, unfair claims, secrecy and consequently suspicion, and altogether general misunderstanding especially among the general public; and this provides fertile ground for political propaganda of the worst kind. (The Russian reports of even this latest feat are somewhat contradictory, and this has immediately had the effect among some people of querying the facts or wondering if some ancillary statements have been made deliberately to confuse the issue among other scientists of the world.) Apart from this, secrecy and surreptitious competition must involve wasteful and very expensive duplication of effort. Gone are the days when one could speak with pride, and justifiably take for granted, freedom in science; that is, freedom in research, freedom in discussion and debate, and above all freedom in exposition to the entire world of new scientific findings.

Lord Hailsham, the British Minister for Science, has said that it would be better and more economical to conduct inquiries concerning the Universe on an international basis. Most scientists, including, we think, many in the U.S.S.R., will subscribe to this view. But, as pointed out by Lord Hailsham, British development of full-scale manned space flight would be a misuse of scarce man-power and economic effort. We trust this will not be interpreted as a scientific dichotomy of the nations of the world into the 'haves' and 'have-nots', for that would be following the absurd propaganda of Nazi Germany. Rather would we accept Lord Hailsham's views and couple them with those of Mr. Harold Macmillan, who recently emphasized that scientific research is not confined to that of 'space'. In all branches of scientific research, for centuries, Britain has contributed its share—in some cases the lion's share; and history frequently puts Britain in the vanguard. Moreover, the urge among scientists to collaborate with each other, irrespective of nationality, is continually being emphasized by the large number of international scientific conferences held in many parts of the world.

Nevertheless, one must realize that the 'Iron Curtain' exists, even for scientists; and while that is allowed to continue to affect science, the humanities and other branches of cultural development there is bound to be a threat to the dignity of man and his very destiny.

It was Herbert Spencer who said that: "Science is organized knowledge". To-day, science has evolved well beyond that definition. However, even if we are prepared, in principle, to accept Spencer's aphorism we should modify it to read 'card indexed' rather than "organized". But, alas, the index cards are not all in the same file to which the entire scientific world may add and/or refer.

This makes all the more relevant and significant the comment made by Sir Bernard Lovell, director of the Jodrell Bank Experimental Station, when referring to this, the latest and greatest triumph of the scientists in the U.S.S.R.

"I think it is remarkable that a nation which was largely illiterate only a generation ago has succeeded in this immense task. It has done this because of the singleness of purpose and human sacrifice in material comforts on a scale unknown in the West.

"If the Western civilizations continue to disregard this example of the great results of investment in science, education and research, they will do so at their peril".

We do not advocate copying the methods in education being adopted in the U.S.S.R.; but we would urge that peoples of the world take note of this recent evidence of the result of encouraging science and scientists. That is best done, fundamentally, by giving sound scientific education to all, so that scientists will receive the encouragement they now lack, and when new scientific results are announced, the non-scientists (very much in the majority, and some in powerful authority) will, in consultation with the scientists themselves, take enthusiastic and intelligent note and be in a position to assess wisely and decide sensibly how such results shall be applied to the advancement of mankind.

At present, we seem to be paying only lip-service to the ideal that scientific achievements should transcend all political and racial barriers. For example, on several occasions during recent years, *Nature* has tried, through personnel of the Moscow Academy of Sciences, to persuade scientists of the U.S.S.R. not to persist in hiding their light under a bushel. But either they or their political superiors have so far turned deaf ears to such appeals. If the time does come when we have achieved that ideal, then shall we be able to congratulate those responsible for any notable scientific feat *because they are scientists*, not because they are members of any one nation or subscribers to any particular political creed.

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The theoretical and thermodynamical principles governing the analytical procedures are presented in much detail, and the academic student will welcome those extensive sections dealing with complexans, redox potentials, ionic equilibria, etc.

Much practical laboratory detail is also included in those descriptive parts relating to reagents, apparatus, methods, etc.; this makes the book singularly attractive to the young chemical analyst, who will find a wealth of experience and sound advice presented in a most assimilable manner. For the more mature analyst, there are descriptions of the recent modern techniques, of reagent standards, chelate compounds, a very extensive section on complexan reactions and titrimetry, potentiometric and amperometric methods, etc. The inorganic sections covers every titrimetric reagent normally used in the most up-to-date laboratory, and ends with an interesting note on the application of a miscellany of the more unusual reagents.

The section of the book dealing with organic quantitative analyses is equally comprehensive and detailed, both macro- and micro-techniques being described for the determination of the main elements, specific groups, etc. Various alternative methods are presented; for example, the determination of carbon gravimetrically as carbon dioxide, by acid titration after absorption in baryta, or by determining the change in conductivity of the baryta solution. All the usual non-metallic and metallic elements are covered, as are also the distinctive groups, nitroso, azo, thiocarbonyl, methoxy, alkyl, etc.

The volume ends with an excellent section covering titrations in non-aqueous solvents, a subject which is still one of the most attractive fields for those interested in pure research. The theoretical approach is again clear; aprotic, protogenic, protophyllic and amphiprotic solvents are discussed in relation to the Brönsted–Lowry–Lewis theories. Visual fluorescent, photometric, conductimetric and potentiometric indicator systems are described for various non-aqueous titrimetric determinations, and typical reaction phenomena are given graphical representation.

There is no doubt whatsoever that an authoritative, detailed and scientifically prepollent work of this nature will find its way on to the bookshelves of all institutions dealing with either the practice or teaching of analytical chemistry and will retain its status as a unique an alytical vade-mecum for many years to come.

D. T. LEWIS

FREE RADICALS IN AROMATIC SUBSTITUTION

Homolytic Aromatic Substitution

By G. H. Williams. (International Series of Monographs on Organic Chemistry, Vol. 4.) Pp. vii+133. (London and New York: Pergamon Press, 1960.) 45*s*. net.

INTEREST in homolytic, as distinct from heterolytic, reactions dates from the recognition by Prof. D. H. Hey in 1934 that the nuclear phenylation reaction, discovered some ten years earlier by Gomberg and Bachmann, did not conform with current ideas of aromatic substitution in terms of reactions by electrophilic and nucleophilic reagents. He postulated that this reaction represented a new type of aromatic substitution, involving electrically neutral free radicals. Further reactions displaying the same characteristics were soon discovered and were intensively studied. Homolytic substitution has since become a subject of considerable theoretical and practical interest.

In recent years, thanks to new and refined physical techniques, organic chemists have been able to accumulate reliable quantitative data on aromatic substitution processes of all types and this new knowledge has often necessitated a revision of theoretical concepts. The appearance of this monograph by Dr. Williams, a collaborator of Prof. Hey for many years, is timely, and it represents a valuable contribution towards a more comprehensive understanding of aromatic substitution generally.

The author subjects to critical scrutiny the various theoretical treatments, based on wave mechanics, which have been applied in attempts to rationalize the observed phenomena of aromatic substitution. Particular emphasis is placed on those aspects germane to the problems of orientation and reactivity in homolytic substitution. The limitations and deficiencies of these theoretical treatments are exposed. Thus, some unwarranted assumptions are made; for example, the failure to take cognizance of the intrinsic reactivity and polar characteristics of free radicals. Evidence, based on partial rate factors, is adduced to show that all homolytic substitution reactions exhibit some partial ionic character.

The reactions surveyed include homolytic arylation, both inter- and intra-molecular, alkylation, hydroxylation, acetoxylation, benzoyloxylation, amination and amidation reactions. In the case of mercuration, the author concludes that the case for a homolytic mechanism has not yet been definitely established.

Not unnaturally, in this rapidly advancing field, there are controversial issues and divergence of views among the various schools, British, American and German. The author projects his own views, but still manages to present a balanced statement, commendably free of any polemical bias.

J. IDRIS JONES

THE CARTESIAN MEDITATIONS

Cartesian Meditations

An Introduction to Phenomenology. By Edmund Husserl. Translated by Dorion Cairns. Pp. xii + 157. (The Hague: Martinus Nijhoff, 1960.) 9.50 guilders; cloth bound 13.25 guilders.

ALTHOUGH it would be idle to deny that Edmund Husserl did otherwise than stand—in a certain restricted sense—upon the shoulders of Descartes, yet the break-through to phenomenology as a rigorous system of pre-suppositionless philosophy was an achievement of the master himself. In the end it was almost independent of Cartesian influences. Hence the paradox of the title *Cartesian Meditations* and all that flows from them. But it was one of those symbolic events of history which brought Husserl to the Sorbonne in February 1929 to deliver a couple of lectures, afterwards elaborated into the work now under review. He truly meditated in Cartesian terms, producing out of this exercise his transcendental phenomenology.

This is scarcely the place to discuss the content of these lectures—that has already been attempted in a review of the German script some years ago. The immediate aim must be limited to welcoming such a scholarly and meticulous English translation as is now available in the present book. Anyone fortunate enough to possess Vol. 2 of *Husserliana* can follow for himself (since the pagination is carefully co-ordinated throughout by marginal brackets) the exact course of this transliteration, and the subtle changes of mood inherent in Husserl's thinking. In fact, the reader has the advantage of a transcription from the text now called *Typescript C*, where, as in most cases, this comes nearer to the author's works which were published during his life-time. Thus, it is possible for English-speaking scholars not only to recapture what happened in Paris more than thirty years ago but also to study it in the setting of the lecturer's habitual mode of presentation as his own thoughts developed. Our debt to Dr. Dorion Cairns is great, and only a little less to the publishers for making this classic of epistemology more widely accessible.

To follow Husserl's 'concluding word' in the original is very difficult. Here, it becomes a little easier, and its message for science more capable of assimilation. It is something like this: our practical daily life is naïve, engulfed in a series of happenings which are just there (an element of brute fact), and continue quite impersonally. It should be noticed that this immersion includes the act of valuing, something which it has become customary to exclude from the natural sciences, but which modern thought seems to be bringing back in a species of subjectivism akin to that of Husserl. This tendency is important for the biological disciplines wherein objectivity is less coercive than in the domain of mathematics. Yet for Husserl, all science is naïve to some degree, metaphysics included. But metaphysics as such are allowable under certain conditions.

The meditations end most appropriately with the well-known Augustinian exhortation: "Do not wish to go out; go back into yourself. Truth dwells in the inner man".

F. I. G. RAWLINS

BIOPOESIS

Aspects of the Origin of Life

(International Series of Monographs on Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, Vol. 6.) Edited by M. Florkin. Pp. viii + 199 + 5 plates. (London and New York: Pergamon Press, 1960.) 30*s*. net.

MOST of the papers given in 1957 at the Moscow international symposium on "The Origins of Life" (see *Nature*, 180, 886; 1957) were precirculated; corrected texts, together with the discussion, were published in 1960. The symposium was planned along tolerant lines, and many of the papers dealt with a range of themes that, though interesting and important for an understanding of present-day manifestations of life, had no necessary connexion with its origins.

In all symposia of this type, viruses and nucleic acids loom large—apparently for the reasons that lead shopkeepers, whatever they may ostensibly be selling, to run a line in ball-point pens as well. Here we have a usefully pruned version of the symposium; the clutter of papers on ephemeral fashions in biochemistry (which move more sluggishly than women's fashions but equally irrationally) has gone and the relevant papers remain.

There are five papers on the probiotic environment. These show so much interesting conflict that it is clear that astronomical and geological evidence leaves ample latitude for speculation. Even the widely accepted view that the primitive atmosphere was anaerobic may be questioned because the amount of ferric iron and oxidized sulphur in sediments shows that more oxygen passed through the atmosphere at a very early stage than is present in it now. Four papers deal with processes that may be analogous to those that formed organic matter initially. As the complexity of the mixtures subjected to various agencies—ultra-violet light, electric discharges, heat, etc.—increases, by the inclusion of hydrogen sulphide, hydrogen cyanide and other probable primitive effluvia, a fascinating range of products, with affiliations to most groups of molecules now important in biochemistry, will be made. Few theories of biopoesis should be excluded on the grounds that the substrates could not have been available.

The remaining papers deal with the evolution of enzymes, the role of asymmetry, photochemistry, and other themes. They are well selected, and one's only regret is that modesty led the editor to exclude his own excellent paper on the frequency with which substances that are used for one purpose in primitive organisms find a different application as evolution proceeds. If this were more generally recognized it would discourage dogmatic attempts to extrapolate back from present-day biochemistry to the primitive state of affairs.

N. W. PIRIE

Vacuum Technology Transactions

Proceedings of the Sixth National Symposium, also cumulative Author and Subject Indexes of 1954–59 Symposia. (American Vacuum Society Inc.) Under the Editorial Supervision of C. Robert Meissner. Pp. xvi + 335. (London and New York: Pergamon Press, 1960.) 120*s*. net.

THE symposium of the American Vacuum Society has become an annual event of international importance in the field of vacuum technology at which leaders in the subject from scientific and technological institutions, throughout the world, gather to read papers on their most recent researches. These papers are presented in detail in the *Vacuum Technology Transactions* which are usually published a few months after the symposium. The material covers almost every aspect of vacuum technology, from fundamental work of mainly academic interest to descriptions of the vacuum systems of the most recent particle accelerators and thermonuclear devices. The 1958 and 1959 volumes, however, show a noticeable increase in technological work directed towards the attainment of ultra-high vacuum in large machines. This is particularly true of the 1959 transactions, which include technical data associated with the design of the vacuum system for the Model *C Stellarator*, which will undoubtedly prove of great use to those facing similar problems. In addition to presenting the papers read at the conference, the 1959 *Vacuum Technology Transactions* contains a cumulative index of papers read at the previous symposia. The index is in chronological order, groups the papers in order of subject and is presented together with an author index arranged in alphabetical order, by year of presentation and page number. The combined index will be welcomed by those wishing to make a survey of recent research in a particular branch prior to embarking on work within the same field. The 1958 and 1959 *Transactions*, in keeping with the previous volumes, will be of considerable value to those who were not fortunate enough to be able to participate in the symposia and to those who wish to retain detailed information for future reference.

An Introduction to Electronic Theory of Organic Compounds

By H. L. Heys. Pp. 236. (London: George G. Harrap and Co., Ltd., 1960.) 16*s*. net.

THE writing of an elementary text-book is an art which is beset by difficulties. This is particularly true when the subject is the electronic theory of organic reactions. The evidence for electron shifts in molecules depends upon a wide variety of chemical and physical observations, and for an understanding of the significance of such measurements at least a thumbnail sketch of each method is desirable.

In developing his account of the subject the author has been aware of this need and he has been largely successful in his treatment of the problem. He has covered the main sections of the theory with an admirable conciseness and clarity, and the simpler aspects of any problem are clearly described without neglecting the minutiæ which may cause difficulties.

I am not, however, entirely in agreement with the author in devoting more than 20 pages to the Bohr and Sommerfeld treatments of the atomic orbitals, a discussion which seems to have no relevance to the remainder of the book. Moreover, some details included in the text do not seem to be in agreement with recent information. Thus, the idea that the two carbon-carbon bonds of ethylene have different energies is a theoretical concept rather than an experimental observation (p. 49). Also (p. 87) the value of 190 kcal./gm. atom $C_{(S)} \rightarrow C_{(g)}$ (^3P) has never been seriously supported.

The book is well produced with clear diagrams and a generous index. The only errors in production noted by the reviewer are 'C' for 'Cl' (last line of p. 78), a spacing error (line 10, p. 106) and the loss of a bond in tropolone (XXV, p. 110). These can be easily put right in the next edition.

R. I. REED

The Veterinary Annual, 1960

Edited by W. A. Pool. Second year. Pp. xxiv + 359 + 32 plates. (Bristol: John Wright and Sons, Ltd., 1960.) 42*s*.

THE literature now being published on every aspect of veterinary science is so voluminous, and it records so many important advances in knowledge, that everyone who has to try to keep pace with it will welcome the appearance of a second volume in this authoritative series. For it gives us, under the able editorship of W. A. Pool, an epitome, selected by 35 specialists, of the important work done since the publication of the first volume of the series in 1959.

The field covered by veterinary research is nowadays very wide, and even a brief study of these pages will show how closely modern veterinary research is integrated with the work of other branches of science. Though the book is mainly for the veterinarian, it reflects the many facets of the study of comparative medicine and surgery, and it will for this reason interest all who are concerned with disease, whether they be medical men, especially perhaps those who are co-operating with the veterinarian, or chemists or others who provide us with so wide an array of drugs and other compounds used in the battle with disease. The nomenclature of these compounds is, as the editor puts it, 'harassing' to many who have to try to understand their composition and uses, and welcome features of this book are the appendixes to the articles on drug therapy and on insecticides and acaricides, which give, together with the chemical names, the trade or proprietary names used for them in different countries.

For those who are interested more in the general aspects of progress of veterinary work there are special articles on veterinary medicine in the United States, the Netherlands and France, written by experts in these countries, an article on veterinary medicine in the British Colonial Territories, two others on the challenge of *Brucella* and on the poultry industry and—graceful reminder of the interests of youth nowadays—a well-written and practical article on "The Child, the Pony and the Veterinary Surgeon". The Secretary of the Royal College of Surgeons contributes an article on legal matters affecting the veterinary profession, and there are chapters on new drugs and appliances and a list of new English veterinary books and new editions.

This book, like its predecessor, should be in every library concerned with the study and control of disease, and it is to be hoped that nothing will prevent the appearance of future volumes in the series.

G. LAPAGE

GENERATION OF ELECTRICITY WITHOUT THE USE OF ROTATING MACHINERY

By Dr. K. H. SPRING
Central Electricity Generating Board, London

A COURSE of lectures on direct processes for the generation of electricity has recently been given at University College, London, under the above title. An introductory survey was given by me. The main object of the lectures was to give an idea of the scientific principles and possibilities, rather than to describe the engineering of devices.

The principles of many of the direct processes have been known for a great many years, but the recent rise in interest is probably attributable to several factors. It seems likely that advances in efficiency above about 40 per cent of the heat engines (steam or Diesel) used for large-scale power generation will be achieved only with extreme difficulty. There is a possibility that some of the new processes may ultimately permit a major advance in generation efficiency to be made, and also lead to a lowering of capital costs. New requirements for electric power for military and other specialized uses, such as operation in remote sites or in space-vehicles, have created a demand for compact, simple, and in some cases silent, devices in which the relatively low conversion efficiency so far attained is not of great economic consequence. A further factor is that in recent years advances in technology and physical understanding have enabled new classes of materials with interesting electrical properties to be developed.

The classical effects of an electric current are magnetic, chemical and thermal, and, in the reverse process of generation, it is only the first of these—moving a metallic conductor in a magnetic field—that has been used at all widely. A number of generation processes can now be listed, some of them based on direct chemical and thermal effects. The first three (or possibly four) of these may ultimately be developed sufficiently to supplant or supplement present methods of large-scale generation, but the others are likely to be of specialized application only.

(1) The Fuel Cell

This is an electrochemical device in which the processes occurring are almost the converse of those in electrolysis. Fuel (usually in the form of a gas) is fed to one electrode, and an oxidant to the other. The electrodes are separated by an ion-exchange membrane or an electrolyte which ensures that only ions can move from one to the other, balance being maintained by the flow of electrons, constituting the current, in the external circuit. Because the free chemical energy is released by a directed flow of ions, and not by degradation into a random thermal motion of molecules, the fuel cell acts isothermally and escapes the Carnot limitation inherent in all heat engines. Consequently, the apparent thermodynamic efficiency may be very high—more than 90 per cent. However, as soon as any current is drawn from the cell there are ohmic and polarization losses which lower the open-circuit voltage, just as they do in a primary battery. The fuel cell is usually operated at a current density that gives an efficiency in the region of 60–70 per cent.

Apart from various exotic fuels and oxidants that may be used for special purposes, hydrogen and oxygen (or air) are most easily ionized and combined at temperatures around 20–200° C., and the work of Bacon at Cambridge, and of Shell and other companies, is well known. For central power generation, hydrogen even if impure is too expensive a fuel, and for economic parity with conventional methods it would be necessary to use the cheapest possible gas based on total gasification of coal, or on re-formation of oil products. The use of such a gas requires further development of a high-temperature fuel cell, using a molten salt electrolyte which is stable to carbon monoxide and dioxide, rather than the simple aqueous alkaline electrolyte of the Bacon cell. Research on such cells is being actively pursued in many countries.

(2) Magnetohydrodynamic (MHD) Generation

This is the name which is now employed to denote direct extraction of electrical energy from a fast-moving conducting fluid passing through a magnetic field. The principle is similar to that of an electromagnetic flowmeter which measures the voltage developed between the ends of a conductor moving in a magnetic field. A gas which has been raised to a temperature high enough for some thermal ionization to take place, say 2,500° K., may be used as the working fluid, the conductivity being raised still further by 'seeding' with a low work-function material such as potassium. The upper temperature is limited by the combustion process itself, and also by problems of combustion chamber and duct materials. At the highest temperatures envisaged only a fraction—less than 1 per cent—of the molecules are ionized. The possibility of generating power at appreciable efficiencies, therefore, depends on the transfer of kinetic energy from the uncharged particles to the ions and electrons which alone interact directly with the magnetic field, and thence by some mechanism to an external circuit. Very high gas velocities, of the order of Mach 1, are also needed. Supersonic working would allow higher efficiencies to be attained, but would pose difficult aerodynamic problems.

Because of their high mobility, most of the current in the plasma is mediated by electrons rather than ions and in a continuously acting device electrodes must be provided to maintain charge equality. It is difficult to conceive of electrodes that will emit for long periods in an atmosphere charged with combustion products, and a system of pulsed or cyclic working which will permit inductive coupling may be necessary. The use of electrodes may be feasible in a clean working fluid which is heated indirectly, for example, by a nuclear reactor. Further research may suggest ways in which ionization can be obtained at lower temperatures by non-thermal means. This would be helpful from the point of view of materials, but would be less attractive thermodynamically.

(3) Thermionic Generation

This implies the 'boiling-off' of electrons from a heated cathode surface to a nearby, cool, anode. (A very small current may be obtained from a diode valve with the heater emitting, but with zero anode voltage.) The separation between the electrodes must be extremely small in a vacuum diode (about 0·01 mm.) to minimize the effects of space charge in limiting the current. Such a small separation may not be practicable on a large scale, and attention has therefore been devoted to alternative methods. Most of these are based on the injection of ions into the inter-electrode space. Cæsium in contact with a hot cathode surface readily ionizes, and such cæsium diodes are sometimes described as plasma thermocouples, by analogy with the more usual solid-state thermocouples.

A thermionic generator is a heat engine, and its efficiency is limited to that of a Carnot cycle operating between the temperatures of the cathode and anode. The former should therefore be as high as possible, consistent with the desired life of the diode. In practice, the thermal radiation loss from the cathode to the anode is a factor seriously limiting efficiency. The use of crossed electric and magnetic fields which guide the electrons from one electrode to the other may reduce space-charge effects and also permit geometries which minimize radiation losses. The voltage developed by a diode depends on the difference in work-function between the cathode and anode. One advantage claimed for cæsium injection is that it reduces the anode work function. Development of maximum power depends on proper matching of the load.

(4) Thermoelectricity

Both the theory and practical applications of thermoelectricity have made tremendous strides in the past few years—due in large measure to Joffe. The usefulness of a thermoelectric material depends on three factors—the thermoelectric (Peltier) coefficient α, and the electrical conductivity σ, which should be as large as possible; and the thermal conductivity K, which should be as small as possible. The 'figure of merit' $Z = \alpha^2\sigma/K$. For metals, in which the electrons are nearly free, α is low—only a few μV./deg. C., but in some semiconductors α can be much higher. The three factors contributing to Z are not independent. To obtain the highest value of Z in a semiconductor, the material is prepared, either p- or n-type, by 'doping' to optimize α to a value in the region 200 μV./deg. C. It is found that the highest value of Z which can be achieved depends on the temperature at which the material operates, and ZT gives a truer impression of usefulness. (T is the absolute temperature.)

The value of σ/K is nearly fixed, being determined by the Wiedemann–Franz ratio in metals. In semiconductors the crystal lattice contributes to the thermal and electrical conductivities; it seems likely that it will not be possible to reduce the lattice (or 'phonon') contribution to less than that of the electrons, which would limit ZT to 4, corresponding to 37 per cent of Carnot efficiency for a given temperature-range. In the most advanced materials now available, it is several times higher, and the best values of ZT are about 1 for both p- and n-type materials, corresponding to about 15 per cent of Carnot.

Moderate voltages may be generated by arranging alternate p- and n-type materials which are thermally in parallel, but electrically in series.

(5) Thermomagnetic Generation

If two coils, of which one (the primary) is carrying a current, are wrapped around a core of ferromagnetic material, the flux linkage with the secondary will change if the permeability of the core changes. Thus, an alternating voltage may be induced in the secondary if the core is temperature-cycled around the Curie point. In a sense this may be regarded as a form of parametric amplification of the signal in the primary, the required energy being provided thermally. Gadolinium, with a Curie point of 27° C., has sometimes been suggested as a useful, if expensive, material to use where a source of heat a few degrees above normal ambient temperatures is available. There has been some controversy over the efficiency of the generator. It appears that quite high Carnot efficiencies can be obtained in the immediate neighbourhood of the Curie point, but a range of, say, 20° C. between source and sink may correspond to only 3° on the specimen. This, coupled with the fact that, to cover any large temperature-range, cascading is needed, makes thermomagnetic generation in practice compare very unfavourably with thermoelectric.

(6) Ferroelectric Generation

It is possible to generate voltages of the order of 1,000 by making use of the variation with temperature of the permittivity of a ferroelectric material, such as barium titanate. The material is made the dielectric of a capacitor, which is charged at its Curie temperature at which its permittivity is maximal, through a small battery via a holding diode. If the temperature of the dielectric is changed, the capacitance falls, and the voltage rises, since the diode prevents loss of charge. The permittivity of a dielectric under electric stress is nearly constant at all temperatures below the Curie point, so it is necessary to cycle it from the Curie point to a temperature above it. Again, this method can be regarded as a form of parametric amplification of the input signal supplied by the battery. Circuits have been developed so that, at any instant, a number of capacitors are in different phases of the thermal cycle, and only a small initial charging is needed: this forms a convenient method of generating high voltages for use in space vehicles.

(7) Photovoltaic Converters (Solar Cells)

The earliest type of photovoltaic generator is represented by the selenium photocell, which had a conversion efficiency of about 0·2 per cent when exposed to sunlight. Its modern counterpart, of silicon, has been developed largely as a result of research on transistors. Its action depends on the penetration of radiation to a p–n junction, so the cell is in the form of a disk of n-type material, on the top of which is diffused an extremely thin p layer.

The photovoltaic cell somewhat resembles the thermocouple in embodying *p–n* junctions; but the physical mode of operation is rather different. The former depends on photons penetrating to the junction with sufficient energy to raise electrons from the valence band to the conduction band. Since the forbidden energy gap (activation energy) of silicon is 1·1 V., the radiation must be shorter in wave-length than about 1μ. However, because some electrons are attached to impurity atoms, and are therefore more nearly free, there is some response at wave-lengths beyond the quantum cut-off. The efficiency of present solar cells is about 15 per cent to sunlight, so that 150 W./m.^{2} may be generated at sea-level in clear weather. As the present cost is about £10,000/m.^{2}, only very specialized applications can be considered. Cells based on more advanced materials such as gallium arsenide and cadmium telluride may possibly attain efficiencies of about 25 per cent.

(8) Piezoelectric Converters

When magnetic or electric fields are applied to certain materials there are dimensional changes, and the converse is also true. Thus, when pressure or shear forces are applied to some types of crystal (for example, quartz) a potential difference is created, and this is the principle of operation of some types of gramophone pick-up. Recently, synthetic materials have been developed suitable for the generation of very high voltages and one of the best now available is 'PZT', a solid-solution ceramic of lead titanate–zirconium titanate. Its piezoelectric coefficient is such that a voltage gradient of about 10 kV./in. is produced for a pressure of 1 ton/in.^{2}. The maximum pressure that can be withstood for repeated rapid stressing is in the range 1–5 ton/in.^{2} so that voltages of 20 kV., suitable for petrol engine ignition systems, can be produced from quite small pieces of material by direct pressure and without the intermediary of coils or breakers. Piezoelectric generation is a method for converting mechanical to electrical energy, and therefore is not in competition with other methods for direct generation from heat or fuel. Nevertheless, it is likely to revolutionize the design of electric ignition systems on vehicles.

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MECHANISMS OF EXPRESSION OF GENETIC INFORMATION

By WENDELL M. STANLEY, JUN., and PROF. ROBERT M. BOCK

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DURING several years past, the overall pattern of protein synthesis has become fairly well established. This pattern indicates that the genetic information inherent in the chemical structure of deoxyribonucleic acid (DNA) is in some way incorporated into the structure of ribonucleic acid (RNA), which then possesses the capability, in the presence of suitable precursors, of guiding the polymerization of specific sequences of amino-acids into protein[1]. We have considered mechanisms by which the DNA may be able to carry out such communication mediated by RNA with the rest of the cell.

It is well documented that a molecule of DNA is composed of two strands, one of which is the reciprocal of the other in a base-pairing sense[2]. For this reason, the information in a single DNA molecule is actually repeated, once in each strand. However, it may quite well be that the cell is only able to 'recognize' one of these information strands, at least so far as the polymerization of an RNA molecule is concerned. This would be the net result of either of the two following mechanisms: (1) RNA synthesis is directed by the two-stranded DNA duplex, but can proceed in one direction only; or, (2) RNA synthesis is directed by a single DNA strand, but only one of the strands is capable of serving as a template. This type of restrictive mechanism might result from the same sort of chemical restraints which give the enzyme polynucleotide phosphorylase its unusual specificity with respect to the polymerization of cytidylic residues as compared to guanilic residues[3].

A second alternative, which is functionally equivalent to the above, is to suggest that both DNA strands are capable of coding RNA, but that one of the two RNA strands produced cannot code protein, either because it is left in a form vulnerable to cellular ribonucleases, or because it becomes stabilized in a non-functional ribonucleoprotein complex. This alternative can also be applied to the case in which both RNA strands are capable of coding protein, but with the additional condition that one of the product proteins be regarded as non-functional or non-resistant to proteolysis. This suggestion, which states that one of the two possible RNA strands derived from a DNA molecule cannot make a 'good' protein, is quite logical when one considers that a point mutation in the original DNA duplex will necessarily change both the RNA strands and consequently both their possible product proteins. To expect that evolutionary selection would preserve both proteins in a functional form is not reasonable, in view of current knowledge on the manner in which amino-acid sequence directs protein structure.

The last and most attractive alternative for the communication of information contained in DNA is to postulate that each strand of the DNA duplex directs the synthesis of an RNA molecule complementary to itself. The difference between this alternative and that already discussed arises when we consider what the cellular environment can do with these two reciprocal RNA molecules. We suggest that one of these molecules, on separation from its DNA parent, encounters a specific protein which has both a tendency to aggregate with molecules like itself and a high affinity for certain regions in the

RNA. This affinity may be explained by the ability of this protein to recognize a certain specific sequence of bases repeated at intervals along the RNA molecule. However, it is apparent that mutations affecting this sequence would not destroy its recognizability so long as the general character of these sections is preserved. Thus, evolution might well have modified these repeating regions into sections of similar character the function of which would not be dependent on an absolute sequence. In any event, the RNA-protein complex so formed 'crystallizes' to form microsomes or ribosomes—structures which permit the exposure of the coding bases functional in the sequential ordering of specific amino-acids. This 'crystallization' of the ribosomal protein with its RNA may well be the basis of the mechanisms for the separation of RNA from its DNA parent. (If a similar specific affinity of an aggregating protein occurred for a strand of DNA, this could provide a mechanism by which the small, single-stranded DNA viruses select one strand for building into the nucleoprotein and exclude the other.)

The second RNA molecule, possessing a base sequence reciprocal to that of the molecule already discussed, will not have this affinity for the ribosomal structural protein. However, it could possess an affinity for a second type of protein, which may well be the protein which binds soluble RNA (*S*-RNA) and which is characterized by having the activity of the amino-acid activating and transfer enzymes. However, due to the lack of interaction of this type of protein with itself, this ribonucleoprotein (RNP) is not compact and resistant to cellular hydrolytic enzymes. It is therefore cleaved at all those points where the protein attachment does not protect it, and thus gives rise to a large number of RNP molecules. It is postulated that this family of small RNP molecules can now be acted upon by enzymes which have evolved in the cytoplasm. Certain of these enzymes will trim away parts of the ends of the bound RNA, others will link these modified RNP molecules with nucleotides such as 5-ribouridilic acid, and others will add nucleotides such as cytidylcytidyladenylate to the 3′-end and to the 5′-end nucleotides such as guanidylate, giving rise, finally, to a molecule which has the properties of the amino-acid activating enzyme – *S*-RNA complex, and which further contains within its nucleotide structure those base sequences which are reciprocal to certain regions of the ribosomal RNA. Thus, this *S*-RNA is automatically equipped with nucleotide sequences capable of pairing both with the enzyme activating the amino-acid and with that section of the ribosomal RNA corresponding to the reciprocal sequence of bases which specifies the particular amino-acid.

This hypothesis provides a clear and internally consistent mechanism for the efficient utilization of both strands of information of the DNA, and, further, a mechanism for the synthesis of both soluble and ribosomal RNA. It predicts phenomena which should be usable in testing the hypothesis ; namely, the synthesis of *S*-RNA should not occur without the dependent synthesis of ribosomal RNA; and, assuming equivalent efficiency or 'turnover numbers' of the two complementary DNA strands (this RNA could be modified in quantity but not in quality by the efficiency of the appropriate protein in stripping the RNA from its DNA parent), the induction of a specific family of RNA, such as occurs upon the virus infection of a bacterial cell, should lead to the production of ribosomal and *S*-RNA, the next neighbour patterns of which when tested with isotopically labelled phosphate should follow the same reciprocal rules that have been found for double-stranded DNA[4]. This hypothesis may also offer reasons for various phenomena which have not as yet been satisfactorily explained. For example, it is known that there exist organisms which do not seem to have any of their DNA molecules in common[5], but which still appear to be able to synthesize similar, if not identical, proteins. Using our postulated mechanism of information transfer, this would be explained by assuming that those regions of the DNA responsible for the sections of RNA concerned with the recognition of ribosomal structural protein could differ markedly between two organisms, and thus, even though their equivalent DNA molecules carried the same encoded message, the DNA composition could vary within wide limits. Thus, the same 'code' between nucleic acid and protein can be used in all organisms, without demanding that their DNA molecules be identical. A second problem which may be partially answered is that of the development and intracellular recognition of DNA molecules derived from individual chromosomes. We suggest that each chromosome has its own general repetitive base sequence ; this sequence again being that responsible for the sections of its RNA which have the function of recognizing the ribosomal protein. This would provide a definite distinction between DNA molecules produced in differing chromosomes; a distinction which may be used by the cell to retain DNA molecules within their proper chromosomes through an interaction with the chromosomal protein. This postulate could be tested by the banding, in a high-resolution density gradient[6], of the DNA isolated from organisms with a small number of chromosomes, and observing whether the number of bands is correlated with the number of chromosomes or linkage groups.

We possess little information on the nature of the precursors of the elaborate genetic forms we see to-day—too little to permit any detailed speculation on the early stages of evolution. Nevertheless, the reader may enjoy, as we have, speculating on the way in which a short primitive polynucleotide double strand, one-half with an affinity for peptide which aggregates readily, and the other strand with an affinity for a poorly specific peptide catalyst of polymerization reactions, could provide a base upon which mutation could play until more elaborate forms of life resulted.

After we submitted this manuscript, Prof. G. H. Echols kindly brought to our attention an earlier proposal of one of the above mechanisms for producing template RNA and amino-acid-transferring RNA[7].

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THE 2-METRE UNIVERSAL REFLECTING TELESCOPE OF THE GERMAN ACADEMY OF SCIENCE, BERLIN

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ON October 19, the Government of the German Democratic Republic placed at the disposal of the German Academy of Science a 2-m. universal reflecting telescope, which was designed and built by VEB Carl Zeiss of Jena, and inaugurated on that day in the newly erected Karl Schwarzschild Observatory in Tautenburg, near Jena.

The 2-m. universal reflecting telescope is outstanding as a type and represents the first and largest of its kind in the world. Embracing four different optical arrangements, the new telescope provides for the entire field of present-day astronomical and astrophysical work and the problems which are likely to arise in the future. The optical systems are interchangeable and conveniently adaptable to certain specific problems of observation and, in each instance, afford the same high performance as would a correspondingly large telescope individually built for the respective type of observation.

Thus, for investigation of extra-galactic objects, such as the structure of the Milky Way, as well as for the compilation of routine astronomical observations, the new telescope embodies the Schmidt system with the 2-m. spherical main mirror and 1·34-m. correction plate, with a 4-m. focal length and an aperture ratio of 1 : 3. In this form the Schmidt system of the telescope represents the largest in the world. It permits photographs 24 cm. × 24 cm. to be taken of portions of the stellar regions about 11 square degrees in size.

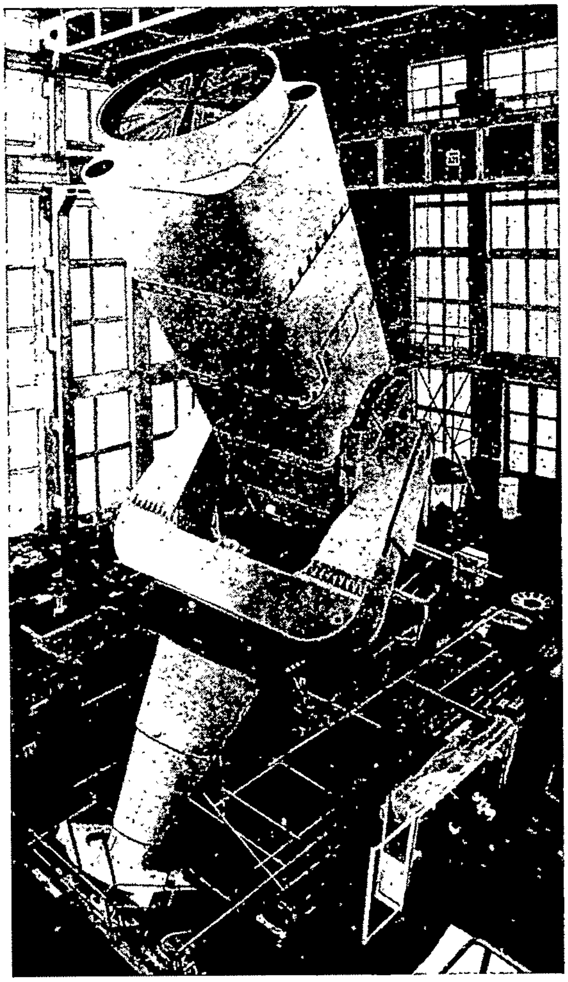

Fig. 1. The 2-m. universal reflecting telescope in the assembling department for astronomical telescopes of VEB Carl Zeiss, Jena

For the individual examination of cosmic gas clouds, distant spiral nebulæ and other faint objects in the universe, the quasi-Newtonian system with its high light-gathering power is employed, for which the full aperture of the reflector is used. In this case a small lens-correction system is interposed immediately in front of the image plane in order to compensate for the errors which a spherical concave mirror produces when used in this way.

With the quasi-Cassegrain system of the new reflector telescope, spectrographic investigations of fixed stars may be carried out down to the faintest luminosities, predominantly by photo-electric photometry and spectrography. Owing to the effective 20-m. focal length of this system, the examination of single stars is possible even in regions of greatest stellar abundance. In the quasi-Cassegrain system a super-hyperbolically deformed convex mirror 400 mm. in diameter is used with the 2-m. concave mirror.

The quasi-coudé system, with the same optical arrangement, but with another convex mirror of greater light-gathering power, and with a 92-m. effective focal length, permits, with the stationary focal image and with spectrographs for the highest dispersions, the study of the fine structure of the spectral lines of stars. Through one of the two bearing journals of the telescope tube, and through the large horseshoe mount, the optical path is conveyed via other plane deflecting mirrors down to the lower end of the main axis of rotation, which is aligned parallel to the Earth and is diverted into the spectrographs located in well-isolated rooms in which constant temperature and humidity are maintained. Astronomical and atomic physicists are using this equipment for research in which both are interested. Indeed, the cosmos affords them research facilities far beyond those available in experimental laboratory activities.

Mechanically, the telescope consists of a square-sectioned tube of about 10 m. length on a horseshoe mounting (Fig. 1). The moving parts of the telescope weigh 65,000 kgm., and therefore special arrangements, including a hydraulic bearing, are used for following the movement of the stars with the necessary accuracy. The telescope floats on an oil film 0·05 mm. thick and is moved with the required accuracy by means of a precision worm-gear 2,160 m. in diameter, the teeth of which deviate from the mean theoretical interspace by less than 0·5 sec. of arc.

The movement of the 26,000 kgm. tube about the declination axis, as also about the polar axis, is via a 'Selsyn' transmitter conveyed to a central control desk and several side desks, whence the instrument may be set entirely electrically for the astronomical co-ordinates of celestial bodies. By means of a number of compensation devices the adjustments and seatings of the optical parts of the telescope in different positions are preserved at maximum accuracy under varying temperature conditions.

The 2-m. main reflecting mirror, weighing 2,370 kgm., as well as the correction plate of thickness 38 mm., and the different auxiliary mirrors, were cast by the VEB Jenaer Glaswerk Schott and Gen., which also attended to part of the preparatory work. What must be looked upon as outstanding performances in the province of astronomical optics are the correction plate, made of glass transparent to ultra-violet radiation and the two Cassegrain and coudé-counter mirrors, the mirror surfaces of which depart from a sphere by no more than 0·05 mm.

The Zeiss spectrographs, which are still in the manufacturing stage, will be equipped with diffraction gratings capable of producing stellar spectra up to a maximum length of 1,500 mm. At the inauguration of the Tautenburg Observatory, the Soviet Academy of Science presented to the Observatory one of these diffraction gratings, in addition to those produced by VEB Carl Zeiss.

The 2-m. universal reflecting telescope and all its appurtenances are housed in an observatory dome 20 m. in diameter and of a total weight of 175 tons, built by VEB Carl Zeiss. The building is heat-insulated, thus effectively preserving the night-temperature within the dome in day-time, despite solar radiation and other heat effects to which the dome may be exposed. Adverse fluctuations of temperature are avoided in this way, or are at least considerably diminished.

While work with this new 2-m. universal reflector telescope has been successfully begun at the Tautenburg Observatory, three more reflector telescopes of the same dimensions are in the process of construction in the Jena Zeiss works at Jena, two for the U.S.S.R. and one for Czechoslovakia. These instruments will benefit by the experience which had been collected during the development, construction and testing of the Tautenburg telescope.

A CHEMICAL BASIS FOR THE CLASSIFICATION OF ANIMAL VIRUSES

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BESIDES satisfying the intellectually tidy, an efficient taxonomy is useful and necessary because it emphasizes resemblances which make worthwhile generalizations apparent, and makes minor differences easier to understand. The possible objection that viruses may not form a single natural taxonomic group need not inhibit attempts to define one. Several systems have in fact been proposed to classify animal viruses[1,2] but have not proved very suitable, and virologists have generally agreed on the need for more data.

However, the earlier systems were based mainly on habitat at the level of the intact animal (that is, host-range, type of tissue usually affected, host-response and type of lesion produced, or vectors), and this now seems a major fault, since many objections can be levelled at such a primary subdivision[3]. For example, 'obviously' different viruses (foot-and-mouth disease and cow-pox viruses) can produce somewhat similar lesions, albeit at opposite extremities, of the same animal. Host-animal reactions may vary with the one virus (for example, chickenpox and herpes zoster[4]), and are a very complex expression of growth at the cellular level. Viruses can often be adapted to unusual hosts without much difficulty. Grouping by known hosts seems an arbitrary procedure, since many hosts may be unknown and thus only a few of the creatures potentially infected are selected; again 'obviously' different viruses (for example, myxoma and yellow fever viruses) can both depend on a similar vector (mosquitoes). Tissue tropism can be genetically very unstable; poliovirus can mutate in one step from neurotropic-plus-viscerotropic, to purely viscerotropic, and back again[5], and host-range variants of encephalomyocarditis virus are easy to obtain by a single growth cycle[6]. Cultured cells normally resistant to intact poliovirus can nevertheless support maturation of virus if infected with ribonucleic acid extracts of infected cells[7]. Adenoviruses have been found in stools[8], and might therefore legitimately be called 'enteroviruses', along with poliovirus.

It is therefore very doubtful whether habitat, as defined above, is at all suitable as a primary criterion for classification of animal viruses, although, as discussed below, it may be useful at another level of emphasis.

All systems of biological classification depend on mutationally stable criteria; indeed, the closeness of all biological relationships might in time be judged by the number of mutations which separate them, although this reasoning is easier to accept for the present when applied to sub-species and to interrelated micro-organisms. The difficulty with viruses is that they are genetically unstable when judged by human time-scales, appearing in a state of rapid evolution, so that a classification denoting species and genera is difficult to apply[9]; attempts at a quantitative appraisal of the genetic differences without emphasizing one criterion more than another are likely at present to run into difficulties for lack of data.

What is needed to provide a lasting system for the classification of viruses is to obtain some primary and secondary sub-divisions which are not too arbitrary and which ought to be genetically quite stable. It is the purpose of this article to emphasize the need for genetic stability, and to point out the utility of a formal system based on lines of thought already familiar to many virologists, the high genetic stability of which, however, is possibly not fully appreciated as a classification criterion.

Nucleic Acid as Primary Criterion

The basis of the system proposed is the chemical structure of the 'virion'[10], that is, the largest free-existing infective unit which can be defined as a single virus particle (usually the infective unit occurring naturally in largest numbers). It is primarily concerned with the type of nucleic acid contained, since this is the one component always essential for the production of infective virus; the chance of interconversion of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) by simple mutation is vanishingly small, if not impossible, so that this criterion should be quite stable. Another advantage is that this criterion must apply to all viruses, yet in an exclusive way. Present genetic concepts demand that a virus must contain either RNA or DNA or conceivably both; if the psittacosis group is excluded, the existence of both in any virus is doubtful, but the possible presence of RNA in a DNA-containing virus (for example, the pox group) need not affect the classification proposed. The only ambiguity may arise in the hypothetical case where the same total of information can be carried by either DNA or RNA in a single form acceptable to a host cell, thus giving two phases for the same virus. Fortunately consideration of this objection can be deferred until it can be raised experimentally. It is submitted that the terms 'RNA virus' and 'DNA virus' are terminologically improper; to avoid the clumsy phrases 'ribonucleic acid-containing virus' and 'deoxyribonucleic acid-containing virus', the terms 'ribovirus' and 'deoxyvirus' are suggested.

Essential Lipid as Secondary Criterion

The secondary sub-division uses the criterion of ether sensitivity, suggested for classification purposes by Andrewes and Horstmann[11]. Destruction of infectivity by lipid solvents (which appears to be correlated with sensitivity to low concentrations of detergents[12,13], and in the case of Newcastle disease virus[14] with sensitivity to lipase) presumably reflects the presence of fatty components essential for infectivity. Those viruses insensitive to ether and detergents either do not contain lipid (for example, poliovirus[15]), or their lipid content is tightly bound or not essential (for example, vaccinia[16]). The genetic stability of this criterion is less certain than that of difference in nucleic acid content, but two facts suggest that it is non-trivial and that it should be fairly stable (that is, not readily lost by non-lethal mutation): (*a*) the ether-sensitive particle of influenza virus breaks down completely when the lipid is removed[17]; (*b*) among riboviruses, the sensitivity to ether correlates with maturation at the cell wall[18], suggesting a widespread mechanism involving lipid materials without which the viruses cannot become infective.

In practice and excepting the pox group, viruses appear either to be quite stable to ether, or to be very sensitive (provided that they are tested in an accessible state and not protected within a mass of tissue), making it a fairly easy criterion to apply. Those ether-resistant viruses so far examined (for example, poliovirus[19], adenovirus[20], polyoma virus[21]) have a strong and regular lattice-like (clathrate) substructure, whereas the ether-sensitive viruses are less rigid[22], tending also to flatten more in electron microscopy. Accordingly, the terms 'clathrovirus' and 'lipovirus' are suggested for the ether-resistants and ether-sensitives respectively, to apply to the infective form only (as some lipoviruses, for example, herpes virus[23], may contain clathrate substructures).

Consideration of Major Groups

When these two types of separation are made, and the viruses the positions of which are known then arranged in each of the four major subdivisions in order of size, a considerable degree of order results (Table 1), and the viruses are brought together in groups which are often immunologically related. Four principal chemical relationships are therefore used: nucleic acid type, content of essential lipid, size and immunological character; it will be noted that 'chemical' is here used in its widest sense, and that among entities of the size of viruses there is little difference between chemical content and fine morphology. There will, of course, be gaps, many known viruses cannot yet be placed, and the placing of some viruses (marked with a query) is based on rather slender data, but the position of most is reasonably well based for at least one member of a serological group.

It will be noted that the pox-viruses are treated as a special case, and this is for two reasons. First, separation into ether-sensitive and ether-resistant has the effect of separating myxoma/fibroma from the vaccinia and avian-pox groups[11], and, although there are some reservations about their relationships[24], recent work[25,26] shows their closeness by reactivation and serological data. Secondly, vaccinia nucleic acid clearly replicates in the cytoplasm[27], whereas the nucleic acid of all other viruses adequately studied (herpes virus[28], adenovirus[29], encephalomyocarditis[30] and fowl plague[31]) replicates in the nucleus. The pox viruses therefore seem metabolically more autonomous than smaller viruses, and there is a strong case for regarding them as a distinct group intermediate between the smaller viruses and the ornithosis group.

Because of its inherent genetic stability the nucleic acid type is preferred as a primary criterion, and, because of its observed genetic instability in some cases, immunological character is set last, but the relative stability of the other characters is not predictable. The viruses also come together in groups of similar habitat, and it may be that this admittedly useful grouping, while unsuitable as a primary criterion, could be applied as a tertiary one.

Deoxyviruses are generally larger than riboviruses although overlap does occur. Lipo-riboviruses tend to be larger than clathro-riboviruses. Thus some generalizations can be made from this system, since the primary and secondary criteria also separate other properties, and this should help the understanding of the structure and growth mechanisms of new viruses if these criteria can be applied.

Table 1. A Classification Scheme for Animal Viruses based on Nucleic Acid Content, Sensitivity to Ether or Detergents, Size and Serological Relationships

						Diameter (mμ)
ANIMAL VIRUS	DEOXYVIRUS (DNA-containing)	Pox-virus	Ether sensitive	Myxoma Fibroma		
			Ether resistant	Animal pox * (vaccinia, etc.) Avian pox * (fowlpox,etc.)		200–300
		Smaller deoxyvirus	Lipovirus (ether sensitive)	Herpesvirus	Simplex *B* pseudorabies	120–150
				Measles ? Rinderpest ? Canine distemper ? } *		
			Clathrovirus (ether resistant)	Adenovirus *		70–90
				Rabbit papilloma		40–50
				SE polyoma		40–50
	RIBOVIRUS (RNA-containing)	Lipovirus (ether sensitive)		Myxo-virus	*NDV*, mumps, parainflu. *	80–120
					Influ. *A*, swine influ., fowl plague *	70–80
					Influ. *B*	
				Rous sarcoma ?		70–80
				Vesicular stomatitis ?		70–80
				Certain arthropod-borne viruses	*A* (*EEE*, Semliki forest, etc.) *	40–50
					B (West Nile, Murray Valley, etc.) *	30–50
		Clathrovirus (ether resistant)		Vesicular exanthema, Reovirus (*ECHO* 10)?		50–70
				Certain entero-viruses	Poliovirus * *ECHO* 8 Coxsackie *A*7, *A*9, *B*1, *B*4, *B*5 Talfan, Teschen	27–32
				EMC, ColSK, *MM*, Mengo *		27–32
				Foot-and-mouth disease		22–25

* Serological group. Data on vesicular exanthema from Shaffer and McClain (personal communication)

Conversely, other properties can help classification when the criteria cannot yet be applied. For example, the large size of measles virus[32], the formation of large intranuclear inclusions and its intracellular mode of growth all suggest that it is a deoxyvirus. Rous sarcoma[11] and vesicular stomatitis viruses are both lipoviruses; there is evidence, not firmly based, that both are riboviruses, and their continuous mode of release[33,34] strongly supports this idea.

At the moment there appears little evidence to contra-indicate the classification of Table 1, although other arrangements are possible; for example, one may regard size as more important than ether sensitivity and promote it to secondary criterion, but the large differences in size among the smaller viruses makes deciding the border-line arbitrary and inconvenient. From a practical point of view, determination of reaction to ether is much easier than determination of size. Good evidence against the proposals would be the finding of strong serological relationships between the main groups differentiated in Table 1.

Correlations with Growth Properties

The separation into four main groups accords well with growth properties, where these are known. In fundamental replication mechanism (that of the nucleic acid) one must expect deoxyviruses to resemble one another more than riboviruses, and vice versa, although a possible exception to this is the small single-stranded DNA-containing phage[35], the replication of which may resemble a ribovirus. The deoxyviruses (vaccinia, herpes and adenovirus as examples) are slow to mature and to be released, and form marked inclusion bodies, but the riboviruses mature much faster and do not form marked inclusions.

The lipo-riboviruses (for example, vesicular stomatitis, influenza, Western equine encephalomyelitis, fowl plague) appear to be matured at the cell wall[18], whereas the clathro-riboviruses mature internally (for example, poliovirus, encephalomyocarditis, foot-and-mouth disease, vesicular exanthema), probably in the cytoplasm. This differentiation does not apply to the deoxyviruses, which all mature internally, but in this case the core of a lipovirus (herpes), although being formed in the nucleus, receives a final coat (and therefore is matured ?) on entering the cytoplasm[28], whereas a clathrovirus (adenovirus) appears to be completed in the nucleus[29].

It is interesting that viruses capable of causing proliferative lesions, even if finally necrotic, occur in all groups except the clathro-riboviruses; hæmagglutinins of one sort or another are also found in all groups except the lipo-deoxyviruses, indicating that neither of these phenomena is related to a particular virus type, and is therefore *per se* of small value for classification purposes.

Means of including more Viruses

It may be felt that it is impractical to determine the nucleic acid present in a large number of viruses in order to classify them, even if one selects only a single member from each serological group. However, this information is urgently necessary, since it is an essential preliminary to understanding the most fundamental part of their growth processes, representing probably the most important single datum, and providing a most useful guide to selecting models for study.

The most direct way of determining the nucleic acid is to examine the purified particle, but this is probably also the most lengthy, as some viruses, particularly the small thermolabile ones, are difficult to purify. Fortunately, there is at least one apparently conclusive and fairly direct short cut. This is to obtain the nucleic acid in an infective form susceptible to nucleases (usually as the free acid), and then to determine its sensitivity to the two species of nuclease. This has now been done with poliovirus, encephalomyocarditis, mengo, Eastern equine encephalomyelitis, Semliki forest, West Nile, Murray Valley encephalitis and foot-and-mouth disease viruses among the riboviruses, and polyoma[36] among the deoxyviruses, and also for the DNA-containing bacteriophages T_2 (ref. 37) and $X174$ (ref. 38); some viruses have so far proved resistant to successful extraction, but may yield to improved techniques.

A more readily applied, if less direct, method[39] is to test the growth-inhibitory effect of 5-fluorodeoxyuridine, which should specifically inhibit DNA synthesis. This compound inhibits growth of a deoxyvirus (vaccinia) but not of a ribovirus (poliovirus), and gives promise of reliable results provided that it can be confirmed with more known examples and that it is used with a host cell which it penetrates successfully (that is, in which it inhibits a known deoxyvirus). This is a very simple test, and there is a strong case for all virus laboratories to run it through their collection of virus strains, as the information to be obtained is of primary importance. A preliminary screening of new clinical isolates, for example, with 20 per cent ether and 5-fluorodeoxyuridine, might speed typing and economize in antisera.

Classification of all Viruses

It is tempting to follow this scheme to its conclusion and include all viruses in the one classification, since superficially plant and bacterial viruses seem separated from animal viruses only by host range. This would have the effect, satisfactory from a structural point of view, of placing all bacteriophages and Tipula iridescent virus alongside the adenoviruses, and plant viruses (for example, tomato bushy stunt virus[40]) with poliovirus; the embarrassment of deciding the correct 'kingdom' for plant viruses which grow in insects (and vice versa) would be avoided. In fact, the fundamental likeness in all biological replications and cellular metabolism makes it nowadays legitimate to question seriously whether the usual separation into plant, animal and bacterial viruses is justified, because this classification may depend only on relatively trivial differences in the virus coating. Convincing evidence of this would be growth of a virus in a host of the 'wrong' kingdom; this might be achieved by unusual manipulative techniques, provided that there is no fundamental impediment. Alternatively, evidence for such an impediment would itself constitute most important information.

Conclusions

In summary, a taxonomy for animal viruses based on type of nucleic acid and essential labile lipid content appears less arbitrary and genetically more stable than those previously suggested, and may provide a useful means of ordering the host of viruses now newly coming to light. The typing of the nucleic acid, a factor of primary importance, is likely to be easier than was hitherto supposed, requiring not very accurate assay procedures but needing some system in which infectivity can be detected; it merits wide consideration. Such a taxonomy might in time include all viruses.

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OBITUARIES

Prof. H. R. Dean

PROF. HENRY ROY DEAN, professor of pathology in the University of Cambridge since 1922 and Master of Trinity Hall during 1929–54, died on February 13, a few days before his eighty-second birthday.

Head-boy of the School at Sherborne, he gained first-class honours and, later, an honorary fellowship at New College, Oxford. He qualified from St. Thomas's Hospital, and was then elected to a senior demyship at Magdalen College, Oxford. Thereafter, on a travelling fellowship, he worked in Berlin with Wassermann. In 1912, he became professor of pathology and bacteriology at Sheffield; and in 1915 professor of pathology and dean of the Medical School in Manchester. He was appointed professor of pathology at University College, London, in 1922 but, before taking up this post, moved instead to Cambridge as professor of pathology there.

Dean's published contribution to medical research was not voluminous, but it laid solid foundations for much of our present knowledge of immunology. His work on complement continued the classical line of Bordet and Wassermann. His work on the precipitin reaction made a quantitative approach to this subject possible. His description of the pathology of anaphylactic shock in man has not been

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surpassed. His realization of the essential unity of the antibody reactions helped to bring order into a confused field. His papers, like those of his great predecessors, are classical in quality, commendable to students.

His second great contribution to medical science was the quality and nature of his teaching. Forty years ago, he was a very great teacher, who insisted on the interdependence of all branches of pathology and medicine. He made his students eager to know more and more about more and more. Pathology he regarded as one great subject, of which bacteriology, immunology, virology, hæmatology and morbid anatomy were all interdependent parts, and liable to suffer from individual elevation to specialist rank. He taught pathology as the true basis of all medicine and as especially valuable for the insight it gave into normal function. He was, thirty and forty years ago, a brilliant lecturer; but his greatest effect was obtained when demonstrating in the laboratory.

His creation of the Part II and Part I Pathology Triposes in Cambridge has benefited many generations of Cambridge medical graduates, and, through them, the whole of medicine.

As a principal leader in the Cambridge Medical School, as Master of Trinity Hall and, during the two difficult and anxious years which preceded the Second World War, as vice-chancellor, Dean's energies were necessarily devoted chiefly to administration. Outside Cambridge, he had many activities; at various times, and for many years at a time, he was chairman of the council of the Imperial Cancer Research Fund, secretary of the Pathological Society of Great Britain and Ireland, a member of the Royal Commission on the University of Durham. He served on the General Medical Council as representative of the University of Manchester during 1919–22, and of the University of Cambridge during 1942–57.

He received several honorary degrees—LL.D. of the Universities of Aberdeen and Western Reserve, honorary D.Sc. of the University of Liverpool. He was a Corresponding Fellow of the New York Academy of Medicine.

Dean was no dilletante, but he was well informed on a very wide range of subjects. To accompany him on a wine-buying expedition for the College; or to handle and appreciate some of the silver he had collected; or to inspect his seedling viruses in Trinity Hall; or to climb mountains with him, or swim with him, or to follow the boats in Mays or Lent races, or sit with him on boards and committees, or hear him quote Horace with a conscientiously archaic pronunciation, or just to drop in for a chat with him—these were all memorable experiences, and for the memory of them one is grateful. But one is chiefly grateful for the example of staunch, objective honesty of outlook on everything that came within his ken, and for the irrepressible humour of his commentary on it. An anonymous contributor to *The Lancet* has said "I owe it to him that I have had such an interesting life". Many others would acknowledge the same debt.

In 1908 he married Irene Wilson, who died in 1959, after being his close partner and perfect counterpart throughout a long joint career. Mrs. Dean shared the affection and regard in which he was held by many hundreds of younger people whom they helped, encouraged, guided and entertained.

E. T. C. Spooner

Prof. Jerome T. Syverton

Jerome T. Syverton, whose death occurred on January 28, began an outstanding, active career in microbiology as an instructor in bacteriology at the University of North Dakota in 1928. Born in Courtenay, North Dakota, in 1907, he entered the University of North Dakota in 1923, obtaining the A.B. degree in 1927 and the B.S. degree in 1928. He graduated from Harvard University School of Medicine in 1931, and after an internship and assistant residency in medicine at Duke University Hospital in 1931–32, he became an assistant in pathology and bacteriology at the Rockefeller Institute for Medical Research, New York. There he obtained basic knowledge in virology under the guidance of Dr. P. K. Olitsky, and in 1932 became a member of the faculty of the University of Rochester School of Medicine and Dentistry.

Except for a sabbatical leave in 1942 at Vanderbilt University School of Medicine as visiting associate professor of pathology and bacteriology, Syverton's fundamental and pioneering work involving, for example, tumour viruses and multiple viral infections of single animal cells, as well as the development of his outstanding skill as a teacher and student adviser, was done during 1934–47 while in the Department of Bacteriology at the University of Rochester. For his outstanding research in virology, he received the Lilly Award in Bacteriology and Immunology in 1938. During 1944–46 he was on active duty in the United States Navy as a visiting investigator at the Rockefeller Institute, and member of Naval Medical Research Unit 2 in the Pacific Theatre. In 1947 he became professor and head of the Department of Microbiology at Louisiana State University School of Medicine, and since 1948 had been professor and head of the Department of Bacteriology at the University of Minnesota.

During his academic career Syverton's enthusiastic and vigorous approach to professional life, with simultaneous devotion to his family and friends, set an extraordinary example for students and associates. His graduate and postdoctoral students during twelve years at the University of Minnesota alone numbered more than 65. His productivity as an investigator was remarkable, and during 1957–58 he was recipient of the Commonwealth Fund Award for Creative Work. Eighty-seven articles describing his own work and 119 in conjunction with his students and associates were published between 1933 and 1960. His stature in science and academic medicine was evidenced by his membership of many scientific advisory committees, and his enthusiastic support of science at an international and national level was widely recognized and appreciated. The world's scientific community has lost a creative and productive investigator, the educational community an enthusiastic teacher, the community of his colleagues and associates a true friend, and his family an affectionate and lovable father.

William F. Scherer

Prof. B. Davison

Physicists and mathematicians connected with the British and Canadian atomic energy projects have learned with regret of the sudden death of Boris Davison on January 24, while still a very productive mathematical physicist engaged on problems of neutron transport.

He was born in Gorky on July 10, 1908, and educated in Russia. He graduated from the University of Leningrad in 1931, and was engaged in hydrology for about seven years thereafter. Through his English grandfather he had a tie with England, and when in 1938 he was requested either to renounce British nationality or leave the U.S.S.R. he elected to go to England. He worked for a short period with Prof. L. Rosenhead, but his health broke down. After a considerable period of convalescence he joined the atomic energy research team at the University of Birmingham under Prof. R. E. Peierls, in 1942. His powers as a mathematician enabled him to make many important contributions, particularly to neutron transport theory, a subject which was to occupy most of his attention for the rest of his life. For some of this work he was awarded the Ph.D. of the University of Birmingham in 1944.

His connexion with Canada started when he joined the Montreal Laboratory of the joint United Kingdom–Canadian Atomic Energy Project in 1943. His association with the late Dr. G. Placzek was a very fruitful one, and he accompanied Drs. Placzek and Mark to Los Alamos. He moved to the new Chalk River Laboratory as a member of the United Kingdom staff in the Theoretical Physics Branch and in 1947 returned to England to work at Harwell. In 1954 he joined the staff of the Computation Centre in the University of Toronto, lectured in numerical analysis and reactor physics and was appointed associate professor of physics in 1960. For the past six years he served Atomic Energy of Canada, Ltd., as a consultant. His knowledge, experience and talent have contributed in many ways to the technical success of the Canadian Atomic Energy programme.

In 1957 his book on *Neutron Transport Theory* was published: it established him publicly as the authority in this subject to which he brought a remarkable combination of analytical power and precision, an uncommon facility in numerical analysis and physical perception. Not only has he expounded the methods of other mathematicians but he has also delineated carefully the boundary of the region within which they can be effectively applied. In his preface he was generous in acknowledging assistance, but those who knew him know well his patience and the exacting standards he set for himself.

Much of Davison's work was concerned with improving mathematical representation of nuclear processes in reactors and with the associated computational problems. He was a ready inventor of algorithms for computing and for avoiding pathological conditions in large calculations.

He was a gentle, shy man, painstaking, kind and generous in helping his fellows, but intolerant of pretence. His intellectual life was not confined to the subject of his specialty. He had a broad, modern interest in science and he liked poetry.

He is survived by his wife Olga.

W. H. WATSON

NEWS and VIEWS

The First Manned Earth-Satellite, 1961μ

THE spaceship-satellite *Vostok*, launched from the U.S.S.R. at 06.07 U.T. on April 12, carried the first man to make a journey in orbit about the Earth, Major Y. A. Gagarin of the Soviet Air Force. The satellite, which weighed 4,725 kgm. (10,417 lb.) excluding the final-stage rocket, was projected into an orbit inclined at 65° 04′ to the equator, and having an orbital period of 89·1 min. Its height above the Earth varied between 175 km. at perigee and 302 km. at apogee. The satellite, designated 1961μ1, made one revolution about the Earth: its deceleration by retro-rocket motor began at 07.25 U.T. and the cabin was safely and successfully brought to Earth in the U.S.S.R. at 07.55 U.T., close to the pre-determined area. The satellite's radio transmissions were on frequencies of 9·019, 20·006 and 143·625 Mc./s. It is reported that on his historic flight Major Gagarin was able clearly to see coastlines, mountain ranges and large cities, and that to him the Earth appeared light blue in colour against a very dark sky. The final-stage rocket, 1961μ2, which separated from the satellite, remained in orbit for several days.

Major Yuri Alekseyevich Gagarin

The Henry Draper Medal: Dr. M. Schwarzschild

DR. MARTIN SCHWARZSCHILD, professor of astronomy, Princeton University, has been awarded the Henry Draper Medal of the U.S. National Academy of Sciences for his work in the field of stellar evolution. Dr. Schwarzschild was born in Potsdam, Germany, in 1912, and took his Ph.D. degree in astronomy at the University of Göttingen in 1935. He was a Research Fellow at the University of Oslo during 1936–37 and at Harvard College Observatory during 1937–40.

In 1940, he went to Columbia University as a lecturer on astronomy and was appointed assistant professor in 1944. Joining the faculty of Princeton University in 1947 as a professor of astronomy, he was appointed to the Eugene Higgins chair of astronomy in 1950. Dr. Schwarzschild is widely known as the director of the Project Stratoscope, in which the surface of the Sun was photographed from an unmanned balloon some fifteen miles above the Earth's surface. Analysis of the data obtained from these photographs contributed much towards the understanding of the mechanisms of heat transfer from the centre of the Sun to its surface. For this work Dr. Schwarzschild and two of his colleagues received the Newcomb Cleveland Prize of the American Association for the Advancement of Science in 1957. Also of great importance are Dr. Schwarzschild's computations of the evolutionary track of stars and his study of their internal composition. He is a faculty member of Princeton's James Forrestal Research Center Program in Advanced Physics, and a member of the U.S. National Academy of Sciences.

Theoretical Physics at Birkbeck College : Prof. D. Bohm

PROF. DAVID BOHM, whose appointment is announced to the new chair of theoretical physics at Birkbeck College, London, is well known among physicists as the originator of the Bohm–Pines theory of collective motions in a plasma. He is known to a wider public for his views on the philosophy of science, particularly his criticisms of the interpretation of quantum theory advocated by the school of Niels Bohr. Prof. Bohm, who was born in the United States, was educated at Pennsylvania State College, California Institute of Technology, and the University of California. During the Second World War he worked (for the U.S. Atomic Energy Project) on the electromagnetic separation of isotopes. After the War he became assistant professor at Princeton, and later professor successively at Sao Paulo, Brazil, and the Technion, Haifa, Israel. Since 1957 he has been on the staff of the University of Bristol. He is a physicist of great inventiveness and imagination, whose stimulus and leadership have set several others on the pathway to success in theoretical physics.

Harvard Museum of Comparative Anatomy

PROF. ERNST MAYR has been appointed director of the Harvard Museum of Comparative Zoology as from July 1. Founded by the great nineteenth century naturalist Louis Agassiz, the Museum is one of the very few major centres of natural history research in the United States. The present director, Dr. Alfred S. Romer, who has guided for the past fifteen years the Museum's growing eminence as a centre for the study of animal life, past and present, will continue to teach and do research at Harvard. Both Romer and Mayr hold Alexander Agassiz professorships at the Museum. Prof. Mayr has made important contributions to evolutionary theory during the past thirty years.

Staff College for Further Education

MR. W. J. RICHARDS, director of the Royal Radar Establishment, Malvern (see *Nature*, 172, 277; 1953), has been appointed the first director of the proposed new Staff College for Further Education by the Governing Body, set up by the Minister of Education last autumn under the chairmanship of Lord Fleck. Mr. Richards will take up his new appointment in July. The aim of the College is to create a national centre at which principals and other selected staff from colleges of technology and commerce can exchange ideas and experience with senior staff from industry, local and central government, the universities and overseas, and where they can jointly study the aims, ideas and the growth of education in the light of the rapid advances in science and technology. A capital fund of £100,000 has been contributed by industry to meet the initial expenses of founding the College, and the Minister of Education has undertaken to contribute an annual grant towards the running expenses. The College is to be entirely residential and a search is being made for suitable premises, preferably in the Midlands, around which the College can be developed.

Harkness Fellowships of the Commonwealth Fund

THE following have been included in the recent appointments to Harkness fellowships of the Commonwealth Fund: J. N. Ball (Liverpool), zoology; J. P. Cornford (Trinity, Cambridge), sociology; J. E. Everett (Clare, Cambridge), geophysics; B. M. Funnell (Trinity, Cambridge), oceanic geology; J. V. Greenman (Gonville and Caius, Cambridge), theoretical physics and applied mathematics; J. M. Hollis (New College, Oxford), philosophy; D. H. P. Jones (Christ's, Cambridge), astronomy; M. C. Scott (Birmingham), nuclear physics; J. J. Turner (King's, Cambridge), infra-red spectroscopy; E. G. Wilson (King's, Cambridge), physics. The following were included in the awards from Australia and New Zealand: N. Sneath (Melbourne), engineering; J. J. Veevers (Sydney), geology. Ten fellowships have been awarded to candidates from France, Italy and the Netherlands.

Commonwealth Students in British Universities

IN a written answer in the House of Commons on April 11, Mr. B. R. Braine, Joint Under-Secretary of State for Commonwealth Relations, stated that in July 1959, 3,457 students from the Commonwealth overseas were studying, in United Kingdom universities, the applied sciences to which paragraph 55 of the report of the Commonwealth Education Conference relates. In the academic year 1959–60, this number was 3,338, but figures for 1960–61 are not yet available. More places are expected to become available for such students as the total number of places in United Kingdom universities increases, but the exact number of additional places which would be taken up in 1961–62 cannot be forecast. In 1958–59, 177 students from the Commonwealth overseas were enrolled for full-time research at United Kingdom universities in agriculture and forestry; 130 in dentistry; 1,475 in medicine; 1,608 in technology; and 67 in veterinary science; for 1959–60, the corresponding figures are: 202; 145; 1,477; 1621; and 93. On April 13 a written answer by the Minister of Education, Sir David Eccles, gave the number of Commonwealth students in colleges of further education in England and Wales as 7,203 in the current session, compared with 6,493 in 1959–60. In the present session, the number of Commonwealth students in technical colleges is 477, an increase of 25 on 1959–60.

Employment of Scientists in Government Services

MOST of the March issue of *State Service*, the journal of the Institution of Professional Civil Servants, is occupied by the annual report of the Association; but the general secretary, Mr. Stanley R. Mayne, directs particular attention to the difficulty experienced by the Ministry of Aviation in filling posts at principal scientific officer-level as an example of the reluctance of scientists to enter the Scientific Civil Service. The press advertisements only produced five applications, and only one of these was judged worth interviewing, and he was not good enough to be selected. The Department had four other candidates, of whom three were selected and offered appointment but the only applicant from industry declined the offer. Three other scientists were afterwards interviewed and two were selected; one of these has already resigned. The annual report for 1960, which records a membership of 53,923, quoting the figures for recruitment of scientists from the recent report of the Civil Service Commission which are causing the Commissioners grave disquiet, is critical of the value of information collected by the Civil Service Pay Research Unit other than as a broad basis for discussion.

Valuation and Rating of Scientific Institutions

THE annual report for 1960 of the Parliamentary and Scientific Committee (Pp. 22. London: Parliamentary and Scientific Committee, 1961) contains a concise summary of the addresses and discussions during the year and of Dr. J. Killian's speech at the annual luncheon. It refers also to the European Parliamentary and Scientific Conference sponsored by the Committee, with the financial support of the Council of Europe, the Organization for European Economic Co-operation and the Foreign Office, in London, during March 21-23, 1961. The Rating Sub-Committee has again considered the position of scientific societies, and at a meeting on December 5 it was agreed to seek amendments to the Rating and Valuation Bill to secure that hereditaments given mandatory 50 per cent rating relief under clause 8 (1) include all hereditaments properly established for scientific purposes, and that the power given to rating authorities to reduce or remit the payment of rates chargeable in respect of such premises should be transferred to the Ministry of Housing and Local Government so that there should be central decision. It was also agreed to seek similar mandatory 50 per cent relief for all organizations qualifying for freedom from income tax under section 449 of the Income Tax Act, 1952 (relating to research associations).

Smithsonian Institution

THE annual report for 1960 of the United States National Museum is as usual a well-illustrated, bulky volume giving details of much curatorial work, an impressive account of research undertaken by, or in collaboration with, the staff, and a list of donors and their gifts covering nearly 2,500 accessions (Pp. vi + 175. Washington, D.C.: Government Printing Office, 1960). The modernization programme started about a decade ago continues in all departments, and the construction of the Museum of History and Technology is in progress and will be completed by March 1962. The building has been designed so that the collections will be shown under the best known modern conditions, and also maximum flexibility within the structure for future development has been a prime consideration. In geology events such as the development of jaws and the transition of life from water to land have been demonstrated by diagrams and models. Among the unusual specimens displayed are a 14-ft. skeleton of the predatory Cretaceous fish *Xiphactinus*, and a slab from New Mexico showing the crowded bones of the amphibian *Buettneria*, entrapped in an evaporating Triassic swamp.

The British Leather Manufacturers' Research Association

THE annual report for 1959-60 of the British Leather Manufacturers' Research Association records a membership of some 75 per cent of the productive industry with a net increase in membership of 15 and a total income of £70,134, including a grant of £21,900 from the Department of Scientific and Industrial Research (Pp. 70. Egham, Surrey: British Leather Manufacturers' Research Association, 1960). An effluent liaison officer and assistant analyst have been appointed to recommend and demonstrate modifications in leather-making processes designed to reduce effluent problems, and a new radioisotope research laboratory has been built, particularly for the study of chrome complexes in leather by radioisotope techniques. The report of the Director of Research refers to the maintenance of fundamental research on the constitution and properties of collagen and other constituents of hides and skins, the chemistry of vegetable tanners and the mechanical and elastic properties of collagen and leather fibres. In the enzyme unhairing of hides a bath-treatment at 80° F. for three days ensured consistent removal of short hairs with the long hairs, and small-scale trials indicate that no great difficulties should be experienced in vegetable tanning. An investigation of the factors in chrome-tanning processes which are important for satisfactory dyeing has commenced, and the leather properties which are required for good acceptance of pigment finish and good coverage are being investigated; investigations are being made on suede leather with the view of improving the colour fastness, particularly of black suede. Work on the adaptation of semi-chrome upper leather for moulded-sole footwear continued, but mechanical engineering work was discontinued and engineering work will be limited to chemical engineering topics, including effluent problems. Work on the deterioration of leather by heat, moisture and perspiration has led to substantial advances in an understanding of the deterioration of leather by perspiration; but in work on waterproofing of leather the study of laboratory testing methods now has priority, and an extensive testing programme in connexion with sheepskins and sheepskin leathers has been necessary. Besides outlining the programme of research, the report includes a list of publications during the year.

The Cawthron Institute

THE annual report for 1959-60 of the Cawthron Institute, Nelson, New Zealand, records some changes in the scope of the Institute's research activities, and its facilities for investigating the more fundamental aspects of plant nutrition have been extended by new equipment (Pp. 35. Nelson, N.Z.: Cawthron Institute). The Director's report reviews the progress of pasture research, hop research and tomato research at the Institute and of the chemical investigation on the constituents of the tobacco leaf responsible for variations in quality. Besides a list of publications, the report includes five short special articles. E. T. Chiltendon describes investigations on bitter pit in apples ; E. B. Kidson, chemical investigations on tomato plants in water culture ; D. J. Stanton, further experiments on the treatment of tomato seedlings with *n-m*-tolylphthalamic acid ; T. B. C. Christie, the effect of fertilizer applications on the *Phytophthora* disease of hops ; and R. J. Monk, work on alkaloids and smoking quality of tobacco.

The Brotherton Library, Leeds

The annual report of the Librarian of the University of Leeds, for the session 1959–60, records holdings at July 31, 1960, of 479,157 volumes and 179,215 pamphlets, of which 334,099 volumes and 117,306 pamphlets are in the Brotherton Library (Leeds: The Brotherton Library, The University. Pp. 12. 1960). Accessions during the year totalled 15,842 volumes, 3,281 pamphlets and 31,907 periodical parts. Loans to readers numbered 116,739 and inter-library loans 1,814 books borrowed and 3,449 lent. The report refers briefly to the extension of the Brotherton building proposed in the University Development Plan published in May 1960 which contemplates incorporation of an additional bookstack for 400,000 volumes, together with further reading rooms and larger administrative quarters, and a separate building for undergraduates with space for 800 readers and 30,000–50,000 volumes. The proposals are being studied and, meanwhile, a further investigation of undergraduate use of library services is being made.

Raw Materials of Ceramics

In *The Museums Journal* of March 1961, Mr. P. J. Adams describes an exhibition which was arranged to mark the occasion of the Seventh International Ceramic Congress and suitably modified will form a permanent feature at the Geological Survey and Museum, London. The exhibition is divided into three sections reflecting the main branches of the British ceramic industry: (1) pottery and whiteware; (2) structural clay-products; and (3) refractories and technical ceramics. Each section consists of a simple wall-type display with a single waist-high bench for the geological and ceramic specimens and a back-screen for narrative text, geological maps, photographs and diagrams. The nature, geological occurrence and methods of exploitation of the raw materials are briefly described and their processing and utilization are summarized.

Selenium Poisoning

Selenium poisoning is known to be a disease of domestic animals in the United States. Wheat, corn and milk produced in western and southern districts of the United States contain small quantities of selenium. Animal and vegetable foodstuffs from the United States make up an increasing part of the diet of Japanese school-children and this has led to investigations into experimentally produced selenium poisoning in various animals (*Yokohama Med. Bull.*, 11, No. 5; October 1960). The investigations were carried out by Hiroshi Tsuzuki, Kazuhoshi Osawa and Takashi Hosoya of the Department of Hygiene, Yokohama University School of Medicine, Yokohama, and showed that selenite had the greatest toxicity among animals. It disturbed the body weight, tibial length and physical strength of animals when it was given intraperitoneally and orally. Inhaled selenium vapour disturbed physical functions, but the toxicity varied because of varying degrees of selenium in the air. Metallic selenium, which was given orally, disturbed digestive functions temporarily and greatly influenced gain in weight and physical strength; it suppressed the development of tibial length only slightly. Selenium in ointment, rubbed on the skin of animals, caused a remarkable dermatitis, and a decreased gain in weight, but there was only slight suppression of tibial length and physical strength. Generally, females were more disturbed physiologically and developmentally than males. The second generations which were born from poisoned mothers seemed to have higher resistance to selenium than other animals.

Internal Carotid Artery Occlusion

In recent years, internal carotid artery occlusion has been diagnosed frequently in young adults. A study by John G. Humphreys of the Department of Medicine, University of Toronto, and T. H. Newton, Department of Radiology, University of California, of seventeen patients of less than 40 years of age with carotid occlusion shows how the underlying mechanism accompanying the occlusion may differ from that in older patients in whom the cause is atherosclerosis (*Brain*, 83, Part IV ; 1960). There were thirty-three older patients whose carotid occlusions occurred after the age of 40. There was little difference in the sex incidence of the younger group compared with the predominance of males in the older group. Eleven of the seventeen patients in the younger group either had lesions adjacent to the carotid artery or had evidence of a generalized systemic illness at the time the carotid occlusion was diagnosed. Four of the thirty-three patients of more than 40 years old had similar complicating factors. The complications of both groups included trauma, aneurysm, tumour, pregnancy, and inflammatory diseases. Hypertension, diabetes and atherosclerotic involvement of other major arteries were noted in only one of the seventeen younger patients, but were present in thirteen of the thirty-three older patients. The prognosis for survival following an internal carotid artery thrombosis is better in patients of less than 40 years of age.

Dietary Fats and Cholesterol

In recent years the number of people dying from the sequelæ of vascular diseases has been as great in the Netherlands as highly industrialized countries. The influence of various dietary fats on the blood cholesterol content has been investigated by F. S. P. Van Buchem, J. J. Speelman, A. A. Idema, A. T. Valkema and H. J. Thomasson, and is reported in the recent *Proceedings of the Royal Netherlands Academy of Sciences* (Series C. Biological and Medical Sciences, 44, No. 1; 1961). The twenty test subjects were physically healthy, were mentally defective and were constantly under clinical and dietetic supervision. Before the test diets were given, the spontaneous variations of the serum cholesterol-level of most test subjects were studied for several months. The test diets used only differ from normal diets in that different fats, but in the same amounts, were given. If maize oil was used as a fat, the serum cholesterol-level fell in all of them, but it rose on cocoa fat feeding. The influence of various mixtures of maize oil and cocoa fat was also studied. The phospholipid content of the blood fell also with maize oil, but not so strongly as the cholesterol-level. The influence on the neutral fat differed in the various test subjects. The cause of this rise or fall of the serum cholesterol-level has not been determined.

New Species of Rotifer

FROM a pool (*p*H 6–6·5) on Cawston Heath, eleven miles north-east of Norwich, a hitherto undescribed *Collotheca* sp. with only four coronal knobs has been taken occasionally since 1957. This rotifer takes its place in the list with the two four-lobed members of the genus so far recorded, namely, *Collotheca quadrilobata* (Hood) and *C. monoceros* (Zacharius). H. G. S. Wright, in an article appearing in a recent issue of the *Journal of the Quekett Microscopical Club* (Series 4, 5; No. 13; 1961) regards this as a doubtful identification, the description and figure given by Zacharius strongly suggesting a mutilated *Stephanoceros fimbriatus* (Goldfuss). The new rotifer has the following specific characters; the corona is quadrate in plan and its base not wider than the body, with a flattened truncate lobe at each angle and a prominent lip on the ventral edge. There is no dorsal lobe, there are long cilia on the lobes, and on the dorsal and lateral rims of the corona is an inner series of short inactive cilia and an outer series of cilia in constant flagellatory motion. The long foot is frequently four times, or even more, the length of the corona and trunk combined. Wright proposes the name *Collotheca quadrinodosa* n. sp.

The Brandberg and Other Rock-Shelter Paintings of the Union of South Africa

THE Abbé Breuil's contention that the Brandberg paintings were of vast antiquity and the so-called 'white lady' a portrait of a European has been hotly contested by prehistorians in South Africa. Some years ago the late Dr. van Riet Lowe suggested that the date could equally well be A.D. 1600 or 1600 B.C. Indeed, nowadays, it is becoming more and more difficult to assign any great age to the rock-shelter paintings in the sub-continent. Revel Mason further points out that though some of the painted individuals seem to show Caucasoid features, such features are common among the southern Bantu to-day. Again, the smearing of white paint on the face for certain ceremonial purposes is not unknown. The physical characters shown in the Tsisab paintings, therefore, give no very certain evidence as to racial type, but the clothing, ornaments, etc., depicted fit in with those found among primitive tribes south of the Zambesi. There is no need to suggest relationship with Cretan or Egyptian originals, or to advocate any very great antiquity for this art. Revel Mason is well known among the younger prehistorians in South Africa. Under the auspices of the Archæological Survey of the Union of South Africa, Pretoria, he has published a pamphlet full of coloured illustrations dealing with his discoveries of a new series of rock-shelter paintings in the Brandberg, south-west Africa and another series at Waterberg, Northern Transvaal. While the whole is only a few pages in length, it is an attractive little work which adds to our knowledge of South African rock-shelter art.

Scientists Working in Antarctica

THE Special Committee on Antarctic Research of the International Council of Scientific Unions now maintains a register of scientists wishing to work on Antarctic expeditions of other nations. The Committee is merely acting as a centre which may put suitable scientists from other countries in touch with national committees which can use their services to advantage. Further arrangements for the employment of individuals are then a matter of negotiation between the expedition organization and the person concerned. Men with previous polar experience would be most suitable. Registration forms are available from the Assistant Secretary, Royal Society, Burlington House, London. W.1.

Ford Foundation Grant to the Zoological Society of London

THE Zoological Society of London has received a further grant of 95,000 dollars from the Ford Foundation to supplement the grant of 550,000 dollars announced in May 1960. Both these grants have been made to support a programme of research in animal reproduction. The original grant of 550,000 dollars from the Ford Foundation was to endow a series of research fellowships, and the appointments of Dr. Peter Jewell, Dr. Lorna Kellas and Mr. David Kirby to the first three fellowships were announced in February of this year.

Royal Geographical Society Award for Women

As the result of a bequest from the late Lady Dorothy Mills, Fellow of the Royal Geographical Society, an award of £1,000 has been established, to be known as the Lady Dorothy Mills Award. The Award will take the form of a studentship involving some element of adventurous travel or exploration, by land or air, tenable for one or more years within the limits of the sum available. Applicants (of any nationality) must be women over twenty-one and preferably under the age of thirty years. They must be Fellows of the Royal Geographical Society. The study which forms part of the candidate's programme may be in any field of science or the humanities, provided that it has some geographical content or aspect. Further information can be obtained from the Director and Secretary, Royal Geographical Society, 1 Kensington Gore, London, S.W.7. Applications must be submitted by February 12, 1962.

International School for Research and Education in Physics

AN international school for research and education in physics has been established at the University of Uppsala. The school is being jointly sponsored by the International Atomic Energy Agency, the United Nations Educational, Scientific and Cultural Organization and the Central Committee for Swedish Technical Assistance. The school is offering one-year courses, the first of which will start on September 1 and finish about July 1, 1962. The aim of the school is to provide possibilities for individual participation in qualified experimental research work in one of the various fields of physics under experienced guidance. The school is open to participants from all countries and a number of fellowships will be available. Further information can be obtained from the International Seminar, Institute of Physics, University of Uppsala, Uppsala, Sweden.

National Agricultural Advisory Service

RECENT changes in the National Agricultural Advisory Service include the following: Mr. J. Butt-Evans, at present county advisory officer for Durham, to be county advisory officer for Yorkshire (North Riding), in succession to Mr. W. S. Rayfield, who was recently transferred to headquarters; Mr. G. P. Chater, at present hops adviser in the West Midland Region, to be director of the Rosemaund Experimental Husbandry Farm, Preston Wynne, Hereford, in succession to Mr. E. L. Jones, who succeeds Mr. W. R. Smith as one of the two livestock husbandry officers for Wales.

American Geophysical Union : Officers

THE triennial elections of the national officers of the American Geophysical Union have recently been held, and the following have been elected for a three-year period from July 1: *President*, Dr. Thomas F. Malone, director of research, Travelers Insurance Co., Hartford, Connecticut; *Vice-president*, Dr. George P. Woollard, professor of geophysics, Department of Geology, University of Wisconsin; *General Secretary*, Dr. A. Nelson Sayre, senior scientist, U.S. Geological Survey, Washington, D.C.

The Night Sky in May

NEW moon occurs on May 14d. 16h. 55m. U.T. and full moon on May 30d. 04h. 38m. The following conjunctions with the Moon take place: May 6d. 12h., Saturn 3° S.; May 7d. 00h., Jupiter 3° S.; May 11d. 16h., Venus 4° N.; May 20d. 18h., Mars 4° N.; May 22d. 13h., Regulus 0·7° N. In addition to these conjunctions with the Moon, Mercury is in conjunction with Aldebaran on May 15d. 22h., Mercury being 7° N. Mercury is an evening star, and is visible in the west after sunset; on May 31 it sets about two hours after the Sun. Venus is a morning star, but it is too close to the Sun for easy observation. Mars is an evening star, but conditions for observation are becoming less favourable. It sets about midnight; its stellar magnitude is + 1·5 on May 15, and its distance from the Earth on May 15 is 158 million miles. Jupiter rises at 1h. 40m., 0h. 50m. and 23h. 45m. on May 1, 15 and 31, respectively. It is in Capricornus, its stellar magnitude on May 15 is – 2·0 and its distance from the Earth on that date is 440 million miles. Saturn rises at 1h. 20m., 0h. 45m. and 23h. 20m. at the beginning, middle and end of the month, respectively. It is near the border of Sagittarius and Capricornus; on May 15 its stellar magnitude is + 0·7 and its distance from the Earth is 890 million miles. There is one occultation of a star brighter than magnitude 6 visible at Greenwich; May 2d. 23h. 16·6m., 24 Sco., reappearance.

Announcements

Prof. Jean Brachet (Brussels) and Prof. Hans Bauer (Wilhelmshaven) have been awarded the Schleiden Medal of the Leopoldina German Academy of Science, for their outstanding work in the field of cytology.

DR. B. P. PAL, director of the Indian Agricultural Research Institute, New Delhi, has been awarded the first Rafi Ahmed Kidwai Memorial Prize of the Indian Council of Agricultural Research, for his work on the breeding of Pusa wheat varieties.

Mr. E. S. Calvert and Mr. J. W. Sparke, both of the Ministry of Aviation's Royal Aircraft Establishment, Farnborough, have been awarded the 1961 Laura Taber Barbour Air Safety Award, in recognition of their "major contribution to world air safety through joint development of the Line and Bar Approach Lighting System and the Visual Glide Path Indicator now installed and in use at many major airports". The Award is made annually by Flight Safety Foundation Award Inc., of the U.S. Society of Automotive Engineers Inc.

THE Duke of Edinburgh will open the McFadzean Laboratory at the Wood Lane research centre of British Insulated Callender's Cables, Ltd., on May 16.

THE Bradford Institute of Technology is organizing a special short course on "Methods of Polymer Characterization and Analysis" during June 23–24. Further information can be obtained from the Registrar, Institute of Technology, Bradford 7.

THE Biology Department of Brookhaven National Laboratory is organizing a symposium on "Fundamental Aspects of Radiosensitivity" during June 5–7. Further information can be obtained from Dr. A. H. Sparrow, Brookhaven National Laboratory, Associated Universities Inc., Upton, L.I., N.Y.

THE Applied Spectroscopy Group and the Manchester and District Branch of the Institute of Physics and the Physical Society are holding a joint one-day meeting on "Spectroscopic Studies using Time Resolution Techniques", on June 16. Further information can be obtained from the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

THE International Academy of Astronautics of the International Astronautical Federation is organizing a symposium on "Space Flight and Re-entry Trajectories" to be held in Paris during June 19–21. A number of symposia are planned, dealing with the following subjects: trajectories for lunar and interplanetary missions; orbital transfer and rendezvous; near-Earth satellites; dynamics of terminal re-entry. Further information can be obtained from the International Academy of Astronautics, 12 Rue de Gramont, Paris 2e.

THE British Biophysical Society is organizing a meeting at the University of Nottingham during July 26–28. Two symposia are being organized: "Phloem Transport in Plants" and "Electron Transfer in Biological Molecules". There will also be a session for the reading of papers, and a discussion on "International Organization in Biophysics". Further information and application forms can be obtained from the honorary treasurer, Dr. P. M. B. Walker, Zoology Department, University of Edinburgh, West Mains Road, Edinburgh.

AN informal meeting is to be held at the Royal College of Science and Technology, Glasgow, on June 27, under the auspices of the Faraday Society, to mark the centenary of the publication by Thomas Graham of his paper on liquid diffusion, in which he distinguished between colloids and crystalloids. Mr. D. C. Henry, of Manchester, will preside, and speakers will include: Sir Eric Rideal, Prof. J. A. V. Butler, Dr. A. S. C. Lawrence and Dr. A. P. Prosser. Further information may be obtained from Dr. A. J. Hyde, Department of Chemistry, Royal College of Science and Technology, George Street, Glasgow, C.1.

THE BELGIAN ADVISORY COUNCIL FOR SCIENTIFIC POLICY

THE Belgian Advisory Council for Scientific Policy was established by a royal decree of September 16, 1959, which also established a Ministerial Committee of Scientific Policy and an Inter-departmental Commission of officials. The Council, of which Prof. L. Massart is chairman and Prof. M. Dubuisson vice-chairman, is to study, in close co-operation with the universities and equivalent institutions of higher education, the development of these institutes commensurately with the nation's needs and make appropriate proposals, as well as the extension of research; to maintain records of the nation's research services and their work; to propose ways of promoting and co-ordinating scientific research in both the economic and the academic sector; to formulate an overall programme for the expansion of research, and to suggest appropriate means of financing scientific research; to study conditions determining efficiency in research and methods of co-ordinating the application of results; and to advise the Government on Belgian participation in international scientific activities. The Council is assisted by two standing committees, an Industry–University Liaison Committee and a Committee of Scientific Experts, and it has already established working parties on medical research and agronomic research.

The Council's first annual report covers the period September 1959–September 1960, in which the broad lines of an overall programme are first outlined, while in the second part of the report the Council's operations are surveyed and in particular the recommendations it has already made (Pp. 132. Bruxelles: Conseil National de la Politique Scientifique, 1960). The first aim of scientific policy is seen as the development of scientific research and the training of man-power for this purpose, and it is the task of the Council to define with sufficient accuracy the objectives of scientific policy and the means to be used to achieve them in the light of economic policy and resources. This involves the submission of recommendations regarding man-power, institutions and resources, and the report considers in some detail the adaptation of the equipment of Belgian universities to the new needs arising in university expansion and the adaptation of methods of teaching and training.

The Council is particularly concerned about the problems of a reasonable balance between the numbers of young people trained for particular occupations and the number of appropriate openings. As regards institutions, the Council has already endorsed the findings of the National Science Commission, which proposed a five-year plan for expanding the universities' scientific, technological and administrative man-power, higher subsidies for the non-State universities and a plan for expanding the State universities. Certain fundamental reforms in university curricula are put forward as urgent, and a University–National Council Liaison Committee has been established. The Council has also urged recommended implementation of plans recently drafted for expanding the staff of scientific establishments under the Ministry of Education. With regard to finance of scientific research, the Council has recommended the appropriation of 75 million francs, to be allocated progressively during 1961 to new projects as they appear desirable.

Discussing international aspects of scientific policy, the report stresses the importance and efficacy of international co-operation in research, as in the European Nuclear Research Centre, and particularly its value to the smaller countries. Finally, the problem of scientific institutions in the Congo is briefly discussed.

THE WOOL INDUSTRIES RESEARCH ASSOCIATION

NEW LABORATORIES FOR WORSTED PROCESSING

THE new Worsted Processing Block at Torridon, the Leeds headquarters of the Wool Industries Research Association, was opened by the Right Hon. R. G. Menzies, Prime Minister of Australia, on March 22.

Both the chairman of the Association, Mr. Basil A. Smith, and the director of research, Dr. A. B. D. Cassie, have long been concerned with the development of worsted processing research at Torridon. Mr. Smith has been very active in the Association's affairs since 1929, particularly in the field of scouring and combing, and also in that of finance—an important aspect when new projects are planned. Dr. Cassie joined the staff of the Association in 1938 as head of the Physics Department and was appointed director of research in 1950. Much of his own work has been of direct and indirect importance to the worsted industry; for example, his researches on the flow of air through porous plugs led to the development of the Association's fibre fineness meter, now widely used in the industry.

Early Researches

The programme of work being undertaken in the new laboratories inevitably stems from earlier fundamental and applied researches relative to the processes concerned: scouring and drying of loose wool, carding, combing, drawing and spinning. In 1920 very little was known about the physical properties of the wool fibre. The general principles of the exchange of moisture between wool and the ambient atmosphere were understood; and there were methods for measuring the moisture content of wool. But virtually nothing was known about the effect of the moisture content of wool at the various stages of processing, how it changes during processing, and how the moisture content is affected by the relative humidity of the atmosphere. These subjects have been thoroughly investigated, and the findings are now applied in the industry. The first testing room with controlled atmosphere was set up at Torridon; the method used, that of wetting

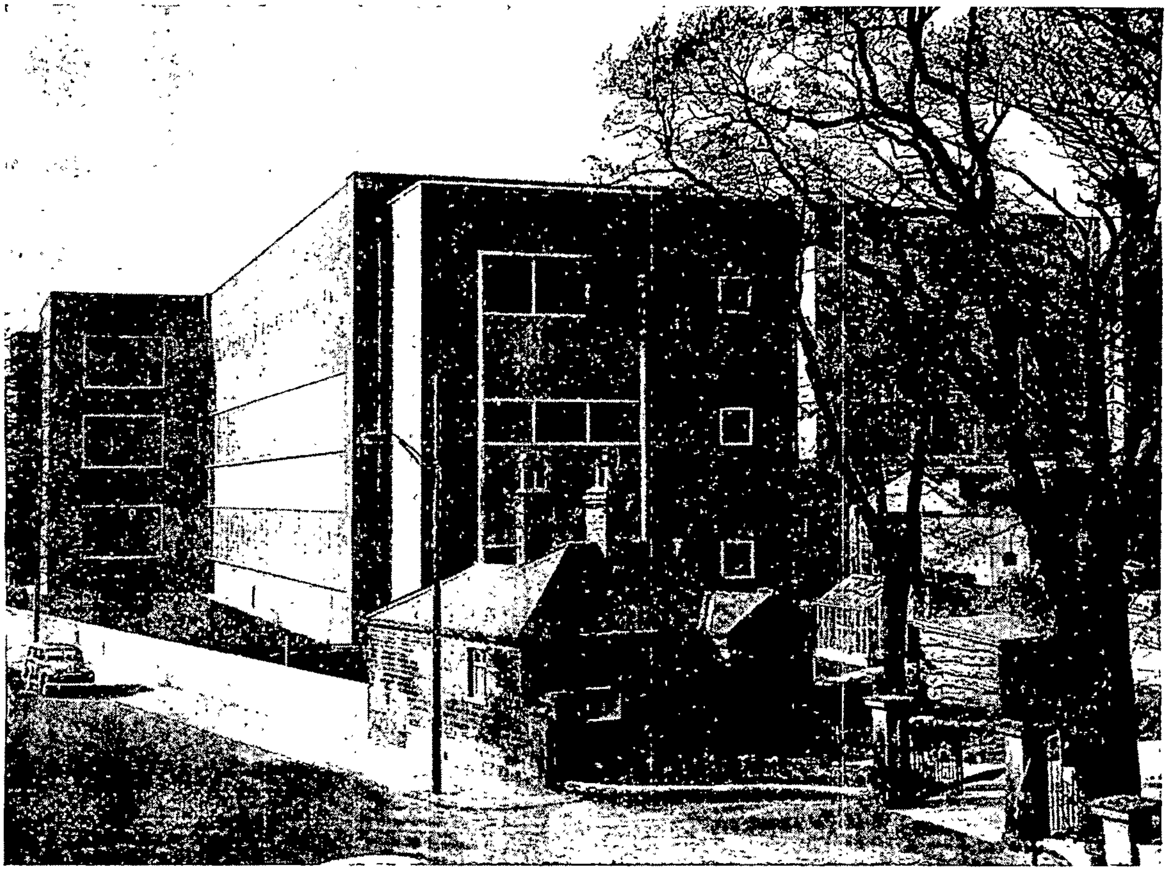

Fig. 1. The new Worsted Processing Block at Torridon, Leeds

and drying towers, was later adapted for commercial control cabinets.

Before 1920, although the industry used several methods for combating the effects of static electricity, little was known about its cause. This phenomenon was studied, therefore, and the findings indicated that the most effective way of dealing with 'static' is to attack it at its source. This knowledge has been used by chemical manufacturers in the development of anti-static dressings for use on wool during processing.

Scouring

The change of *p*H in the liquors during scouring, the use of various alkalis, and the scouring properties of suint (exudation from sweat glands of the sheep) have all been investigated at Torridon since the early 'thirties. In 1953 a complete scouring plant was installed and has now been transferred to the new Worsted Block. Recent research work has led to a greater understanding of the physico-chemical mechanism of scouring, and of the mechanical action. It has been shown that most of the mechanical scouring action takes place not in the bowls but at the point where the liquor is expressed by the squeeze rollers. This type of research has involved the devising of methods for separating and measuring the constituents of the liquors: grease, soap and free fatty acids.

Carding and Combing

The avoidance of fibre breakage is an important requirement in the early stages of worsted yarn production. Investigations have shown that fibre breakage in worsted carding depends largely on the state of the wool coming from the scouring. This finding has stressed the desirability of a gentle mechanical action in scouring. The modern type of air-flow dryer through which the wool passes after scouring, using the principles advanced by the Wool Industries Research Association in 1938, also does much to lessen the entanglement of the fibres.

Progress in methods for the removal of burr (vegetable matter) in carding had at one time been hampered by the difficulty of estimating the amount of burr in a sample. Satisfactory mechanical and complementary chemical methods of estimation have now been developed and the requirements for efficient burr removal are consequently more thoroughly understood.

Research in combing is being directed to the improvement in the thickness regularity of the combed sliver, this having increased in importance with the introduction of the new drawing and spinning techniques mentioned here.

Drawing and Spinning

About eight or ten stages of attenuation had been traditionally used in drawing 'tops' (the product of the comb) to the 'roving' from which the worsted yarn is spun. The development of the Association's roving levelness tester and the early work on roving and yarn irregularity led to the introduction of shorter processes by many firms. An outcome of this line of advance was the invention of the Raper autoleveller, a drawing machine which maintains thickness regularity by automatically taking account of the irregularity of the ingoing material. Mr. George Raper brought his idea to Torridon for early development, and after further development in a mill it was put into commercial production. This system of drawing, in conjunction with the Ambler 'superdraft' system of spinning (developed by Mr. Geoffrey Ambler with the co-operation of the University of Leeds), is now accepted as one of the modern methods of worsted drawing and spinning.

A more recent achievement, used as a part of a shortened drawing system, is the Association's rotating can system, in which twist is inserted by means of a rotating can instead of a spindle. Other investigations in improved methods of worsted drawing are being pursued at Torridon, including the use of closed loop servo-techniques for autolevelling.

An important aspect of the irregularity of the ultimate yarn is its effect on the appearance of the fabric. The Association has pioneered the expression of this irregularity in numerical form, and the problem of linking numerical assessment with visual judgment is still being studied.

Many other projects are in hand, both in scouring and combing and in drawing and spinning. Members' day-to-day problems often lead to some profitable line of research the solution of which benefits the industry as a whole. The use of man-made fibres in blends with wool raises difficulties at various stages of processing. New types of machinery introduce new aspects of old ones. This kind of service to the industry will be greatly facilitated by the increase of machinery and laboratory space in the new Worsted Block.

H. Lemon

APPLICATIONS OF INTERFERENCE MICROSCOPY

A SYMPOSIUM on this subject was held at the Royal Microscopical Society's London headquarters on March 15. The president, Dr. V. E. Cosslett, was in the chair and the audience included a number of visitors from abroad. It is nearly ten years since the first interference microscopes suitable for general use began to appear, and the purpose of the meeting was to take stock of the present position and to review some of the main applications of the instrument in various fields.

In his introductory survey Dr. R. Barer (Oxford) dealt with the instrument, theory of operation and the principles underlying its use for 'weighing' cells. He paid tribute to the work of J. Dyson, F. H. Smith and J. St. L. Philpot, who made the development of modern high-aperture instruments possible, and discussed some of the problems common to all types of interference microscope. The operation of a two-beam interference microscope can be explained by means of a simple vector theory which shows how the optical path difference produced by a transparent specimen can be measured and also demonstrates the similarity between interference and phase-contrast microscopy. The latter can be regarded as a form of interference microscopy in which the two interfering beams are imperfectly separated. This causes the edge of a refractile object to be surrounded by a halo and the contrast of edges and discontinuities is accentuated. These apparent defects of phase contrast are not necessarily a disadvantage for purely observational work; and the image of a cell seen through an interference microscope is often disappointingly 'flat' and lacking in internal contrast as compared with a phase-contrast image. Dr. Barer concluded by giving a practical interpretation of the meaning of optical path difference, the quantity normally measured with an interference microscope. The refractive index of a solution varies linearly with its concentration and since the optical path difference is defined as the product of the object thickness and the difference in refractive index between the object and the surrounding medium, it follows that the optical path difference is proportional to the dry mass per unit area. This is an important quantity in cytology, but present methods are mainly adapted to point-by-point measurements and the integration of dry mass over the area of a cell might be very tedious. The full value of interference microscopy in biology will not be realized until automatic integrating devices, based on rapid electronic scanning and capable of 'weighing' a cell in a fraction of a second, have been developed. This is a technical problem and by no means insoluble.

Dr. K. F. A. Ross (Leyden) described how interference microscopy can be used to measure the mass or solid concentration of cell inclusions. It is necessary to overcome the difficulty that the inclusion is surrounded by a layer of cytoplasm of unknown thickness. One method is to immerse the cell in an isotonic protein solution having the same refractive index as the cytoplasm. The difference in refractive index or concentration between the inclusion and the cytoplasm can then be found. Measurements of this type have been made on the Nebenkern of the locust spermatid, a body formed by the fusion of mitochondria. Values in the range of 21–26 per cent for solid concentration were obtained. Work on lipid droplets in various cells has thrown some light on their physical state. The refractive index of droplets known to contain mainly phospholipids is considerably lower (about 1·42) than that of any pure lipid substance. This suggests that in their natural state these bodies are highly hydrated, a conclusion supported by evidence from polarization and electron microscopy. Finally, Dr. Ross described measurements on different regions of the giant salivary gland chromosomes of *Chironomus*. A number of different methods all showed that the euchromatic regions contained a higher concentration of solids (20–25 per cent) than the heterochromatic regions (10–15 per cent).

Dr. E. J. Ambrose (London) described his work on cell division. The instrument used was a polarizing type of interference microscope with a birefringent wedge mounted in the eyepiece. Time-lapse ciné-films in colour were taken of dividing cells, simultaneously with a coloured image of the wedge. The optical path difference of any part of the cell could be measured by matching its colour against the scale of colours exhibited by the wedge. In some cases it was necessary to compress the cells in order to obtain good optical conditions, but with plant endosperm the cells could be mounted on a thin film of agar on which they were made to spread out by surface tension. Measurements were made on the cytoplasm, nuclear sap, chromosomes, spindle and cell plate. A good correlation was found between regions of high mass per unit area and the development of birefringence. Dr. Ambrose concluded by showing some excellent colour films of cell division.

The three papers in the afternoon session were devoted to non-biological applications. Dr. S. Goldstaub (Strasbourg) first described his work on crystal growth. The interference fringes surrounding a crystal immersed in its mother liquor are in fact lines of equal concentration. In regular growth these fringes tend to cut across the corners of the crystal, so that the crystal surface is not a region of constant concentration. Nevertheless, since the fringes move parallel to themselves during growth, an equal mass of material is deposited over the whole surface. In dendritic growth, on the other hand, the fringes do not cut the crystal surface. Dr. Goldstaub also gave an interesting account of the use of the interference microscope for studying the behaviour of salt solutions surrounding isolated particles of ion exchange resins. The flow of ions from the solution to the resin gives rise to concentration gradients, and hence to fringes around the particles so that the exchange process can be observed directly. A colour film illustrating some of these points was shown.

Dr. R. C. Faust (Imperial Chemical Industries, Ltd., Northwich) discussed the study of optically heterogeneous specimens with special reference to textile fibres. Many fibres are far from homogeneous and some possess a surface layer or 'skin' that differs markedly from the central core. A knowledge of the absolute refractive indices at different parts of a fibre is an important guide to the degree of molecular packing and orientation. A convenient method of observing the variation in refractive index is to immerse the fibre in a liquid of known refractive index and to study the way in which interference fringes are deviated in traversing the image of the fibre. At those points at which the fringe is undeviated the refractive index of the fibre matches that of the liquid. If the refractive index of the liquid is changed by varying the wave-length or the temperature, new match positions are obtained. A map of refractive index variation can thus be built up. In some unstretched fibres the refractive index for polarized light vibrating parallel to the axis remains essentially constant across the fibre whereas for light vibrating perpendicular to the axis the index falls rapidly from the edge to the centre, so that the birefringence is maximal at the edge. After stretching, however, both refractive indices vary together, giving uniform birefringence. Dr. Faust emphasized that the optical properties of fibres vary considerably, depending on the method of manufacture.

A glimpse into the future was provided by the last speaker, M. Marcel Locquin (Paris). He described how microscopes are needed to magnify the very small 'memories' used in machines for automatic documentation and translation. The 'memories' are produced as photomechanical negatives by a process similar to that used for making graticules. Although in most European languages 40,000 phrases are in common use, about 90 per cent of a language can be translated with only 2,000–4,000 phrases. A common word can be represented by one or more letters using speedwriting shorthand. Additional letters are used to represent the dependence of one word on another. The encoded phrases are made into a memory which can be a thousand times smaller than a magnetic type of memory. The memory negative is magnified through a microscope and its enlarged image falls on to a 'question' in the form of a photographic positive. A photoelectric system measures the amount of light transmitted by the overlapping images; if the memory and the question are identical no light is transmitted and various degrees of similarity can be selected according to the light transmitted. Such a system can be used for selecting papers on similar subjects without human intervention. The particular advantages of an interference microscope for this work are that it provides a means of contrast variation and that silver grains in the photographic emulsion are rendered essentially transparent by interference contrast, so that the image is effectively grain-free. However, M. Locquin felt that there were possibilities for reducing the size of the memories still further and looked forward to the day when they would have to be magnified by means of an electron microscope.

R. BARER

THE CARNEGIE TRUST FOR THE UNIVERSITIES OF SCOTLAND

THE fifty-ninth annual report of the Carnegie Trust for the Universities of Scotland, covering the year 1959–60, includes that of the Executive Committee, the abstract of financial accounts for the year ended September 30, 1960, lists of publications during the year from Fellows, Scholars and recipients of grants, and of papers published by the Royal Society of Edinburgh assisted by Carnegie grants, and the usual reports on the work of Fellows and Scholars and on research grants and travel grants awarded during the year*.

In annual and non-recurrent grants in the 1957–62 quinquennial distribution the heaviest items have been more than £18,000 by St. Andrews on the new Biology building, a similar sum by Glasgow on the new Arts building, and more than £23,000 on the new Physical Education Centre ; more than £26,000 by Aberdeen on the new hall of residence, Crombie Hall, and nearly £9,000 by Edinburgh on the new hall of residence at Salisbury Green (Holland House). The Committee is now considering its policy with regard to capital grants for the period 1962–67, and has agreed to ask each university, in formulating proposals for use of the Trust's funds in this eleventh quinquennial distribution, to concentrate on projects which are not supported by the University Grants Committee.

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* The Carnegie Trust for the Universities of Scotland. Fifty-ninth Annual Report for the year 1959–60 submitted by the Executive Committee to the Trustees on 27th February, 1961. Pp. iv+66. (Edinburgh: Carnegie Trust for the Universities of Scotland, 1961.)

Expenditure on the research scheme has risen to more than £117,000, that on postgraduate scholarships and fellowships remaining about the same. During 1959–60 all four Fellows were in the humanities, but 3 out of 5 Senior Scholars and 31 out of 56 Scholars were in science; 19 Scholars, 3 Senior Scholars and 3 Fellows were new appointments. Of 44 research grants, 13 were towards the cost of illustrating research papers with special diagrams, maps or photographs, and 10 were travel grants of special kinds. Seven major travel awards were made, making a total of 40 over the past three years, and the Committee continues to attach great importance to these foreign travel awards. The policy remains clear-cut: a high standard of research achievement must be established; the journey proposed must be based on the intention to carry out research or acquire new research techniques; and no applications will be accepted where the central purpose is to attend an international conference. Awards in 1959–60 include those to E. W. Fisher for comparative studies of the cardiovascular system of the horse, cow and dog, at Pennsylvania; G. F. Hamilton, for studies of the supplementary motor areas of the primate cortex at Michigan; G. Eglinton, to study chemical constituents of the flora of the Canary Islands; N. A. Mitchison, to study cellular aspects of antibody synthesis at Stanford; and R. G. Moorhouse to study elementary particle physics at the European Centre for Nuclear Research, Geneva.

Only seven authors received subventions towards the cost of publishing books, and while the policy of aid for the Scottish learned societies with the cost of publishing their *Proceedings* or *Transactions* continued, the Committee intends to adhere strictly to its new policy of reviewing applications simultaneously once a year, and imposing a ceiling of £2,500 on such expenditure.

NATURE OF SARCOLEMMA, THE STRIATED MUSCLE FIBRE MEMBRANE

By Prof. F. LŐRINCZ and Dr. G. BIRÓ

Hungarian Meat Research Institute, Budapest 2

THERE is a close relationship between the structure of muscle tissue and the palatability of meat, above all, its softness, tenderness, and even its biological value (for example, the ratio of true muscle tissue to connective tissue). Among the tissue components of the muscle cells, the structure and function of the sarcolemma have not yet been examined in such detail as those of other components of the muscle cells.

Jones and Barer[1], Rózsa, Szent-Györgyi and Wickoff[2], Conte and Rieser[3] and Wang[4] have investigated the nature of sarcolemma. Jones and Barer failed to confirm the opinion that the sarcolemma is a sheath composed of fine collagenous or reticular fibrils. By means of the electron microscope it was recognized as a fine membrane on which small spots were seen. According to these authors, it is possible that the small spots might be points of attachment of either connective tissue fibrils or myofibrils. Rózsa *et al.* described the sarcolemma as a thin sheet having a granular surface to which threads are attached. According to their view, these threads actually do not belong to the sarcolemma, but rather to the system of fibrils surrounding the muscle fibres. After a critical survey of the assertions of other authors, Conte and Rieser suggested, as a result of their tests, that the sarcolemma is a protein membrane free of a collagenous or fibrillar structure. It is most likely a lipoprotein, and this is in agreement with the fact that also the mammalian erythrocyte envelope is a membrane of this type. Finally, according to Wang's view, the bipartite nature of the sarcolemma can be recognized. The true sarcolemma is a homogeneous membrane about 0·1μ thick, which is surrounded by the fibrous sarcolemmous envelope.

We have also examined the finer structural conditions of muscle, from both chemical and histological points of view[5,6]. For our experiments reported here, we took samples from the gluteus medius of a cow about 3 years old, a quarter of an hour after death. After having freed the samples carefully from the other visible tissue elements, that is, the adipose and coarse components of the connective tissue, the samples were transversely cut up into small pieces measuring about 3–5 mm. in thickness and disintegrated in sterile physiological solution of sodium chloride utilizing a Waring blender. Our purpose was to obtain isolated muscle fibrils.

On examining the disintegrated tissue under a microscope, a phenomenon formerly not experienced

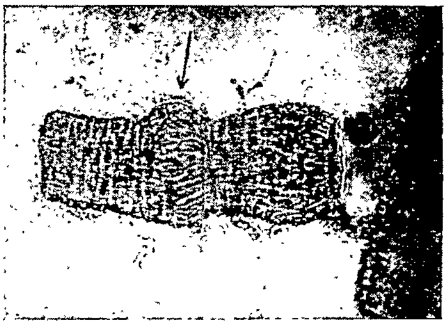

Fig. 1. (× 200)

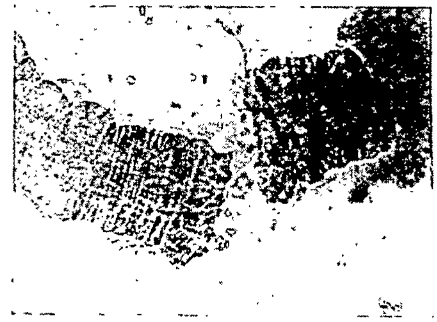

Fig. 2. (× 200)

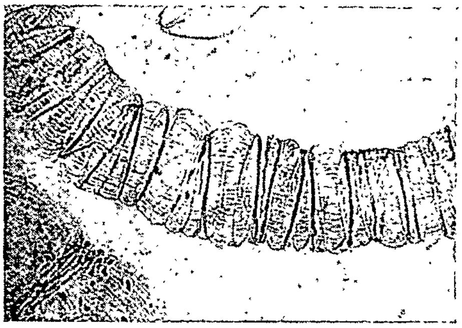

Fig. 3. (× 200)

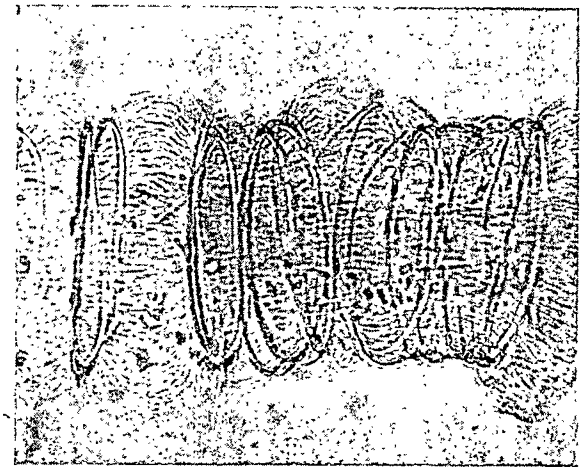

Fig. 4. (× 500)

was seen (Figs. 1–4). The majority of the muscle cells were separated into fibril fragments. However, rather short muscle cell parts which were surrounded by a more refractive connective tissue structure, exceeding in thickness the fibrils and resembling a spiral rim, could be seen. This structure, composed of circular fibres, was interrupted in many places, due to treatment in the homogenizer. As a matter of consequence, the originally presumably regular and dense 'winding' became not only loose but also the fibril bundles, freed from the elastic hold, were also—so to speak—escaping from the tight connective tissue grasp. As the loosened, here and there, ruptured circular fibres slipped towards one another, the fibril bundles appeared between them like a hernia.

Fig. 1 shows a relatively intact muscle cell. At one spot, however, the fibrils are already protruding under the connective tissue 'wire', holding together still concentrically and rather regularly. In Fig. 2 the 'winding' has been damaged more severely, and this phenomenon is still more apparent in Figs. 3 and 4.

In order to clarify the nature of the fibre structure holding together the muscle cells, the muscle tissue disintegrated in the homogenizer was held for 24 hr. at room temperature in 0·2 *N* sodium hydroxide solution, then, after washing in water, placed in hot 0·66 per cent solution of tartaric acid for 30 min. The purpose of this treatment was to remove the muscle protein and the collagen. Fig. 5 illustrates a preparation treated in such a way, on which the faint design of the fibre structure showing the outline of the muscle cell can be recognized. This test seems to verify the collagenous fibrous nature of the structure. This might be the exterior fibrous envelope surrounding the homogeneous membrane of true sarcolemma, as described by Wang.

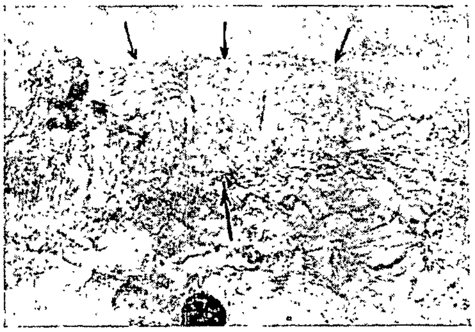

Fig. 5. A fragment of muscle fibre, treated in 0·2 *N* sodium hydroxide solution and then in hot 0·6 per cent tartaric acid solution (arrows). The concentric fibres have disappeared and only their traces can be detected. (× 250)

Fig. 5 presents some fine undulating elastic fibres which presumably belong to the endomysial connective tissue.

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UBIQUINONE (COENZYME Q) AND THE FUNCTION OF VITAMIN E

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VITAMIN E comprises a small group of closely related substances, the most important of which is α-tocopherol. α-Tocopherol is widely distributed in the plant kingdom, has been found in unicellular organisms[1], yeasts and fungi[2], and, so far as we are aware, occurs in all animal tissues. It can be readily observed in even the most carefully prepared 'vitamin E-free' diets[3] based on natural foodstuffs and also in all the tissues of animals reared on such diets, provided that suitable analytical procedures are used. All though it appears likely that vitamin E is a normal constituent of most living things, little is known of its biochemical role, and nutritional vitamin E-deficiency in animals is characterized by a great variety of clinical, physiological and histopathological symptoms, the very diversity of which has usually proved more of an embarrassment than a help in attempts to understand the fundamental processes involved in the deficiency state. Thus chicks may exhibit three different vitamin E-deficiency syndromes (muscular degeneration, exudative diathesis or encephalomalacia), separately or together, depending on the composition of their diet. Some species, such as the rat, are affected by failures in reproduction due to a variety of causes; other species, such as the pig, suffer hepatic disorders; still other species are subject to diseases chiefly affecting vascular or adipose tissue.

It is essential for an understanding of the role of vitamin E that these diverse manifestations produced by the same deficiency should be correlated with function, and we have already made the suggestion that animal tissues can suffer local and specific tocopherol deficiencies that may account for the nature of the syndrome in any given species[3,4].

A great advance was made, if not in our understanding, at least in our appreciation of the complexities of the vitamin E problem, when Schwarz and Foltz[5] discovered that selenium was able, like α-tocopherol, to prevent nutritional hepatic necrosis in the rat. Since then, many other workers have demonstrated that selenium and α-tocopherol have similar physiological effects in preventing some, but by no means all, of the symptoms of deficiency in a variety of animal species, and it is now certain that a fuller comprehension of the function of vitamin E must include an understanding of the role of selenium, both with regard to their similarities and even more to their interesting differences. It seems reasonably clear that at least three types of deficiency state can be discerned in animals: (1) diseases such as the hepatic necroses of the rat, mouse and pig, and the exudative diathesis of chicks, which can be prevented with either tocopherol or selenium, partially or totally; (2) at least some, and possibly all, disorders of reproduction and fertility (as in the rat, chick, and turkey) as well as encephalomalacia in the chick and muscular dystrophy in the rabbit, which can only be prevented with tocopherol; (3) muscular degenerations in species such as the sheep, which may be primarily due to selenium deficiency, although they can be either prevented or ameliorated by tocopherol. To these three conditions (and there are no doubt other intermediate states still to be elucidated) may perhaps be added a further category, namely, diseases of adipose tissue, such as the 'yellow fat' diseases of the cat and mink, in which only tocopherol appears to play a part. Formally the latter may fall within the realm of category (2) above, but because of the unusually clear effects on lipid metabolism in diseases affecting adipose tissue, they may with advantage be separated at present.

Active Metabolite of α-Tocopherol

In this article we wish to examine in further detail the nature of the function of α-tocopherol in animals and present some new evidence concerning its biochemical role. We have shown previously[3,4] that deficiencies of tocopherol and selenium in the rat and the rabbit produce a decrease in tissue levels of ubiquinone (coenzyme *Q*). It was also shown that dietary supplementation or oral dosage with either substance produced marked increases in ubiquinone concentrations in these animals (these effects can also be produced in avian species—to be discussed elsewhere), and we have already discussed[3] some possible connexions between these observations and the physiological effects of vitamin E in animals. In these earlier experiments our general technique was to give doses of α-tocopherol to deficient animals and kill them after 72 hr., after which time ubiquinone was measured in their tissues by methods described previously[6]. Table 1 gives the results of an experiment in which rats were given intravenous injections of 500 μgm. of α-tocopherol and examined after 2 hr. The technique in this and in similar experiments described afterwards was as follows: The animals in each test, randomized into appropriate groups, were anæsthetized lightly with ether. Tocopherol (emulsified in normal saline, containing 'Tween 80') was administered intrajugularly, and the wound was then closed with a clip and the animals allowed to recover. The operation generally took only a few minutes, and during the remainder of the experiment the rats behaved normally. At the end of the appropriate test period the rats were killed. Pilot experiments showed that ether anæsthesia had no effect on ubiquinone-levels, except after prolonged and heavy administration (and then, usually, only in the kidney). In the experiment summarized in Table 1, marked increases in the ubiquinone-levels of heart, liver and kidney were produced 2 hours after injection with tocopherol and, most significantly, the increase that occurred in heart was observed before any increase in the tocopherol concentration in this organ could be detected. (In the Tables, the symbols UQ, UC and T have been used to signify ubiquinone, ubichromenol and tocopherol, respectively.) In subsequent experiments (not shown) the latter phenomenon could sometimes be observed in other tissues, depending on the dose administered and how soon afterwards the animals were killed. As a result of these experiments, it seemed possible that the effect on ubiquinone was produced not by tocopherol but by a metabolite—perhaps a water-soluble derivative—formed from it. Such metabolites are in fact known. Simon *et al.*[7] isolated two water-soluble degradation products of α-tocopherol from the urine of rabbits that had been given large oral doses of the vitamin. These two substances were shown to be 2-(3-hydroxy-3-methyl-5-carboxy)-pentyl-3,5,6-trimethylbenzoquinone (I) and the lactone (II) derived from it. Although these workers seem to have regarded the compounds at that time as excretory products, Mertz and Schwarz[8] showed them to be highly active, *in vitro*, in preventing the respiratory failure that occurs in liver slices taken from rats deficient in tocopherol and selenium, and Corwin and Schwarz[9] have recently found them to be as active as tocopherol in maintaining α-ketoglutarate oxidation in tocopherol-deficient liver homogenates. We obtained a generous supply of lactone (II) from Dr. J. Weichet of Prague

Table 1. EFFECT OF α-TOCOPHEROL ADMINISTERED INTRAJUGULARLY TO RATS

Group and treatment	Heart			Liver			Kidney		
	UQ	UC	T	UQ	UC	T	UQ	UC	T
Controls	128	7	1·7	63	15	0·9	49	3	1·9
2 hr. after injection of α-tocopherol (500 μgm.)	166	8	1·5	74	22	13·7	88	9	5·2

Two groups of six male rats, 4 months old, on diet *E*10*Y*. Results are in μgm./gm. of fresh tissue

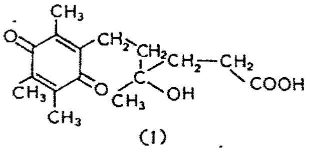
Table 2. EFFECT OF TOCOPHERONOLACTONE GIVEN ORALLY AND INTRAVENOUSLY TO RATS

Group and treatment	Heart UQ	Heart UC	Heart T	Liver UQ	Liver UC	Liver T	Kidney UQ	Kidney UC	Kidney T
Controls	94	7	1·0	32	12	1·0	69	2	1·1
24 hr. after 1 mgm. tocopheronolactone orally	134	8	1·0	33	12	1·2	101	4	1·1
15 min. after 25 μgm. tocopheronolactone intravenously	166	18	1·3	50	19	1·0	81	4	1·3

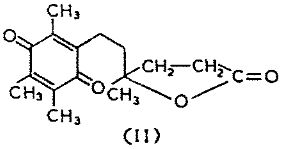
Three groups of six male rats, 9 months old, on diet *E*10*Y*. Results in μgm./gm. of tissue

(and later synthesized it in our laboratories) and examined it for its effect on ubiquinone in a number of experiments, some of which are summarized in Table 2.

It was found to be highly active, even more so than α-tocopherol itself when given in an equimolecular amount, either orally or when given intravenously. Marked increases in ubiquinone could be produced *within* 15 *min.* of injecting as little as 25 μgm. of lactone (II) intravenously into fully grown rats. In view of the likely physiological significance of these two metabolites, therefore, we propose that the name tocopheronic acid be given to compound (I) and that compound (II) be called tocopheronolactone.

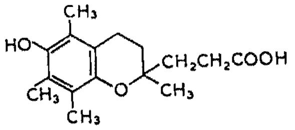


(I)



(II)

We have shown previously[10] that whereas α-tocopherol or selenium can control ubiquinone-levels in most tissues of the rat, only tocopherol is effective in the uterus, a phenomenon that is probably connected with the inability of selenium to prevent gestation-resorption. Since such important differences between tissues exist, it was clearly important to discover whether tocopheronolactone was as effective as α-tocopherol in the uterus and (for allied reasons) in the ovary. Table 3 gives the results of an experiment in which female rats were given oral doses of (*a*) sodium selenite, (*b*) α-tocopheryl acetate, or (*c*) tocopheronolactone, and killed 24 hr. later. Heart, ovary and uterus were analysed. Tocopheronolactone was found to be as active as α-tocopherol in all three tissues, and the experiment again confirmed the inactivity of selenium in uterus. Finally, Table 4 gives the results of an experiment that illustrates the differences in the rates of action of α-tocopherol and its metabolite. Rats were injected intrajugularly with small amounts of each substance (in equimolecular amounts). Groups of rats were killed both after 15 min. and after 120 min. and three tissues were examined in the usual way for effects on ubiquinone and ubichromenol. These were compared with tissues from negative and uninjected controls and also from groups of rats injected with bland saline 'Tween 80' solutions (used as vehicles for the tocopherol and tocopheronolactone). The results show that whereas after 15 min. the tocopherol was just beginning to take effect, the lactone was already fully active at this time in kidney and nearly so in heart and liver. After 2 hr. the effect of tocopherol was maximal, whereas the effect of tocopheronolactone in kidney was already on the wane, although it was still pronounced in heart and liver. An interesting feature of the analyses is that 150 μgm. of tocopherol was obviously insufficient to produce appreciable changes in the tocopherol-levels of the tissues: this quantity given to deficient animals can clearly be rapidly and practically completely metabolized during the 2-hr. period. In this experiment we also studied the effect of the interesting chromanol (III) (also prepared by Dr. Weichet), which is derived from tocopheronic acid. Although this substance was active in increasing ubiquinone in heart, it was quite ineffective in liver and kidney, where, indeed, ubiquinone-levels were actually depressed. These four experiments suggest that tocopheronic acid is either the physiologically active agent produced from tocopherol or that it is closely allied to such a derivative. We have had some indication that tocopheronolactone may itself be converted to another substance *in vivo*. Chromatograms of tissue extracts from rats given fairly large amounts of the lactone (1–2 mgm.) have shown the presence of a non-reducing lipid-soluble substance, chromatographically slower than α-tocopherylquinone in reversed-phase systems and therefore possibly of greater molecular weight, which spectroscopically appeared to be a typical



(III)

Table 3. EFFECT OF α-TOCOPHEROL, TOCOPHERONOLACTONE AND SELENIUM IN RAT HEART, OVARY AND UTERUS

Group and treatment	Heart UQ	Heart T	Ovary UQ	Ovary T	Uterus UQ	Uterus T
Controls	30	0·5	48	18·4	33	0·8
Selenite	57	1·1	76	16·8	37	2·4
Tocopherol	131	4·5	88	61·0	50	6·9
Tocopheronolactone	118	0·6	96	16·5	48	3·2

Groups of nine female rats (5 months old on diet *E*10*Y*) were used. Rats in test groups were given (*a*) 40 μgm. sodium selenite, or (*b*) 10 mgm. α-tocopheryl acetate, or (*c*) 2 mgm. tocopheronolactone, and killed 24 hr. later. Results are in μgm./gm. of tissue

Table 4. COMPARISON OF EFFECTS OF α-TOCOPHEROL AND TOCOPHERONOLACTONE

Group and treatment	Time after injection (min.)	Heart UQ	Heart UC	Heart T	Liver UQ	Liver UC	Liver T	Kidney UQ	Kidney UC	Kidney T
No injection	0	32	9	1·1	25	16	0·4	56	25	8·4
Bland injection	15	33	9	1·2	26	20	0·4	52	20	6·6
Bland injection	120	36	9	1·3	25	18	0·5	60	22	6·9
α-Tocopherol	15	47	5	1·7	42	21	1·8	50	9	9·5
α-Tocopherol	120	85	N.D.*	1·4	58	27	1·7	88	7	9·2
Tocopheronolactone	15	72	1	1·5	38	19	0·4	86	5	6·8
Tocopheronolactone	120	145	N.D.*	1·8	51	27	0·4	56	2	8·2
Chromanol (III)	30	137	N.D.*	1·8	18	11	0·5	50	19	2·8

Male rats (5 months old, on diet *E*10*Y*) were injected intrajugularly with α-tocopherol (150 μgm.) or tocopheronolactone (100 μgm.) or chromanol III (100 μgm.) dissolved or suspended in saline 'Tween 80' solution. Control rats were used with and without injections of bland saline/'Tween 80' solutions as two types of negative control. Results in μgm./gm. of tissue

* N.D., not detected.

substituted benzoquinone ($\lambda_{max.}$ at 259 and 268 mμ.), reducible with borohydride. Its significance is at present quite unknown.

Factors affecting Ubiquinone-levels in the Rat

It has become apparent from work with many groups of rats that tissue ubiquinone-levels in this species are subject to marked fluctuation, particularly in vitamin E-deficient animals but also in normal rats from the stock colony. Although the factors responsible are in the main unknown at present, one such factor is undoubtedly the nature of the diet, especially with regard to traces of vitamin E and selenium, but also with regard to gross composition. The diet (*E*10*Y*) used in our experiments contained very little tocopherol. Its composition was as follows: dried yeast (Marmite Food Extract Co.), 25; casein ('Genatosan', low vitamin content), 10 per cent; lard, 1 per cent; sucrose, 59 per cent; McCollum's salt mixture, 5 per cent; with the addition of 10 I.U. of vitamin A and 2·5 I.U. of vitamin D/gm. of diet. We have found that the use of this diet consistently produces low ubiquinone-levels in the rat, especially in liver. This can be seen from the results (Table 5) of an experiment that deals with another factor having a marked effect on ubiquinone-levels, namely, starvation. In this experiment, groups of rats on 3 diets were examined, in comparison with the controls, after being fasted for 18 hr. One group was fed diet *E*10*Y* for 6 months, the second group received the same diet supplemented with 100 mgm./kgm. of α-tocopheryl acetate, and the third group was on the adequate stock colony diet. Starvation increased ubiquinone markedly in the stock colony animals, less so in the other groups, but significantly in all livers. These observations are of especial interest since cholesterol synthesis is known to be markedly depressed in liver during fasting, and both the ubiquinone side-chain and cholesterol are derived from a common precursor, mevalonate. Bucher[11] has discussed some of the factors that might be concerned with the inhibition of cholesterol synthesis in fasted animals. Although these are far from clear, it appears that the process leads to a concurrent increase in ubiquinone (the amount of the latter in tissues being, of course, fairly small compared with the amounts of cholesterol). It should be noted that other factors have also been found to increase ubiquinone in the rat: these include uncoupling agents such as thyroxine and dinitrophenol[12] and substances that inhibit oxidation without uncoupling of phosphorylation[13], such as barbiturates (fuller details of this work will be presented elsewhere). In spite of the multiplicity of these effects, the possibility that they influence some common point in metabolism, of which the increase in ubiquinone may be only one aspect, must be considered.

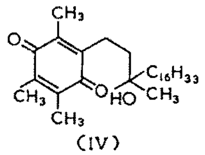
Table 5. EFFECT OF STARVATION ON UBIQUINONE- AND UBICHROMENOL-LEVELS IN VITAMIN E-DEFICIENT AND ADEQUATELY FED RATS

Group	Treatment	Heart UQ	Heart UC	Heart T	Liver UQ	Liver UC	Liver T
E-deficient	Non-fasted	37	15	1·8	15	11	0·4
	Fasted	38	15	1·8	23	5	0·4
E-def. + E	Non-fasted	58	8	6·2	28	13	10·5
	Fasted	60	4	5·0	34	8	5·0
Stock diet	Non-fasted	39	N.D.*	3·3	83	23	11·5
	Fasted	66	N.D.*	9·8	121	37	9·1

For details of diets, see text. Results in μgm./gm. of tissue.
* N.D., not detected.

α-Tocopherylquinone

The discovery of physiological activity for tocopheronolactone inevitably raises anew the problem of α-tocopherylquinone. This substance (IV), the irreversible oxidation product of α-tocopherol, is only present in traces in animal tissues (see Diplock *et al.*[10]).



(IV)

Many workers, searching for evidence of a reversible oxidation-reduction reaction of α-tocopherol in tissues, have examined this quinone for activity of vitamin E and reported it to be inactive[14]. However, there seems superficially to be no obvious reason why it should not be as easily degradable to tocopheronolactone as α-tocopherol itself, and if this is so it also should be effective in increasing ubiquinone-levels. Accordingly, we prepared a specimen of α-tocopherylquinone and purified it until it was rigorously freed from any traces of reducing material and was chromatographically homogeneous. When it was given orally to vitamin E-deficient rats (10 mgm. to each animal), it increased ubiquinone-levels markedly, in the same way as tocopherol. Moreover, analysis of the tissues showed conclusively that no conversion to tocopherol had occurred, nor could the presence of tocopherylquinone itself be detected.

It seemed therefore that the reason for the lack of vitamin E activity of α-tocopherylquinone might reside in the inability of the animal to store or transport it. In order to study this possibility, the quinone was examined in a rat gestation-resorption assay test in the following manner. Virgin female vitamin E-deficient rats (4–5 months old) were mated and given 5 mgm. of the quinone (dissolved in propylene glycol/10 per cent ethanol) intraperitoneally and 5 mgm. of the quinone orally (in 'Tween 80' emulsion) every day between the 7th and 13th days of pregnancy. This dosage was completely effective in preventing fœtal resorption, proving that tocopherylquinone has vitamin E activity if given in adequate amounts to rats during gestation. We have recently become aware of the demonstration by Mackenzie and Mackenzie[15], using virtually identical methods, of the vitamin E activity of tocopherylhydroquinone, which these workers predicted by a process of reasoning quite different from ours. Their belief, however, that there is an essential difference between the quinone and the hydroquinone would now appear to be invalid: any quantitative discrepancies between the activity of quinone and hydroquinone can probably be explained by differences in the absorption and transport of the two substances. As a matter of history, the discovery of vitamin E activity for the quinone confirms the prognostication of Smith, Renfrow and Opie[16] made in 1942, when they found high biological activity for α-tocopheramine (which gives the quinone on oxidation). We have tried, so far without success, to demonstrate comparable activity for either tocopheronolactone or chromanol (III) (given intraperitoneally at 4 and 3 mgm./day respectively from the 7th to the 13th day of pregnancy) in the rat gestation-resorption test. In view of the solubility characteristics of the former and the rate at which it seems to be excreted, maintenance of adequate levels throughout the gestation period might be expected to be difficult and such levels may not have been attained with the small quantities of the lactone at present available to us.

Vitamin E and Lipid Peroxidation

It is now possible to discuss, in somewhat better perspective, the important question of the relation between the total biological function of vitamin E and its role as a lipid antioxidant. It has been the view of many workers that in fact the two functions are identical, but we believe that this view can no longer be accepted. We have shown previously[10,17] that the effect of tocopherol and selenium on ubiquinone is probably independent of any antioxidant role that vitamin E might have, and this is now further supported by the finding that tocopheronolactone is likely to be the active vitamin E metabolite controlling ubiquinone-levels. There is, of course, much nutritional and physiological evidence that undoubtedly suggests that α-tocopherol does play an important part in fat metabolism, protecting oxidizable lipids from destruction and perhaps preventing the accumulation of potentially toxic compounds in tissues. It seems reasonable to accept that α-tocopherol functions as a conventional lipid antioxidant, for at least some of its physiological effects can be duplicated by non-physiological antioxidants. However, in recent years, partly because of the many and persistent failures to demonstrate a more specific role for α-tocopherol in animals, it has proved tempting to explain the total role of vitamin E in terms of its antioxidant properties and to suggest that all the nutritional requirements for vitamin E can be satisfied by a physiological lipid antioxidant.

It is this hypothesis that we now wish to examine in some detail. Much of the evidence on which it is based stems from the demonstration that respiring mitochondrial preparations[18,19] and whole homogenates[20] exhibit a phenomenon usually referred to as 'lipid peroxidation' and that this can be inhibited by the *in vitro* addition of α-tocopherol, antioxidants, or certain metallic ions such as those of cobalt and manganese. Dietary supplementation with tocopherol has been shown, in a few instances, to reduce tissue peroxidation; and certain characteristic symptoms of vitamin E deficiency, such as rat erythrocyte hæmolysis[21] and impairment of ascorbic acid synthesis in rat liver[22] are always accompanied by an increase in peroxidation, and both are reversed *in vitro* by α-tocopherol. Lipid peroxidation itself appears to be poorly understood and is obviously a simple term for a complex process. *In vivo* peroxides and *in vitro* peroxidation (the latter after incubation of tissue preparations at 37° for periods varying between 15 min. and 1 hr.) are usually determined by adding thiobarbituric acid (TBA) and measuring the red colour produced. It is usually considered that the TBA-reacting substance is malonaldehyde[23]. The *in vitro* peroxidation is considered to be a 'development' of a process that has already produced changes in the tissues, and it is known to be greatly enhanced by the presence of ascorbic acid, which catalyses many peroxidations. Kitabchi *et al.*[22] have shown that peroxidation in tissues is mainly microsomal in origin, the malonaldehyde being produced by a reaction between a microsomal lipid and a metal ion or an intermediate formed from gulonolactone or ascorbic acid. They suggest that peroxidation is closely related to the impairment of ascorbic acid synthesis that occurs in vitamin E deficiency.

We have already discussed certain aspects of the antioxidant theory[5] and have shown that study of the tocopherol concentrations in different tissues leads to the conclusion that there is no real relationship between these concentrations and the extent of lipid peroxidation. For example, the rat adrenal contains several times as much tocopherol as any other tissue in the body and yet peroxidizes very easily[24]. Moreover, in most tissues of the rabbit[19] and also in the rat[24], there are no significant differences between the *in vivo* peroxides of vitamin E-deficient and adequately fed animals. It has been suggested[19] that the TBA reactants found in the tissues of adequate animals do not represent lipid peroxidation products; but this appears to involve a circuitous argument, and, as we show below, they are in fact indistinguishable from the 'peroxides' of deficient animals. It is agreed by all workers who have investigated the phenomenon that whereas *in vitro* lipid peroxidation can easily be prevented in liver and perhaps muscle by the addition of tocopherol to deficient diets, such an effect cannot be obtained in other tissues. For example, Carpenter *et al.*[20] found that rat testis, a tissue particularly susceptible to the effects of vitamin E deficiency, synthesized peroxides *in vitro*, even when taken from animals given sufficient tocopherol to prevent deficiency. Bieri and Anderson[25] had to feed relatively huge amounts of tocopherol for long periods to prevent lipid peroxidation in rat testis and even then they were unable to effect any appreciable reduction in brain and kidney.

Table 6 summarizes the results of an experiment in which we have studied lipid peroxidation in pregnant female rats from the stock breeding colony and similar rats from the vitamin E-deficient colony. Both groups were mated and pregnancies allowed to continue to the 18th day: this is the time at which rats are normally examined in the standard rat gestation-resorption assay for vitamin E and resorbed

Table 6. LIPID PEROXIDATION IN STOCK COLONY AND VITAMIN E DEFICIENT PREGNANT RATS

Group	Test	Liver	Uterus*	Fœtus	Placenta
Stock colony	*In vivo* peroxides	6 (2)	3 (5)	4 (5)	4 (2)
	In vitro peroxidation†	12 (5)	5 (5)	10 (3)	5 (2)
	In vitro + ascorbate‡	12 (5)	5 (7)	19 (4)§	5 (2)
				(Total uterine contents)	
E-deficient	*In vivo* peroxides	10 (13)	4 (4)	5 (4)	
	In vitro peroxidation†	22 (5)	9 (3)	7 (1)	
	In vitro + ascorbate‡	34 (12)	17 (6)	22 (4)	

Results are given as direct readings of the optical density at 520 mμ, after reaction of 4 ml. of a 2 per cent homogenate with TBA. Each result is a mean followed by number of rats separately tested.

* This tissue could not be homogenized and was used as a fine suspended mince.

† Incubation time of 15 min. for liver, 60 min. for other tissues.

‡ 3×10^{-6} mole of ascorbic acid added before incubation.

§ One discordant result was found in another rat in this group.

fœtuses counted. The rats were killed and liver, uterus, placenta and live fœtuses taken from the stock animals, and liver, uterus and total uterine contents (combined resorbing fœtuses and placentæ) from the deficient group. Individual tissues from each animal were examined for *in vivo* peroxides and *in vitro* lipid peroxidation by methods described in detail elsewhere[21,26]. In all the tissues of the deficient animals, peroxidation increased after *in vitro* incubation, particularly in the presence of ascorbate. In the adequate animals, however, although peroxidation was low in liver, uterus and placenta, the viable fœtuses peroxidized almost as much as the total resorbed uterine contents of the vitamin E-deficient group. It is especially significant that this fœtal peroxidation in the adequate tissues increased in the presence of ascorbic acid (compare liver and uterus, where it did not), suggesting quite strongly that the phenomenon is identical with the 'lipid peroxidation' of deficient tissues. The significance of this experiment is that the viable 18th-day rat fœtus, *by classical definition,* contains sufficient vitamin E for all its needs and the conclusion must be that, at least in this instance, the TBA reaction cannot bear any relation to vitamin E function.

It would in fact appear from the series of experiments now to be described that even the 'inhibition' of lipid peroxidation may not prove to be explicable solely in terms of the reducing or antioxidant properties of α-tocopherol itself. In these experiments, the results of which are given in Table 7, a number of substances were examined for their activity in preventing the *in vitro* peroxidation of vitamin E-deficient rat liver homogenates. The substances tested clearly fall into two groups: (1) those as active as α-tocopherol, which include only thyroxine and, most remarkably, the non-reducing substance, tocopheronolactone, and (2) those with only about one-tenth of the activity of tocopherol, which comprise the ubiquinones, ubichromenol, α-tocopherylquinone, the quinone reduction products (quinols), vitamin A, and menadione. The quinols were obtained from the corresponding quinones by reduction in ethanol with aqueous potassium borohydride and maintained in the reduced state with an excess of reagent (pilot tests showed that borohydride had no significant effect in preventing peroxidation).

The results show that conversion of the quinones to quinols only enhances the activity very little. This may be because the quinones are, in any event, mainly reduced to quinols under the conditions of test: ubiquinone can be reduced by many substrates[27], and both menadione[28] and tocopheronolactone[29] can be enzymatically reduced by diphosphopyridine nucleotide. The evidence, however, is not incompatible with the assumption that the non-reducing quinones themselves may participate in the inhibition of 'lipid peroxidation'. For example, it has not so far proved possible to demonstrate that tocopherylquinone can be reduced either *in vivo* or by *in vitro* biochemical systems[30,31]. It was considered as a possibility that the activity of tocopherylquinone could be accounted for by an enzymatic cleavage to tocopheronolactone under the conditions of incubation: however, when tocopherylquinone was pre-incubated for 60 min. with a liver homogenate and then re-tested for its effect on peroxidation in a fresh homogenate, only a small enhancement of activity could be demonstrated.

Another interesting observation from Table 6 is that vitamin A has 'antioxidant' activity, as measured by the 'lipid peroxidation' test, although it is a substance that can scarcely be considered as a conventional antioxidant: it is difficult to see why the addition of an unsaturated lipid like vitamin A should protect against peroxidation (although we have observed similar effects at a higher concentration with substances such as nerolidol, farnesol and geranyllinalool).

Since vitamin A is an important constituent of liver, which has not only been extensively used for studies of peroxidation but also provides a good deal of the evidence for the effect of vitamin E, it seemed important to examine its influence on 'lipid peroxidation' in this tissue. Three groups of male rats were placed, at the age of 21 days, on a diet deficient in both vitamin E and vitamin A. After a month, one group was transferred to the same diet supplemented with 10 I.U. of vitamin A/gm., the second group to the same diet supplemented with 40 I.U. of vitamin A/gm., and the third group remained as vitamins A- and E-deficient controls. After a month on these diets, the rats were killed and livers and brains pooled for studies of their 'lipid peroxidation'. As Table 8 shows, there was indeed a marked 'antioxidant' effect in both tissues containing vitamin A: this is doubly remarkable since, as we show elsewhere[26], the *vitamin E* concentration in liver deficient in vitamins A and E may be several times higher than in control livers deficient only in vitamin E (because of the well-established antagonistic effect of vitamin A on tocopherol). The same is true of brain; and, in fact, in this tissue the 'antioxidant' effect of dietary vitamin A is at least as great as we have ever obtained with dietary vitamin E (see also Bieri and Anderson[25]). It can be concluded, therefore, that vitamin A must also be considered as a factor in reducing 'lipid peroxidation' as measured by the TBA test, particularly since the addition of tocopherol to homogenates protects vitamin A from destruction.

Table 7. EFFECT OF α-TOCOPHEROL, TOCOPHERONOLACTONE AND SOME QUINONES ON INHIBITION OF *in vitro* LIPID PEROXIDATION IN LIVER HOMOGENATES

	Per cent reduction in peroxidation (TBA test)	Amount of active substance used (moles)
α-Tocopherol	94	5×10^{-7}
	c. 50	$2\text{–}4 \times 10^{-8}$
Substances as active as α-tocopherol		
L-Thyroxine sodium salt	93	5×10^{-7}
	c. 50	2×10^{-8}
Tocopheronolactone	79	1×10^{-6}
	c. 50	2×10^{-8}
	36	1×10^{-8}
Reduced tocopheronolactone (quinol)	25	1×10^{-6}
Substances with about one-tenth or less of the molar activity of α-tocopherol		
α-Tocopherylquinone	40	2×10^{-7}
	9	2×10^{-8}
α-Tocopherylhydroquinone	25	2×10^{-8}
Ubichromenol 50	32	2×10^{-7}
Ubiquinone 30	20	4×10^{-7}
Ubiquinone 35	31	4×10^{-7}
Ubiquinone 40	23	4×10^{-7}
Ubiquinone 45	13	4×10^{-7}
Ubiquinone 50	19	4×10^{-7}
Ubiquinol 45	0	4×10^{-7}
Ubiquinol 50	8	4×10^{-7}
Menadione	76	5×10^{-7}
Vitamin A alcohol	44	2×10^{-7}
Vitamin A palmitate	81	1×10^{-6}
Vitamin K_1	56	1×10^{-7}

Table 8. EFFECT OF DIETARY VITAMIN A ON 'LIPID PEROXIDATION' OF RAT LIVER AND BRAIN

	Peroxides† (E at 520 mμ)					
	Liver‡			Brain‡		
Level of dietary A, I.U./gm.	0	10	40	0	10	40
In vivo test	4	2	2	2	1	1
Incubated at 37°	17	21	13	42	37	28
Incubated with ascorbate*	51	38	27	118	120	91

* 3×10^{-6} mole of ascorbic acid in 4 ml. of homogenate.
† Each result is a mean of two determinations.
‡ Liver incubated for 15 min., brain for 60 min.
For details of diets, see text. Results are given as direct readings at 520 mμ of the TBA reaction.

The picture of 'lipid peroxidation' is still further complicated by considerations of another series of experiments (summarized in Table 9), in which tocopheronolactone and tocopherylquinone were examined for their activity in preventing erythrocyte hæmolysis in rats. This hæmolysis, which is produced by incubation with dialuric acid, is an early and characteristic symptom of vitamin E deficiency in the rat[32] and can be completely reversed if the cells are pre-incubated *in vitro* with α-tocopherol or if the animal is given vitamin E orally some time before the blood is taken. Many other substances, some of them non-reducing, can also inhibit hæmolysis[33] and this inhibition is always accompanied by a decrease in the lipid peroxidation (thiobarbituric acid reaction) of the erythrocytes[21]. As Table 9 shows, tocopheronolactone and tocopherylquinone were both negligibly active when given orally ; but the latter substance, in strong contrast to the former, was found to be quite active *in vitro* both in inhibiting hæmolysis and in reducing peroxidation. Now, lipid solubility *per se* is not a factor that determines the *in vitro* activity of substances in the hæmolysis test : we have shown[33], for example, that ubiquinone 50 is inactive, whereas thyroxine affords complete protection[21]. It is, moreover, unlikely (judging from the results in Table 7) that tocopherylquinone is more easily reduced to a quinol on the surface of the erythrocyte than tocopheronolactone would be. While these results are not easy to interpret, they provide still further evidence that the relation between the effect of antioxidants and 'lipid peroxidation' on one hand and between 'lipid peroxidation' and the thiobarbituric acid reaction on the other needs to be reconsidered.

Table 9. EFFECT OF TOCOPHERONOLACTONE AND α-TOCOPHERYLQUINONE ON RAT ERYTHROCYTE HÆMOLYSIS, *in vivo* AND *in vitro*

Substance	*In vivo* potency (D-α-tocopheryl acetate = 100)	*In vitro* potency (DL-α-tocopherol = 100)	Protection, *in vitro* against peroxidation
Tocopheronolactone	< 10 (if any)	< 1 (if any)	Nil
Tocopherylquinone	< 3 (if any)	*c.* 10	complete
Tocopherylhydroquinone	< 3 (if any)	—	—

Possible Functions of Vitamin E

Although it is still difficult to present a unified concept that will explain satisfactorily the role of vitamin E in animals, several developments in the past two or three years signify a new approach to the problem that will certainly prove to be most productive. In our opinion, the present position may be summarized as follows: Vitamin E must have at least two functions, which at present have to be considered independently, but which may prove to be closely related. One is concerned with the metabolism of unsaturated lipids, in which role its antioxidant properties probably play a part : however, the connexions between this function and the phenomenon that occurs in tissues and is termed 'lipid peroxidation' seems to us, for many reasons, still to be obscure and may yet prove to be an example of a biochemical truism. It is perhaps not altogether surprising that this should be so. It is only necessary to examine the findings by Carpenter *et al.*[20] and Kitabchi *et al.*[22] on the relation between tocopherol and ascorbic acid synthesis to appreciate the subtleties that might be involved in the TBA reaction. Thus, tocopherol increases the synthesis of ascorbic acid, which itself catalyses fatty-acid peroxidation and inhibits its own synthesis[22] : tocopherol inhibits malonaldehyde production, but ascorbic acid increases it markedly[22] : and, contrarily, ascorbic acid enters into a potent synergistic relationship with tocopherol in preventing the hæmatin-catalysed peroxidation of unsaturated fatty acids[34] : the possibilities of feedback mechanisms are obviously many and intricate.

The second function of vitamin E, in which the active substance is almost certainly a metabolite of α-tocopherol, possibly tocopheronolactone, results in the biogenesis of ubiquinone. Two key questions immediately present themselves : How far is it possible to understand the physiological and histopathological symptoms of vitamin E deficiency (with the exception of those relating in the narrow sense to lipid antioxidant effects) in terms of ubiquinone deficiency ? Is the effect of tocopherol on ubiquinone a unique effect on its synthesis or only a secondary phenomenon resulting from a more fundamental process elsewhere ? Although answers to these questions must await further work, it is reasonable even now to suggest that at least some of the disturbances in respiration, oxidation, and tissue (particularly embryonic) differentiation in vitamin E deficiency states may be explicable in terms of the effect on ubiquinone-levels. In the rat, and probably in other species, there is an alternate mechanism, involving selenium, which also influences biosynthesis of ubiquinone. The two mechanisms appear to be parallel, since in most tissues of the adult rat, as shown elsewhere[26], tocopherol does not increase ubiquinone-levels if sufficient selenium is present and *vice versa*. In two organs, however, the two mechanisms are plainly of unequal importance. In uterus, the selenium-dependent process does not appear to be present at all, whereas in liver, where selenium exerts a remarkable influence on ubiquinone compared to that of tocopherol[8,10,26], the selenium-dependent mechanism is apparently preponderant. Such a difference between the effects of selenium and tocopherol is also found in the livers of avian species (to be discussed in a separate communication).

The existence of such alternate biochemical systems and the possibility that they may be of unequal significance in different tissues might account for the observed differences between the physiological effects of selenium and tocopherol in animals. It is at this stage not possible to say whether all animals require a supply of both tocopherol and selenium. The answer to this question may not only vary from species to species but also may differ at different periods during the life-cycle. Thus the rat needs tocopherol specifically for reproduction (and perhaps during very early life), but during adult life its needs could conceivably be met by selenium. According to Nesheim and Scott[35] the chick has an absolute requirement for selenium even if tocopherol is supplied, and a similar position may exist in the lamb. Both Draper *et al.*[36] and, more recently, Crider *et al.*[37] have concluded that the rat does not need tocopherol if certain antioxidants are provided in the diet and that substances such as DPPD (N,N′-diphenyl-*p*-phenylene diamine) and ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) can prevent reproduction failure in the rat.

We have analysed lard-casein diets identical in composition with those used by these workers, however, and have found them to contain enough tocopherol to supply about 40–70 μgm. per week to each rat. These amounts cannot be considered insignificant. Furthermore, as we have demonstrated elsewhere[26], ethoxyquin added to these so-called 'vitamin E-free' diets (especially those containing considerable amounts of vitamin A[36,37]) effectively spares tocopherol in rat uterus and in fact raises it to levels not incommensurate with it having some biological activity. Bieri *et al.*[38] have likewise suggested that the chick does not need tocopherol; but we have found that the diets used by these workers also contain appreciable amounts of tocopherol and that animals fed on them show significant tissue concentrations (to be published). There is no doubt that many of the discrepancies in these investigations—as well as the continued use of diets incorrectly believed to be free of vitamin E—stem largely from the use of inadequate analytical methods: only the more recently developed chromatographic procedures can deal satisfactorily with the small quantities of tocopherol relevant to investigations of vitamin E-deficient animals. It would seem that, if the absolute requirement of both the rat and the chick for α-tocopherol is to be determined, there is no alternative to the use of wholly synthetic diets. (In this connexion, even the methyl linoleate urea complex (Hormel Foundation), used by Crider *et al.*, has been found to contain about 3 μgm./gm. of α-tocopherol (and, in addition about 5 μgm./gm. of ubiquinone) which amounts are consistent with its method of preparation. The concentrate used by Draper *et al.* may have contained similar quantities. These figures illustrate some of the difficulties in work on vitamin E.)

Returning once more to the biochemical function of tocopherol, two points of interest need to be mentioned. First, is there any connexion between tocopherol and cholesterol synthesis? This much-neglected topic presents itself afresh in view of the contrasting effects of fasting on ubiquinone-levels and cholesterol synthesis, already referred to. There is already an area of confusion here: many workers[39,40] have shown that tocopherol has a marked hypocholesteræmic effect in the rabbit, but other workers[41] could not demonstrate this in the rat, whereas the results of Gray *et al.*[42] suggest that tocopherol might increase cholesterol synthesis in the rat. Secondly, is it possible to correlate the effect of vitamin E on ubiquinone with the demonstration by Corwin and Schwarz[9] that the decline in the oxidation of α-ketoglutarate by vitamin E-deficient liver homogenates can be prevented by *in vitro* addition of α-tocopherol, tocopheronolactone or menadione? Neither vitamin K deficiency nor menadione supplementation affects ubiquinone-levels in the rat (unpublished observations; see also Morton and Phillips[43]) and, furthermore, Corwin and Schwarz found dietary selenium to be inactive in restoring oxidation. It is possible, therefore, that although the effect of dietary selenium and tocopherol on hepatic necrosis may be related to their common role in ubiquinone biogenesis or processes leading to it, the effect of tocopherol in restoring respiratory decline in slices and of the ketoglutarate system may be due to yet another function of tocopheronolactone.

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PHOTOCHEMICAL SYNTHESIS OF AMINO-ACIDS AND A NEW TRANSAMINATION PROCESS BY TRANSFER OF FREE AMINO-RADICALS

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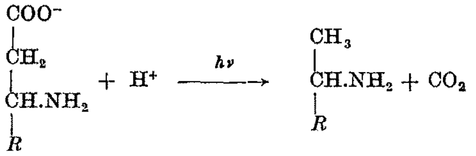
RECENT investigations conducted in our Institute[1-3] have shown that it is possible to synthesize amino-acids by ultra-violet irradiation of solutions containing a ternary organic compound together with a nitrogen compound, such as potassium nitrate, sodium nitrite, hydroxylamine and ammonium hydroxide. All the various steps of the photochemical process have been elucidated; the action mechanism of each of them has been established exactly, and a rational, co-ordinated interpretation has been deduced.

For reasons of brevity we report in Table 1 only a few examples of the syntheses accomplished by us.

Further investigations, which will be published in full in *Gazzetta Chimica Italiana*, have given results which we regard as interesting.

Amino-acids in acidified aqueous solution, when exposed to ultra-violet rays, undergo various changes, above all (scheme 1):

(1) Decarboxylation:

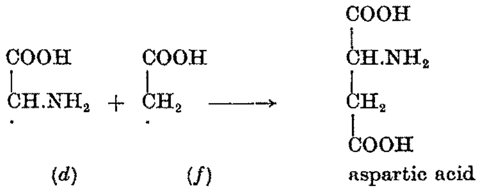


$$\begin{array}{l}COO^-\\ |\\ CH_2\\ |\\ CH.NH_2\\ |\\ R\end{array} + H^+ \xrightarrow{h\nu} \begin{array}{l}CH_3\\ |\\ CH.NH_2\\ |\\ R\end{array} + CO_2$$

Scheme 1

(2) Radical decomposition by homolytic degradation, for example, scheme 2:

(3) Recombination of radicals, with formation of new amino-acids: the free radicals may recombine in the most varied ways, for example, scheme 3:



$$\underset{(d)}{\begin{array}{l}COOH\\ |\\ CH.NH_2\\ \cdot\end{array}} + \underset{(f)}{\begin{array}{l}COOH\\ |\\ CH_2\\ \cdot\end{array}} \longrightarrow \underset{\text{aspartic acid}}{\begin{array}{l}COOH\\ |\\ CH.NH_2\\ |\\ CH_2\\ |\\ COOH\end{array}}$$

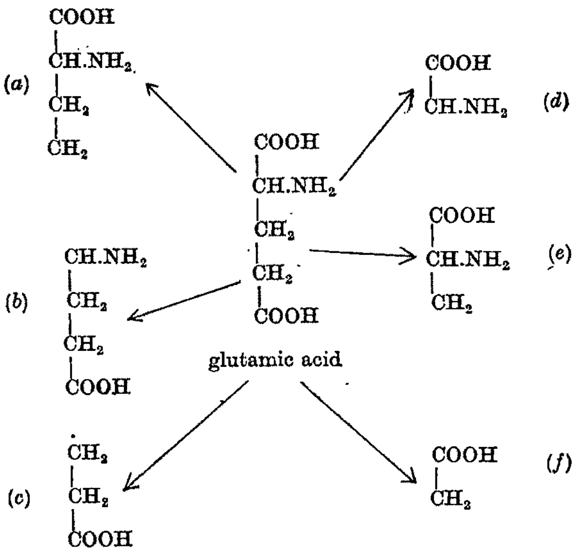
Scheme 3

Thus the radicals containing the amino-group may become 'transamination agents', in that they can determine the transfer of properties of the amino-group from one amino-acid to another of different molecular structure.

If the solution containing the amino-acid is irradiated in the presence of a properly chosen organic compound, capable, in its turn, of yielding free radicals, it is possible to direct the transamination process toward the formation of determinate synthesis products.

Table 1

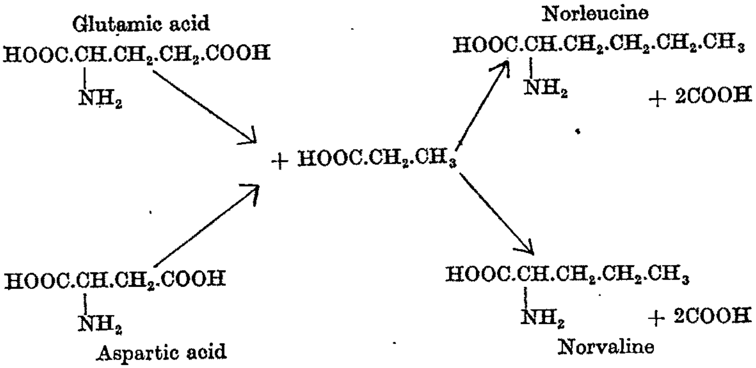
Organic substance irradiated with ultra-violet rays	Nitrogen compound present in the solution	Amino-acids obtained
Glutaric acid	KNO$_3$, KNO$_2$, NH$_2$OH	Glutamic acid, glycine, β-alanine, γ-aminobutyric acid, aspartic acid
Citric acid	KNO$_3$, KNO$_2$, NH$_2$OH	Glycine, β-alanine, serine, aspartic acid, γ-aminobutyric acid
Glucose	KNO$_3$, KNO$_2$, NH$_2$OH	Serine, glycine, aspartic acid



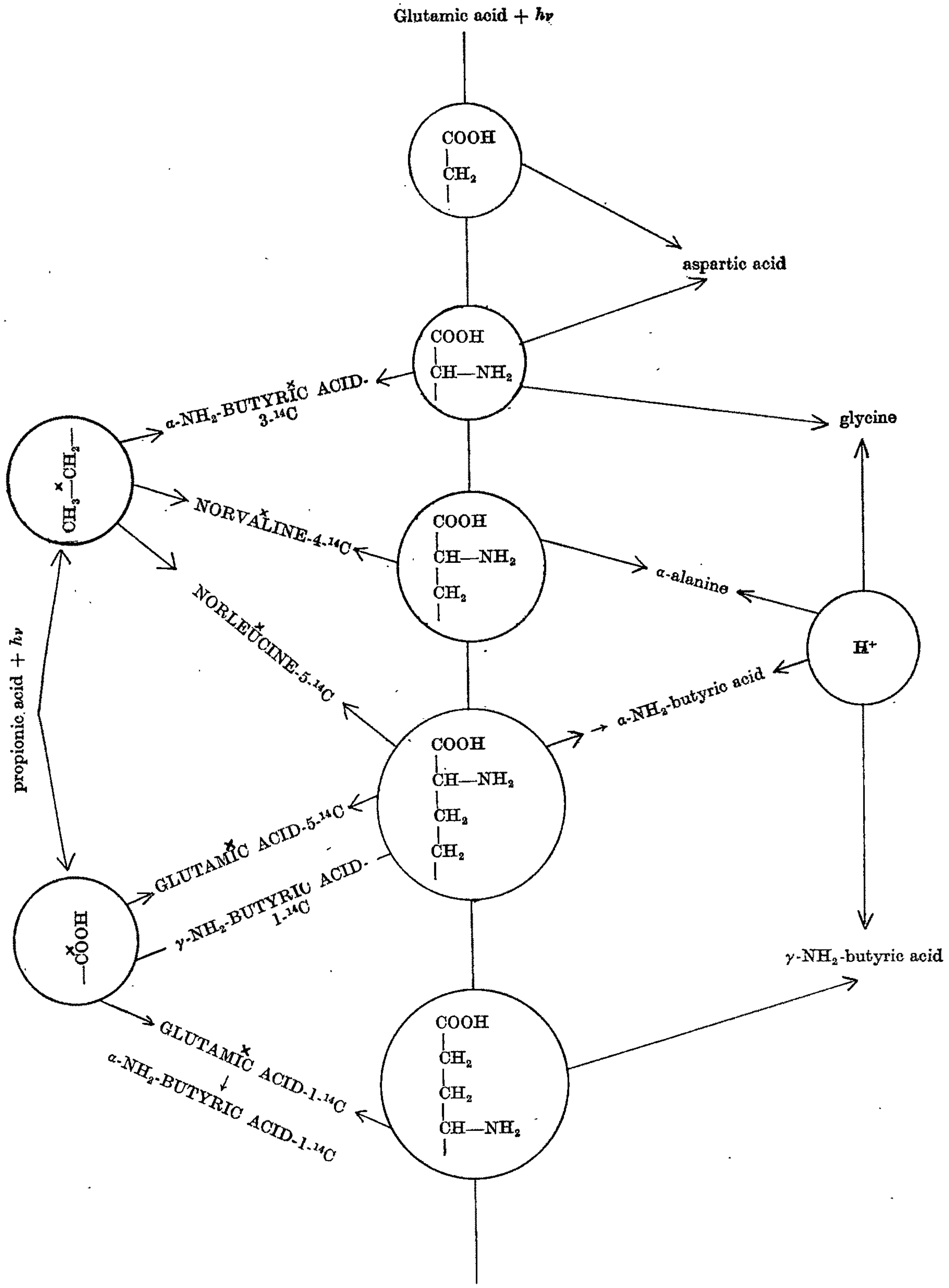
Scheme 2

For example, we have synthesized norleucine by irradiating glutamic acid in the presence of propionic acid, and have synthesized norvaline by irradiating aspartic acid in the presence of propionic acid

The scheme of the reactions involved is shown in scheme 4:



Scheme 4



Scheme 5

Capital letters, radioactive compounds; small letters, non-radioactive compounds. +, radioactivity from propionic acid-1-^{14}C; ×, radioactivity from propionic acid-2-^{14}C.

(As has been experimentally established, the two —COOH radicals form oxalic acid.)

In order to demonstrate the mechanism of the process described we have employed both the chromatographic detection of the synthesized compounds and the isotopic tracer method. For the latter purpose we have used two types of propionic acid labelled with carbon-14 in positions 1 and 2, respectively ($CH_3.CH_2.\mathit{C}OOH$ and $CH_3.\mathit{C}H_2.COOH$).

In all cases we have obtained a variety of amino-acids; but the examination of the autoradiographs obtained from the chromatograms has shown that the major part of the radioactivity is concentrated in the norvaline coming from aspartic acid and propionic acid-2-^{14}C, while the norvaline coming from propionic acid-1-^{14}C is completely inactive. Similar results have been obtained with the norleucine coming from glutamic acid + propionic acid. We have therefore succeeded in demonstrating the effect we sought.

We report the recombination scheme for the radicals obtained by exposing to ultra-violet rays the solution of glutamic acid + propionic acid-1-^{14}C and -2-^{14}C (Scheme 5). In the scheme the radicals are indicated, for simplicity, with an interrupted valence bond, whether they are actual free radicals formed by homolytic decomposition or ionic-type radicals due to heterolytic decomposition.

The new transamination process appears to be much more efficient than the conventional one, on account of the variety of amino compounds to which it can give rise.

As an example we may compare the possibilities of the system glutamic acid + oxalacetic acid within the framework of conventional transamination with those of the analogous system glutamic acid + succinic acid within the framework of radical transamination. In the former case only aspartic acid can be obtained, whereas in the latter case nine amino-acids can be formed, namely, aspartic acid, α-aminopimelic acid, γ-aminopimelic acid, α-aminoadipic acid, γ-aminoadipic acid, α-alanine, glycine, α-aminobutyric acid and γ-aminobutyric acid.

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MEASUREMENT OF FUNGICIDAL EFFECTS IN FIELD TRIALS

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DURING field trials on the control of coffee rust (*Hemileia vastatrix* B. et Br.) at the Coffee Research Station, Ruiru, Kenya, special methods were devised to obtain maximum sensitivity by minimizing the effects of localized, natural variability in rust, attack intensity. These methods are of general application, and may be adapted for use with other diseases. The very considerable local variation of rust attack found is, perhaps, remarkable if, as has been generally supposed, spore dispersal is predominantly by wind. However, recent research[1-3] has shown that most air-borne spores probably do not travel far and has indicated rain-splash as important for spore dissemination.

The degree of rust infection is best expressed as the number of spore-producing, individual, infection loci per unit of leaf area and is approximately equal to the number of lesions per leaf if the proportion of overlapping mycelia is low. The relationship to the percentage of infected leaves, which is easier to determine, was examined.

A representative sample of 100 leaves was taken from five trees in each plot of a six treatment, four times replicated randomized block, spraying experiment showing considerable differences in the level of rust attack, and was recorded for the numbers of leaves having respectively one, two, three, etc., rust lesions per leaf. The lesions were generally fairly small and scattered so that undetected overlapping was negligible. The cumulative frequency distribution curves for the percentage of the total number of leaves in each category of rust lesion number per leaf were plotted on a probit scale for each plot (Fig. 1). For a low percentage of leaves infected, a straight line indicating a truncated normal distribution is approximated. As the percentage increases so the truncation is less and the degree of curvature is more pronounced, indicating increasing divergence from normality due to the greater development of the upper 'tails' of the distributions. The relation between the percentage of rusted leaves and the number of lesions per 100 leaves is thus exponential (Fig. 2) instead of sigmoid, as it would have been had the distributions remained normal. Similar results from a locality in a climatically different area showed the same relationship. Thus one measurement may be regarded as a transform of the other.

The percentage of rusted leaves may in fact be a better measure of the effect of rust attack on the tree, as evidence has been obtained of a direct relation to foliage density. Thus, in one trial, the relation between the percentage of rusted leaves in May and the number of mature leaves per mature branch (that is, one on which leaf shedding had commenced) gave a regression coefficient of $-0{\cdot}98^{**}$, that is, for each leaf rusted in May, there was nearly one less present in September. Further, in other investigations, not however concerned with rust, strong correlations between foliage density and crop yield have been found.

In order to obtain a representative sample of leaves, account must be taken of the different leaf densities in different parts of the tree. In the above investigation, a method devised for investigation of fungicide cover, etc., was used. The total number of primary branches (that is, those originating on the trunk) per tree was counted. Every second, third, etc., primary was sampled, the interval increasing with the total number present. From the trunk, a path was traced out along one side of each primary sampled, up one side of any laterial branch or lateral on this and back down the other side to its base and finally back down the opposite side of the primary, so that the whole branch system was systematically traversed. Along this path, every 10th, 20th, etc.,

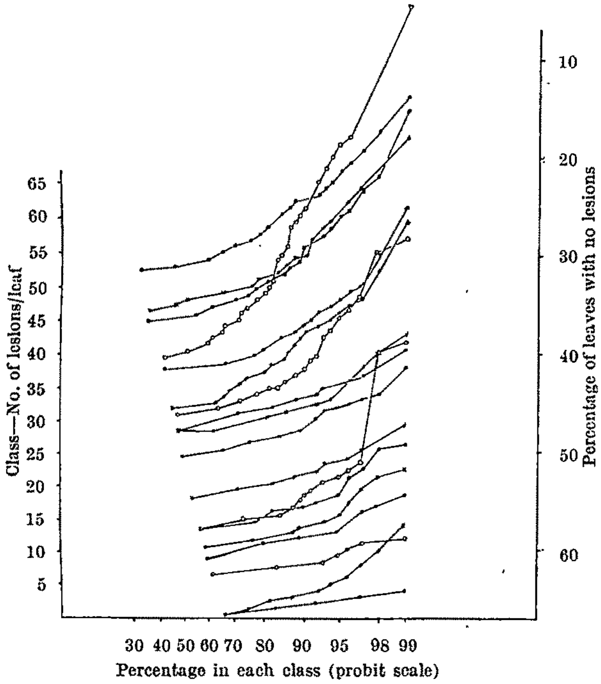

Fig. 1. Probit cumulative frequency distribution curves for eighteen plots of coffee trees, showing the percentage of the total number of leaves examined which occurred in each 'number of lesions per leaf' class. Each curve is displaced upwards to an amount proportional to the percentage of leaves with no lesions, so as to exhibit the change in form of the curve with this latter factor

leaf passed was removed according to the size of the tree, to obtain a sample of 20–30 leaves. This was reduced to 20 by discarding at random.

For field trials a similar, but less laborious, systematic sampling system was used. Five trees in each plot were roughly divided by eye into five radial sectors and five horizontal zones. On the first tree, the lowest primary in the lowest zone was traversed as before, on the second tree the middle primary in the next sector and next zone and so on. Each mature leaf traversed was examined and recorded if attacked by rust. One hundred leaves were inspected on each primary (the adjacent primary being included if necessary), a total of five hundred leaves in each plot. The resulting percentage infection was somewhat biased by over-representation of the lower regions of the tree since these have fewer leaves. Hence some over-estimation of rust attack probably resulted since more rust occurs in this region. As the trials were concerned with comparisons of treatments, this was of little importance, and it was possible for one man to record completely a large factorial trial of 42 plots and 920 trees in a few days.

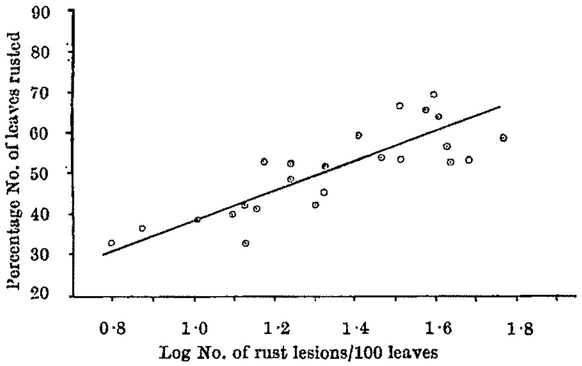

Fig. 2. The relation of the percentage of leaves with rust to the logarithm of the total number of rust leaves per hundred leaves

That incidence of rust in the field is strongly localized is clearly shown by observations made in three field trials. In these, the design could be treated as consisting of plots of two rows of ten trees each. Rust was sampled in each row on alternate, unsprayed trees, the other trees being sprayed. The standard error between the pairs of rows (sub-plots) was considerably less than between plots, being 63·8, 44·2 and 41·9 per cent lower in the three trials. The differences between the plots were, in accordance, very highly significantly greater than would have been expected from the differences between the sub-plots. Correlation between the sub-plots is thus present, the coefficients being 0·877***, 0·522** and 0·490*** respectively. These results suggest a distribution of rust attack by dispersal from centres of infection within the area rather than by mass infection arriving from outside. The correlation between sub-plots suggests that the measurement of rust intensity on a half of each treated plot in a field trial of the effects of fungicides could be used to correct that on the treated half for local variations using covariance, and hence increase the sensitivity of the trial. The effect was measured in the trials, omitting treated trees. The standard errors were reduced by 51·1, 15·1, and 11·9 per cent. The experimental area was divided into three blocks, and some reduction in standard error was obtained from this alone (14·1, 18·3 and 0·5 per cent), but in each case using covariance additional reduction (36·3, 5·7 and 11·8 per cent) resulted. Thus the relative usefulness of the two methods for the reduction of standard error varied from trial to trial, depending on the pattern of local variation in rust intensity.

Covariance analysis, although affording a useful approximation, is not strictly applicable when treatments are actually applied, since the greater the treatment effect the lower the rust intensity and the less the effect of the unsprayed level. The regression coefficient would be expected to range from 1 to 0, varying directly with the percentage of control. This was, in fact, found to be the case when data from one trial were examined critically. The correct adjustment is thus obtained if the rust-levels on the treated halves of the plots are expressed as a percentage of those on the untreated halves. A difficulty arises if the rust-level on individual unsprayed plots falls to a level very considerably lower than is general. Small differences between sprayed and unsprayed halves will then be disproportionately exaggerated and the apparent sensitivity of the trial will be unduly decreased. The use of an index of relative disease incidence, the ratio of the differences between sprayed and unsprayed halves to their sum, which amounts to a simple transform of the percentage, was found useful in such a case.

The degree of disease control exerted by a given treatment or treatment combination over the whole trial is more precisely estimated by summing the rust intensity on the respective sprayed halves of the plots and expressing this as a percentage of that on the unsprayed halves, rather than summing and averaging the percentage reduction of rust intensity on each individual plot, these latter data being reserved to estimate the significance of the differences. Some heterogeneity of variance was found and account was taken of this, sometimes by employing a transformation, but the effects were only slight.

The effectiveness of the methods used may be judged from the results of a factorial trial with three replications in which two fungicides at four concentrations equivalent to $\frac{1}{4}$, $\frac{1}{2}$, 1 and 2 per cent Burgundy mixture were contrasted for application to upper and lower leaf surfaces. Local variation in rust attack was considerable, the coefficient for the untreated plot halves being 56·5 per cent. The linear effect of concentration was very highly significant and differences between the effects of the fungicides estimated as equivalent to those produced by the difference between 1 and 1·3 per cent concentrations were detectable. In another trial with three replications of $\frac{1}{2}$, 1 and 2 per cent concentrations of three fungicides with upper and lower surface applications, differences equivalent to that between 1 and 2·6 per cent in one season and 1 and 2·1 per cent in a second season were detectable.

The observation discussed shows that intensity of rust attack may be estimated from the percentage of rusted leaves per bush, since there is evidence that this is mathematically related to the number of rust mycelia per leaf. It is probably directly related to effects on foliage density, which in turn is related to crop yield.

The sensitivity of field trials on the fungicidal control of rust disease may be considerably increased when local variation in intensity of attack is marked, by assessing this variation from estimates of rust intensity on alternate, unsprayed trees in each plot. The procedure increases the accuracy of the estimates of degree of control. Further, it should reduce the effects of any reduction in inoculum production resulting from a spray treatment on one plot on the inoculum-level in neighbouring plots.

The design was very satisfactory for differentiating between treatments where the aim was to assess the amount of protection against infection afforded by each. If, however, it is required to estimate the effects, especially as regards timing, on the rate of build-up of infection and this is influenced to any considerable extent by the amount of inoculum movement from tree to tree, the method may be unsatisfactory except for estimating 'within tree' effects and the amount of the inoculum movement. A method of dealing with this difficulty might be to use square plots in which the effect of varying the number of trees in a central sprayed group surrounded by unsprayed trees would be determined, and allowance made for the estimated effects of area sprayed on rate of build-up of attack by the disease.

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PREDICTION OF THE EFFECT OF LIGHT-TIME FRACTION ON THE CRITICAL FLICKER FREQUENCY; AN INSIGHT FROM FOURIER ANALYSIS

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THE study of the response of the human eye to flickering light has notoriously produced a number of extremely complex and confusing results. This article shows how at least part of the confusion can be resolved by the application of simple physical principles to the problem. At least two workers[1-3] have commented recently on the particularly chaotic results found in investigations of the relationship between the light-time-fraction (L.T.F., sometimes misleadingly called light-dark-ratio) and the critical flicker frequency (C.F.F., that is, the fastest frequency at which flicker is just perceptible). A sample of these results as summarized by Landis[3] is shown in Fig. 1. We suggest that the confusion can be understood and ordered by considering the Fourier analysis of the stimuli with different L.T.F.'s. In the present case, all we wish to do is to point out that a train of stimuli near fusion (the C.F.F. condition) can be considered to contain two different components: (*a*) a steady component (the first Fourier component) which is the mean intensity of the light and which we shall call I_M; (*b*) a fluctuating component (consisting of the second and higher Fourier components). This we shall call I_F.

The steady term I_M can be considered as a background illumination (or adaptation-level) and must have the effect of masking the fluctuating part, I_F, and thus of reducing the C.F.F. if I_F is held constant. I_F, on the other hand, is the effective stimulus and for a constant I_M, increasing I_F must increase the C.F.F. The crux of our argument is that complementary L.T.F.'s (for example, 0·1 and 0·9, or 0·3 and 0·7) are identical in the amplitude of their flickering components but differ in the amplitude of the steady components; 0·9 L.T.F. is much brighter than 0·1 L.T.F. since its I_M is nine times greater. Two things follow from this. First, we would predict that if the reduction in I_M for short L.T.F.'s is compensated by the addition of a steady light to the flickering light, the C.F.F. for complementary L.T.F.'s should be equal. Secondly, if we knew the effect of changing I_M, while holding I_F constant, we should be able to predict the shape of the curve of L.T.F. against C.F.F. for the uncompensated condition.

Other workers[4-6] have attempted to compensate for the obvious difference in brightness by varying the intensity of the flickering light. In our terms this is an illogical procedure, since, although I_M is now held constant, I_F varies inversely with the L.T.F. It is therefore not surprising that all these workers found C.F.F. higher for 0·1 L.T.F. than for 0·9. If no compensation of any kind is attempted, 0·1 still usually has a higher C.F.F. than 0·9. In this case it is because I_M varies with L.T.F. and the higher I_M masks the constant I_F and hence reduces the C.F.F.

To test our hypothesis we have repeated the experiments reported by previous workers and have managed to obtain most of the forms of relationship reported previously. Then we compensated by adding

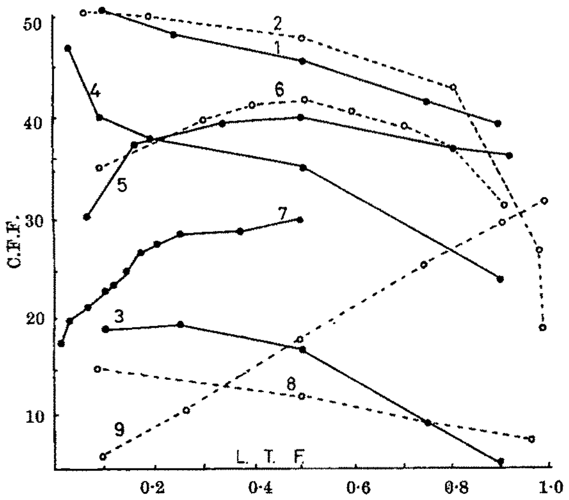

Fig. 1. Landis's (1954) summary of previous data. Effect of L.T.F. on C.F.F. as reported by different investigators. 1, Cobb, P. W. *J. Opt. Soc. Amer.*, **24**, 107 (1934); 2 and 3, Crozier, W. J. and Wolf, E., *J. Gen. Physiol.*, **24**, 505 (1941); 4 and 5, Bartley, S. H., *J. Exp. Psych.*, 21, 678 (1937); 6 and 8, Ives, H. E., *J. Opt. Soc. Amer.*, **6**, 254 (1922); 7, Porter, T. C., *Proc. Roy. Soc. A*, **70**, 313 (1902); 9, Pieron, H., *C. Rend. Soc. Biol.*, **99**, 398 (1928).

It should be noted that curve 9 is not entirely accurate. Pieron gives no percentage light per cycle (L.T.F.) but percentage dark per cycle. With L.T.F. as co-ordinate the curve should be reversed. It is, in fact, entirely compatible with curves 8, 3, 2, for example

a steady light of such an intensity that for all the L.T.F.'s we used the I_M was equal to that of the 0·9 L.T.F. As we expected, this resulted in complementary L.T.F.s having the same C.F.F. no matter what the shape of the uncompensated curve. A selection of our results is shown in Fig. 2. It adds to our satisfaction that in those cases where the uncompensated curves are symmetrical, our compensation has no effect and the curves remain symmetrical.

The bow-shaped symmetrical curve is also to be expected from the Fourier analysis. If one assumes (as one very plausibly may near the C.F.F.) that only the first flickering component of the Fourier analysis is effective, then one would expect the relationship of C.F.F. to L.T.F. to follow the relationship between the amplitude of the first flickering component and the L.T.F. This latter is, in fact, a simple bow-shaped curve with a maximum at 0·5 L.T.F.

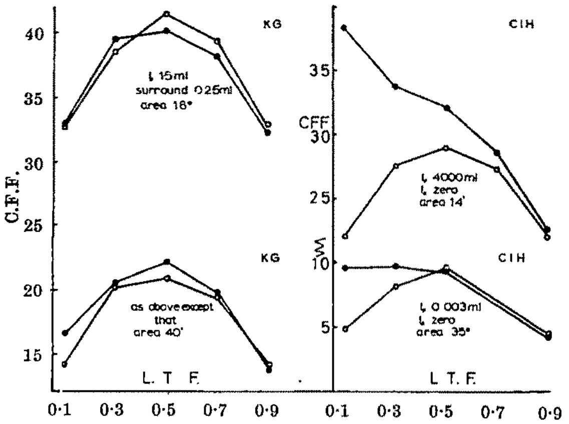

Fig. 2. The effect of changing the light-time-fraction on the critical flicker frequency. Filled circles represent mean C.F.F. with L.T.F. uncompensated for mean intensity. Empty circles represent mean C.F.F. with L.T.F. compensated so that the mean intensity is that of the uncompensated 0·9 L.T.F. This was done by adding a steady light to the remaining L.T.F. stimuli

We have already mentioned that the degree of asymmetry of the uncompensated curve should be predictable if one knew the relationship between I_M and C.F.F. for a given I_F. Complete data on this do not exist, but a very plausible deduction can be made from the very extensive data on the relationship between I_M and C.F.F. when I_F varies with I_M keeping I_F/I_M constant. This is, in fact, the usual way in which the relationship between C.F.F. and stimulus intensity has been obtained, using square waves of variable intensity with no background. Typically C.F.F. increases linearly with log I except for two regions where intensity has little or no effect on C.F.F.; see, for example, Hecht and Smith[7]. The first of these occurs at about 0·001 ml. between the photopic and scotopic regions, the second occurs at very high intensities, about 1,000 ml., where the direction of the relationship may actually reverse. Between these two regions C.F.F. increases markedly with intensity. In the two regions where intensity has little influence on C.F.F., the effect of I_F in increasing the C.F.F. and of I_M in decreasing it must almost exactly balance. In between, the effect of I_F is relatively greater. If we assume that this is because I_M is having a smaller effect in the intermediate region, then we would expect the least degree of asymmetry in the C.F.F.–L.T.F. curves under this condition. The two curves on the left-hand side of Fig. 2 were, in fact, obtained in this region, and the slight effect of area which they show fits the greater effect of increasing intensity for the larger areas. The two regions where intensity has little effect on C.F.F. would be expected to produce the greatest degree of asymmetry since here we have supposed that I_M will be exerting its greatest effect and the asymmetry is due to the change of I_M with L.T.F. in the uncompensated condition. The two curves on the right of Fig. 2 were obtained under these conditions.

There remains the possibility that the regions in which intensity has little effect on C.F.F. are due not to a change in the effectiveness of I_M but to a change in the effectiveness of I_F. However, de Lange[8] has published enough data to make this seem extremely unlikely although his data do not quite cover the necessary intensity range. The present experiments, of course, show that at 0·001 ml. and at 1,000 ml. I_M has a considerable influence on C.F.F., while at 1·5 ml. it has almost no effect.

The application of Fourier analysis to physical systems with a limited frequency response is strictly possible only for linear systems. There is a great deal of evidence that the eye, like most other sense organs, is non-linear in its response. However, there are many non-linear systems where the analysis of square wave impulses can give useful information provided not too much is expected of it. In the present case we have made only the most elementary use of the concepts of Fourier analysis. How far the method can be extended remains to be seen.

We wish to thank the Director-General of Medical Services for permission to publish this paper.

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PHYSIO C [illegible]

LETTERS TO THE EDITORS

RADIOPHYSICS

Some Preliminary Experimental Tests of a Novel Method of Radiating at Very Low Frequencies

For some years the idea of using an island as a radiator of very low frequencies (VLF) has been discussed in this Establishment. A long, narrow island some 20 miles by 1 mile, composed of poorly conducting material, would be ideal for the purpose. A narrow peninsula would provide a somewhat less efficient radiator. There are some similarities with the slot aerial, but the analogy cannot be pressed too far.

Experiments have recently been carried out using a peninsula some 10 miles long. A cable was laid across its root and the ends of the cable earthed in the sea. Power was fed into the mid-point of the cable.

If it is assumed that the island or peninsula is sufficiently narrow for the far-field to be determined by the algebraic sum of the currents on either side of it, then for radiation purposes a standing wave pattern of a half-wave dipole, or a correctly phased series of them, could be produced. Sufficiently low lobes might then be expected from a large enough array to launch very low-frequency waves at an angle suitable for long-range propagation. In the present trials it is quite probable that the major part of the radiation passed well above the receiving sites investigated, and returned to earth at much greater ranges.

Of several sites considered for the preliminary work, the Loch Long/Gareloch area offered the most advantages. Fig. 1 shows the position of the earthing plates $P1$ and $P2$. The earthing plates consisted of thin copper sheets 6 ft. × 3 ft., mounted on wooden frameworks. Three such plates spaced about 50 yards apart were used in each Loch. They were suspended near the surface with the aid of a system of buoys. The cable used was ordinary power cable of which approximately 3,000 yards were laid.

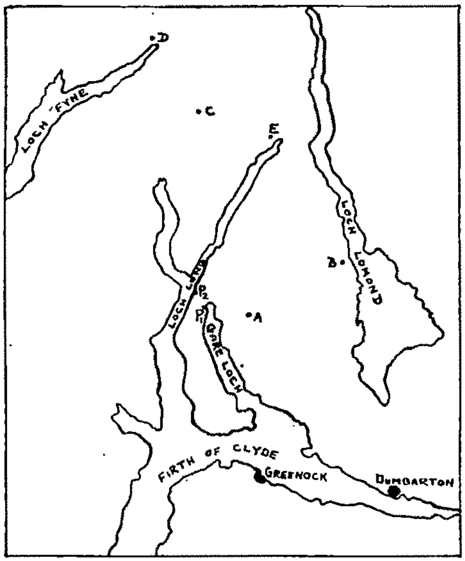

Fig. 1. Trials area

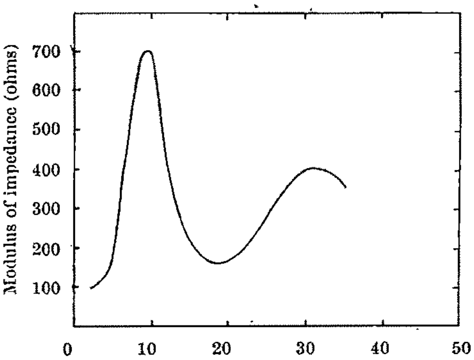

Fig. 2. Total path-impedance at high water

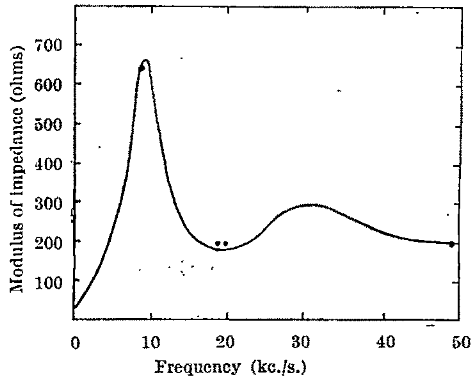

Fig. 3. Total path-impedance. ——, between high and low water ; ●, at low water

The transmitter delivered 50 watts at a frequency of 10 kc./s. The signals were received on a standard communications receiver with a high-Q ferrite loop aerial.

The objects of the initial experiments were to determine the suitability of such a peninsula for a radiator of very low frequencies and to determine its impedance characteristics.

Although the atmospheric noise appeared to be abnormally high, the signal was received at points A, B, C, D and E, shown in Fig. 1. At these points the receiving loop aerial could be used to obtain the approximate bearing of the transmitting aerial, and in each case this bearing appeared to point towards the centre of the sea-path. This was true, even at point A, when the receiving loop plane was rotated about a vertical axis: however, at A, reception was also achieved with the plane of the loop horizontal, which was not the case elsewhere.

It was expected that when the total length of current-path was an odd number of half wave-lengths the impedance would be high, whereas when the path-length was an even number of half wave-lengths the impedance would be low. Impedance minima were therefore expected, due allowance being made for the measured path-length, at integral multiples of 10 kc./s. and impedance maxima at odd integral multiples of 5 kc./s. As shown in Figs. 2 and 3, a maximum occurred at 10 kc./s. and a minimum at 20 kc./s. From subsequent experiments it appears that the effect arises on account of changes in the characteristics of the cable due to the proximity of the ground with which it was in contact.

It is concluded that the experiments clearly demonstrate the feasibility of the present proposals, as genuine radiation effects were observed. Some attention must, however, be paid to reducing the cable capacity to earth and the associated losses.

In conclusion, it should be mentioned that during these first trials, Press reports appeared of a similar investigation in Antarctica which had a rather different objective.

Thanks are due to Messrs. R. Benjamin, A. T. Rawles and K. V. Watt, of this Establishment, for useful discussions, to Messrs. W. R. Carter, W. L. Hibbens and E. G. Richards for assistance in the trials, and to the Admiralty for permission to publish the results.

R. N. Gould

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ASTROPHYSICS

Minimum Range to Artificial Earth Satellites

A prerequisite for ionospheric work with satellites by means of the Doppler method is the knowledge of the behaviour of the Doppler-frequency shift in free space. A free-space condition prevails, if the operating frequency f is much greater than the maximum plasma frequency. It is the purpose of this communication to present in a general form an equation from which the minimum range between a satellite S and an observer P can be determined and to illustrate the magnitudes of higher-order terms.

The first reports which appeared in the technical literature regarding the minimum-range equation generously neglected the effects of the curvature of the satellite orbit. An equation which included the effects of this curvature has been reported[1]. Specific reference to the previous neglect has been made by Brito[2] and in a simplified form by me[3]. Priester and Brito took into account the relative velocity between source and observer, but neither equation was complete. To arrive at a complete expression for the minimum-range equation, I shall first consider a two-dimensional case with the observer at rest ($v_p = 0$) in the plane of the orbit of S. In this case, one obtains the following equation for the time-derivative of the Doppler-shift curve for $v_s/c \ll 1$:

$$\lambda \dot{f}' = -\frac{v_s^2}{R}\sin^2\psi + \frac{v_s^2}{\rho_s}\cos\alpha \qquad (1)$$

where $\lambda = c/f$, c is the velocity of light in free space and f is the transmitter frequency of the satellite, $\dot{f}'$ is the time derivative of the frequency f' received at P, v_s is the orbital speed, R is the range SP, ρ_s is the radius of the orbit of S, $\psi = \sphericalangle \vec{v}_s \vec{R}$, and $\alpha = (\pi/2 - \psi)$. In the limit equation (1) assumes the form (when $\psi = \pi/2$, $\alpha = 0$, $R = R_m$):

$$\lambda(\dot{f}')_{\text{max.}} = -\frac{v_s^2}{R_m} + \frac{v_s^2}{\rho_s} \qquad (2)$$

or

$$\lambda(\dot{f}')_{\text{max.}} = -\frac{v_s^2}{R_m} + \frac{v_s^2}{\rho_s}\cos\xi \qquad (3)$$

if P is outside the plane of the orbit[4], ξ is the angle between $\vec{\rho}_s$ and $\vec{R}_m$ and R_m is the minimum range between S and P. In equations (1), (2) and (3) the second terms on the right represent the components of a radial acceleration toward P.

An earth-based observer at P, who is not located at the poles, is in orbital motion ($v_p \neq 0$) due to the spin of the Earth around its axis. Consequently, the minimum-range equation contains an additional radial acceleration component and can be written as:

$$\lambda(\dot{f}')_{f'=f} = -\frac{v^2_{\text{rel.}}}{R_m} + \frac{v_s^2}{\rho_s}\cos\alpha - \frac{v_p^2}{\rho_p}\cos\beta \qquad (4)$$

where

$$v_{\text{rel.}}^2 \doteq v_s^2 + v_p^2 - 2v_s v_p \cos j,\ j = \sphericalangle \vec{v}_s \vec{v}_p,$$
$$\alpha = \sphericalangle \vec{\rho}_s \vec{R}_m,\ \beta = \sphericalangle \vec{\rho}_p \vec{R}_m$$

with the signs in (4) corresponding to $\alpha, \beta < \pi/2$. It is seen that $\lambda(\dot{f}')_{f'=f}$ which may be different from $\lambda(\dot{f}')_{\text{max.}}$ is equal to the sum of three accelerations. The first term represents the radial acceleration due to a rotation of S with $\vec{v}_{\text{rel.}}$ perpendicular to $\vec{R}_m$. Since R increases for any displacement from $R = R_m$, the sign of the first term is negative. The second and third term represent the components of a radial acceleration for the satellite (v^2_s/ρ_s) and for the observer (v^2_p/ρ_p) in the direction of $\vec{R}_m$.

For $\rho_s = \rho_s = \infty$, including now the relativistic correction, one obtains the minimum-range equation for linear orbits:

$$\frac{\lambda(\dot{f}')_{f'=f}}{\sqrt{1-\left(\frac{v_{\text{rel.}}}{c}\right)^2}} = -\frac{v_{\text{rel.}}^2}{R_m} \qquad (5)$$

For satellite orbits the relativistic correction amounts to about four parts in 10^{10}. For practical purposes equation (4) seems adequate, since this correction corresponds to a quality of measurement equivalent of resolving less than 1 mm. of a 1,000-km. range.

The orbital motion of S and P may not be circular. Moreover, since the Earth is moving around the Sun ($v_G \doteq 30$ km./sec.) and since the Sun is moving in a curved orbit ($v_H \doteq 230$ km./sec.) around a centre of curvature, and since our Galaxy may move in its own curved path, etc., one can, for theoretical completeness, write the minimum-range equation in a form which includes all possible velocity and acceleration components. For $v_{\text{rel.}} \ll c$ this leads to the scalar equation :

$$\lambda(\dot{f}')_{f'=f} = -\frac{v_{\text{rel.}}^2}{R_m} + \sum_i\left(\frac{v_{is}^2}{\rho_{is}}\cos\alpha_i - \frac{v_{ip}^2}{\rho_{ip}}\cos\beta_i\right) + \sum_i(\dot{v}_{is}\cos\gamma_i - \dot{v}_{ip}\cos\delta_i) \qquad (6)$$

where in vector form, $\vec{v}_{\text{rel.}} = \sum_i(\vec{v}_{is} - \vec{v}_{ip})$ and the signs in equation (6) correspond to $\alpha_i, \beta_i, \gamma_i, \delta_i < \pi/2$. i is an index which refers the motion of S (satellite) and P (observer) to centres of curvature in the systems of the Earth, Sun, Galaxy, etc., respectively. In equation (6) both summations contain components of radial and tangential accelerations in the direction of $\vec{R}_m$.

Since the magnitudes of the higher-order terms (Sun, Galaxy, etc.) are less than 10^{-9} times the first term in equation (6) the slope of the Doppler-shift curve can be said to contain detectable and 'undetectable' elements of motion. The cause of the 'undetectable' motions reaches deep into space. For the detectable motions equation (4) seems sufficient. An estimate of the significance of each component of (4) reveals that for S and P orbiting, for example, in the equatorial plane with S at 1,000 km. elevation, the error introduced in R_m by neglecting the curvature of the orbit of S is about -15 per cent, while the error introduced by neglecting the curvature of the orbit of P is about $+0 \cdot 1$ per cent. If the slope of the Doppler-shift curve can be measured to an accuracy better than about 0·1 per cent, the latter contribution cannot be neglected. This does not mean, however, that with less accuracy the contribution due to the radial acceleration of P vanishes. If P is located at either pole of the Earth, $v_p = 0$ and equation (3) is valid for P outside the plane of the orbit of S.

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PHYSICS

Confining a Plasma in Steady Electric and Magnetic Fields

I HAVE suggested that ions may be trapped in an electric potential-well situated in a magnetic field[1]. The basic field configuration proposed was analogous to that in the Penning or Philips ion gauge discharge[2], where electrons are trapped, say, between two cathodes for a comparatively long period before they reach the anode ring situated between the cathodes. For trapping ions, the required potential-well is produced, for example, if a cathode ring is situated between two anode plates, with a magnetic field parallel to the axis of the cathode.

Experimental work was undertaken to study this possibility. The main experimental difficulty in realizing a field configuration suitable for confining ons was due to the fact that ions had to be generated by auxiliary discharges in regions adjacent to, but different from, the trapping region and had to be drawn into the latter region. In the case of Penning discharge, the electron current is initiated in the trapping region itself by field emission from the cathode or by ionization produced by cosmic or other background radiation.

Deuterium was used in these experiments, because the neutrons emitted by D–D reactions served as the indicator for the study of the plasma produced. Recent experiments were carried out at a gas pressure of a few microns of mercury. The voltage used for the discharge varied from 10 to 20 kV., and the discharge current was 5–10 m.amp. The magnetic field available was about 1,700 gauss.

Three main results were obtained as a result of this work: (*a*) The emission of neutrons indicated that high-energy ions were produced in this discharge. (*b*) By scanning the region of the discharge with a neutron detector provided with neutron collimation, it was found that the neutrons were mainly from the discharge region and not from the electrodes. (*c*) The most important result was that there was evidence for confinement of the ions. Because of the uncertainty in estimating the ionic component of the discharge current and the effective ion density, an estimate of absolute efficiency of neutron production was only of dubious value at the present stage of the experiment. Therefore, comparative neutron yields at different voltages were determined, with other parameters kept constant. If there were no confinement of the ions one would expect that the relative yield should follow the theoretical Gamow curve which has been experimentally verified[3-5]. On the other hand, if there were any confinement, the lower-energy ions would be confined relatively more than the higher energy ions, even in this very limited magnetic field. This meant that a graph of neutron yield at different voltages, when normalized at a particular energy on the Gamow curve, should show relatively larger yield at lower energies. Indeed, this was observed (Fig. 1). The solid line shows the variation of D–D reaction cross-section with energy[3], and the dashed line represents the relative yield of neutrons in the present experiments, at different voltages, normalized at 16 kV.

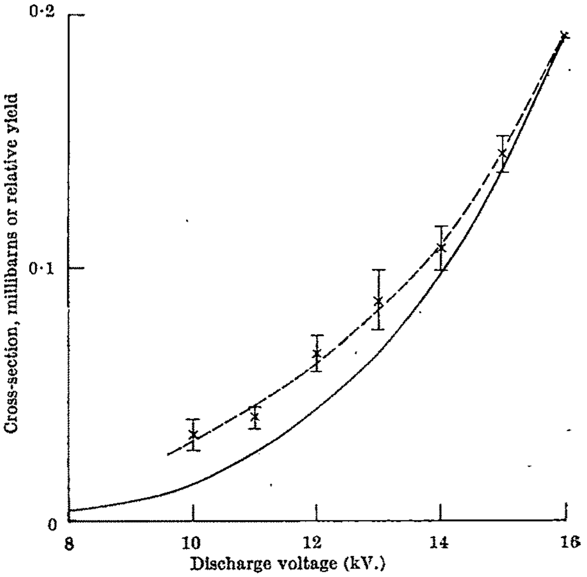

Fig. 1

Work is being continued, and the experimental details will be published elsewhere.

I am grateful to my colleagues Mr. S. S. Samant for assistance in the experimental work, Mr. M. L. Uplekar for the construction of some equipment, and Dr. A. P. Thatte and Mr. S. S. Bhave for useful discussions. I thank Prof. D. Y. Phadke for his encouragement.

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A Proposal for increasing the Beam Output from a Synchrocyclotron

In this communication, the possibility is considered of appreciably increasing the mean output of a synchrocyclotron, using relatively inexpensive equipment and with the expenditure of only a small amount of electrical power.

Suppose that a small dee system, covering the orbits of ions with energies of up to about 5 MeV., is placed opposite the live dee at the centre of the machine. To this small additional dee a high-voltage radio-frequency signal is fed which is in antiphase to that on the main dee. This signal is derived from the main oscillator system after amplification in a separate power amplifier. The auxiliary amplifier is pulsed on at a time slightly before the main dee commences to capture ions from the ion source, and is switched off when the captured ions have gained on the average a few MeV., that is, when the ion bunch is circulating at a radius such that it truly enters the main dee electrode and thus picks up the full dee voltage. It is essential that the auxiliary dee signal is reduced to a very small value as the ion bunch approaches the radius of the small dee edge, to avoid inducing radial betatron oscillations.

In Fig. 1 the assumed variation of the energy gained per turn by an ion is shown as a function of the orbit radius. Three cases are shown: the first curve corresponds to a normal synchrocyclotron, and the second curve to a machine modified in the way here described. The third curve, produced by too large a voltage on the small dee, is probably undesirable, because ions captured into stable orbits at the peak of the curve would afterwards be lost. Obviously, to achieve the required shape of the curve, the auxiliary power amplifier output must be gradually tapered off.

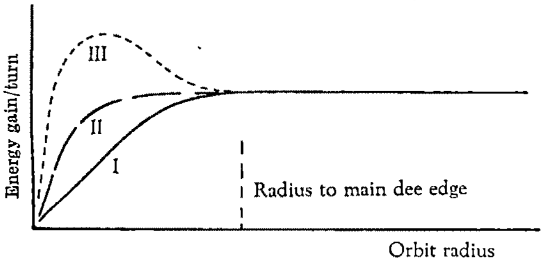

Fig. 1

The manner in which such an arrangement could produce an increase in the intensity of the beam is now considered. First, the energy gained per turn by the ions would be increased, permitting the use of a larger area in phase-space for ion capture. Secondly, the ions would be extracted more rapidly from the region of high particle density near the ion source, with a consequent reduction in the number of ions lost by gas scattering. Thirdly, the production of a more uniform electric field in the central region would cause a reduction in the movements of the centres of the orbits away from the centre of the machine, thereby reducing the amplitudes of the radial betatron oscillations.

The band-width and power requirements of the auxiliary amplifier are quite modest; a power of 1–2 per cent of the main oscillator power is sufficient, and the band-width need only cover the range of synchronous frequencies required by the ion bunch in its passage from the source to the radius at which the auxiliary amplifier is switched off. So that large signals are not induced in the small dee system by the main dee when it is switched off, the feed line should be resistively loaded; but this is usually also required to achieve a sufficiently wide band-width. In addition, a small earthed screen between the main dee and the auxiliary dee is desirable to reduce the electric coupling between the two dees and to improve the shape of the field near the centre of the machine. A typical arrangement is shown in Fig. 2.

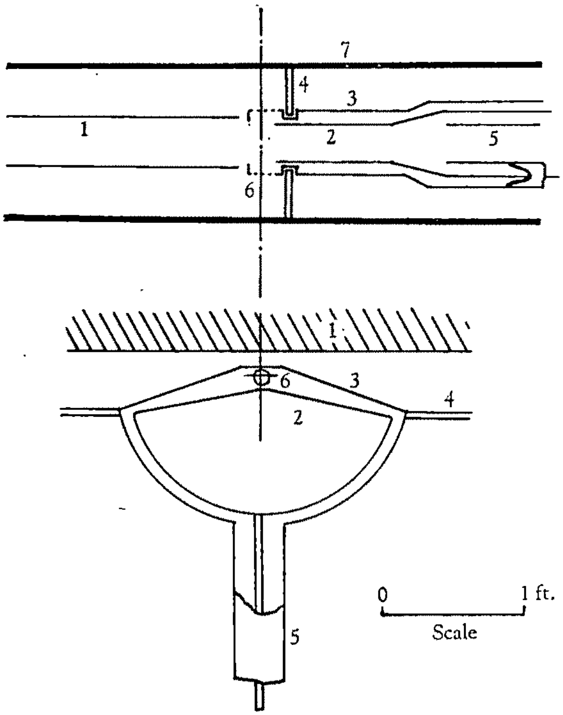

Fig. 2. 1, Main dee; 2, auxiliary dee; 3, auxiliary screen; 4, dummy dee; 5, co-axial feeders; 6, hole for ions; 7, vacuum chamber walls

Preliminary tests of this scheme have been made with the Harwell 110-in. synchrocyclotron, using an 11-in. radius dee with a total operating time of approximately 50 μsec. in each main modulation cycle. The rate of repetition for this machine is 200 c./s. A peak voltage of 4 kV. was used on the small dee, the main dee voltage being approximately 8 kV. peak. Despite a reduction in the vertical aperture of 25 per cent, caused by the small dees, an increase in the beam current measured at 50 meV. of approximately 60 per cent was readily achieved.

I thank the members of the team engaged on this work, in particular P. S. Rogers, for their help, and also J. D. Lawson for helpful discussions.

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Effect of a Resisting Couple on the Rotational Motion of a Rigid Body

In a recent communication[1], Nonweiler demonstrates the proposition that an external couple applied to a rigid body about its instantaneous axis of rotation in the sense opposing its rotation tends to cause the axis to approach the axis of greatest moment (provided that the instantaneous axis is not initially that of a permanent rotation). He refers to the rotational motion of artificial Earth satellites and the manner in which their instantaneous axes of rotation have been observed to approach the axis of greatest moment and suggests that this theorem may be relevant.

It seems to me, however, that if the underlying assumption of Nonweiler's proposition, that the external couple $\mathbf{G}$ is opposite to the angular velocity vector $\mathbf{w}$, is interpreted in the usual sense, $\mathbf{G} = -k\mathbf{w}$ (k, positive), then it does not, in general, apply to bodies under the influence of aerodynamic damping moments. In most practical cases, we are concerned with bodies having axial symmetry, and the damping moment about the axis of symmetry will be quite small compared with the damping moment about a lateral axis; in other words, the spin will tend to be retained whereas angular motions in pitch and yaw will be damped. Thus, whereas the direction of $\mathbf{G}$ will be such that $\mathbf{G}.\mathbf{w}$ is negative, the more restrictive condition $\mathbf{G} = -k\mathbf{w}$ will not apply. The only case to which the theorem as stated is directly applicable is that of a body, such as a sphere, having inertial asymmetry, but equal damping derivatives about three orthogonal axes.

Nonweiler derives the equation:

$$\frac{d}{dt}(h^2/2T) = \tfrac{1}{2}(\mathbf{G} \times \mathbf{h}).(\mathbf{h} \times \mathbf{w})/T^2 \qquad (1)$$

where $\mathbf{h}$ is the vector moment of momentum and T the kinetic energy of rotation. He shows that an increasing value of $(h^2/2T)$ may be interpreted as a movement of the instantaneous axis of rotation towards the axis of greatest moment. Now the term $(\mathbf{G} \times \mathbf{h}).(\mathbf{h} \times \mathbf{w})$ is positive if, and only if, the component of $\mathbf{G}$ in the plane of $\mathbf{w}$ and $\mathbf{h}$ is directed towards the side of the line of action of $\mathbf{h}$ opposite to $\mathbf{w}$. This includes the particular case $\mathbf{G} = -k\mathbf{w}$, but does not include the more general condition that $\mathbf{G}.\mathbf{w}$ is negative, satisfied, in practice, by an aerodynamic resisting couple. For this latter case, the conclusion may be rather different. Instead of the instantaneous axis of rotation tending to approach the axis of greatest moment, it will in most practical cases tend to approach the axis of symmetry, whether this is the axis of greatest moment or not. For example, a long pencil-like object spinning about its axis of symmetry and subjected to a resisting couple would be stable and not unstable as Nonweiler's proposition might imply. The fact that the converse behaviour has been observed on artificial Earth satellites would suggest that they are not in fact behaving as rigid bodies and that the role of internal energy dissipation is a dominant one.

The result for this more general condition may best be seen by resolving the vectors along principal body axes. Thus, writing:

$$\mathbf{w} \equiv (p,q,r)$$

$$\mathbf{G} \equiv (-\lambda p, -\mu q, -\nu r)$$

equation (1) becomes:

$$\frac{d}{dt}(h^2/2T) = \Sigma q^2 r^2 (\nu B - \mu C)(B - C)/T^2 \qquad (2)$$

where (A, B, C) are the principal moments of inertia and the summation is taken over all three components.

This equation could be used to indicate the motion of the instantaneous axis of rotation in any particular case, but no general interpretation can be given. However, for an axisymmetric body, we may put $\nu = \mu$, $C = B$ and equation (2) reduces to:

$$\frac{d}{dt}(h^2/2T) = p^2(q^2 + r^2)(\mu A - \lambda B)(A - B) \qquad (3)$$

In most practical cases, μ will be large compared with λ so that $(\mu A - \lambda B)$ will be positive. The sign of $\frac{d}{dt}(h^2/2T)$ will then be determined by that of $(A - B)$. Thus for $A > B$, $h^2/2T$ will increase and the instantaneous axis of rotation will tend towards the axis of greatest moment, that is, the x axis, which is the axis of symmetry. If $A < B$, $h^2/2T$ will decrease and the axis of rotation will tend towards the axis of least moment, that is, again towards the x axis. Thus it will always move towards the axis of symmetry provided that the condition $\mu A > \lambda B$ is satisfied.

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Anomalous Thermoelectric Properties of Gadolinium Selenide

AN earlier paper[1] has shown a narrow range of composition in the gadolinium–selenium system to exhibit highly interesting thermoelectric figures of merit ($Z = 4{\cdot}5 - 6{\cdot}0 \times 10^{-3}$ at 800° C.) The composition attaining such values lies between the two compounds GdSe and Gd_2Se_3, the characteristics of which have been summarized[2] as follows:

GdSe: NaCl type structure, bonding essentially covalent, very low electrical resistivity, Seebeck coefficient ~ 10 μV./deg. C.

Gd_2Se_3: defect Th_3P_4 type structure, bonding essentially ionic, very high electrical resistivity, Seebeck coefficient ~ 300 μV./deg. C.

High thermoelectric figures of merit are obtained when the preferred composition (~ 90 per cent Gd_2Se_3) has been quenched from temperatures just above the melting point (~ 1,800° C.), but these are lost on remelting or slow cooling from the melt. The parameters (Fig. 1) from which Z is calculated in the preferred composition present several interrelationships which are anomalous in the light of normal thermoelectric semi-conductor theory[3]. A detailed paper, to be submitted elsewhere, attempts to correlate and explain these anomalies.

Examples of the anomalies in the gadolinium–selenium composition are as follows: the increase in Q values with temperature suggests that the material is impurity-degenerate; but this does not conform with the linear or slightly decreasing values of $\varkappa$ which indicate non-degeneracy. The normal comparison between ZT and Q^2/L is not found. Up to ~ 800° C., $\varkappa_{ph}$ is proportional to reciprocal temperature, but above this the relationship $\varkappa = \varkappa_{el} + \varkappa_{ph}$ obviously does not apply. Derivation of $\varkappa_{el}$ from ρ values through the Wiedemann–Franz relationship is inadmissible, because the system is heterogeneous, as indicated by a plot of ρ vs. T. Normal X-ray diffractometry and metallography show, however, the material to be essentially single phase, and indeed when two phases are obviously present the material loses its high Z values.

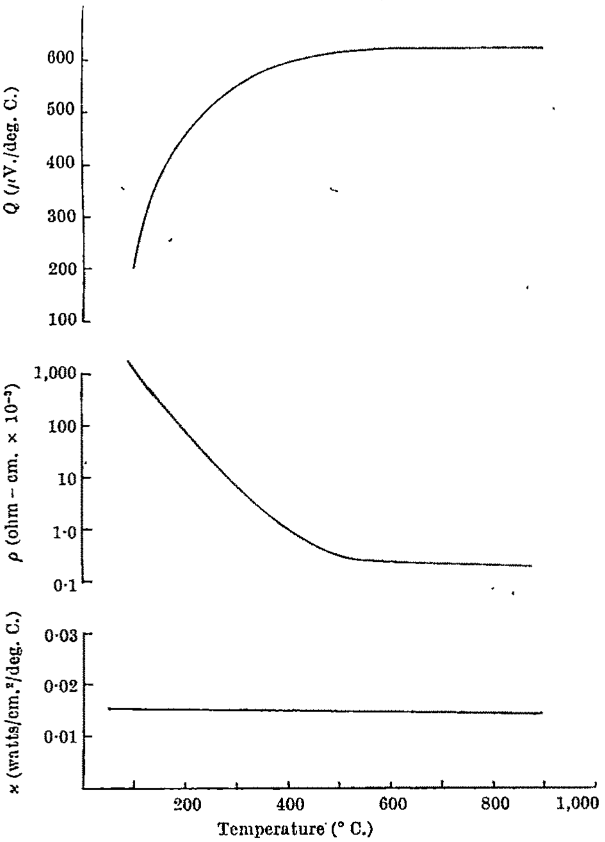

Fig. 1

Duwez *et al.*[4] have recently shown that, under conditions of rapid quenching from high temperatures, metastable and previously unknown intermetallic compounds are found in alloy systems. Similar work has been reported by Russian workers and summarized by Yevseyev[5]. In the present system, it is now believed that, under appropriate conditions of preparation, GdSe and Gd_2Se_3 interact to form such a metastable compound. This metastable product is believed to involve a lattice interaction through which the covalently bound GdSe and the ionically bound Gd_2Se_3 share a common crystal plane boundary. The bonding across such a boundary must be visualized as existing in a highly resonant condition; preliminary calculations suggest a room temperature resonance frequency of $\sim 4 \cdot 6 \times 10^{17}$ sec.$^{-1}$.

The essentially ionic nature of the predominant Gd_2Se_3 component, and the presence of the bounding plane postulated can be expected to present some resistance to the passage of carriers of electrically stimulated charge unless a novel transport mechanism is involved. This we now feel can be rationalized, on the basis of the phenomenon observed, by visualizing electromagnetic waves of thermal frequency—'thermons'—as charge carriers, and these associated with an almost purely inverse temperature-dependent variation in frequency of bonding resonance. Consequently, 'thermons' of a given frequency will not penetrate the resonance barrier until, by thermal vibration, the resonance frequency has decreased to the point at which the incident wave and the resonance oscillation are in harmony. At this point 'thermons', carrying the electrical charge, will avalanche through the barrier and electrical resistivity will drop.

Considering further the function of thermons in terms of Seebeck coefficient, we can visualize their passage through the resonating barrier in terms of oscillator-wave interaction theory, from which it is expected that energy will flow to or from the incident thermons, depending on resonance or otherwise of the bonding frequency (oscillator) and the incident frequency (wave). We believe, therefore, that the remarkably high Seebeck coefficient of such lattice-interacted gadolinium selenide can be attributed to the acquisition by incident thermons of energy from this resonating boundary which is then translated into terms of e.m.f. The behaviour thus presented is similar to that which forms the basis of 'maser' operation and, carrying the concept further on the basis of our most recent work, we believe that, by suitable selection of crystal parameters, materials and temperatures, it is possible so to adjust the resonating boundary frequency that translation from any one electromagnetic frequency into another may be possible.

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Structure of Calcium Soap Fibres

PREVIOUS publications on soap particles have contained conflicting views and evidence as to the orientation of the hydrocarbon chains within the particles. This is true even for a single soap, calcium stearate, and may be due to different methods of preparation leading to different crystal habits and/or structures.

So far as we are aware, no direct evidence from electron microscopy has yet been published concerning calcium soap fibres. We are particularly interested in materials such as are used in lubricating greases, and the samples we have examined were in fact prepared from calcium-based greases made with commercial grade fatty acid. It must be noted, therefore, that although our fibres contained a high proportion of C_{18} fatty acid, some of this was unsaturated and there were also minor proportions of acids of other chain-lengths present.

The method of sample preparation was the simple one of pressing a microscope grid coated with a carbon film on to a smear of the grease. The grid was then removed, washed in *n*-heptane to remove residual oil from the soap particles transferred, and examined in a high-resolution microscope without further treatment. Care was taken to keep the beam current low and to minimize the time of exposure of the sample to the electron beam.

Two types of soap fibre are commonly found in this type of grease: (i) small so-called 'twisted' fibres of widths about 0·06μ, which actually have more the appearance of helically rolled thin ribbons; (ii) ribbons of variable larger dimensions which have no twist.

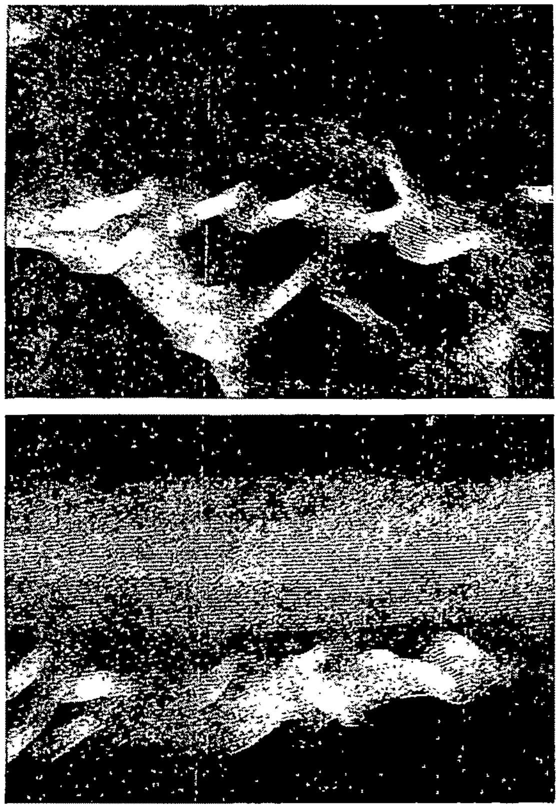

Fig. 1. Electron micrographs of calcium soap fibres showing structural detail (reverse printed for clarity). (× 114,000)

On examining both types at high resolution, striations were seen running parallel to the lengths of the fibres. The spacing of these striations is 42 Å., which, in the absence of an accurate calibration for the microscope, agrees well enough with the long-spacing value of 47·5 Å. derived from X-ray diffraction patterns of the soap. It is concluded that the striations are due to the 'edge on' view of the layers of metal atoms in the soap structure, which thus run approximately parallel to the direction of thickness and lengthwise. Fig. 1 shows the effect in both the straight and 'twisted' types of fibre, and does in fact help to suggest that there may be no marked difference in the structures of the two types. Furthermore, it gives some support for the idea sometimes entertained, that the small 'twisted' fibres result from the splitting up of the larger flat ones. In other micrographs of these grease fibres, edge dislocations have been seen, and traces of moiré fringe effects have been seen in crossed fibres.

Camp and Shuttleworth[1] found somewhat similar detail in sodium laurate fibres produced from aqueous solution, and from it they drew the same conclusions regarding the disposition of the molecules, thus supporting the ideas of Bondi and co-workers[2] on soap fibres generally. They found it necessary, however, to resort to metal shadowing in order to reveal this detail, and they believed that they were seeing agglomerates of shadowing metal nucleating on some unit within the fibre.

It seems likely that not all soaps crystallize in this way. Vold[3], using the same X-ray technique as Bondi, found sodium palmitate to fit in with the older ideas[4], namely, to have its molecular layers arranged perpendicularly to the thickness of the fibre. She claimed indirect evidence that calcium stearate, presumably the monohydrate, also obeyed the older pattern. Stanley[5] carried out some X-ray diffraction work on calcium stearate monohydrate using material re-crystallized from a mixture of propylene glycol and chloroform ; and in the lath-like crystals resulting he also found evidence that this soap crystallized with its molecular layers perpendicular to the direction of thickness.

The fibres with which we are working are formed under very different conditions, and differences of habit and/or structure seem not unreasonable, particularly in view of the impure nature of our material. What is surprising is that the structural detail is more stable and visible in the instrument than might be expected from accounts of other work in electron microscopes on crystalline long-chain compounds. It has not been necessary to resort to metal shadowing as did Camp and Shuttleworth, or to use osmium tetroxide as did Trurnit and Schidlovsky[6] when studying deposited multilayers of soaps. The metal atoms in calcium stearate are, admittedly, heavier than those in sodium laurate ; but they are only half as numerous, and, from simplified considerations of electron scattering power relative to carbon, they are likely to be only about 40 per cent more effective than the sodium atoms. We have noted striations still visible in these untreated calcium soap fibres after exposures of 3½ min. to the beam. The stability may in part explain the visibility.

Supposing that these grease fibres differ only from Stanley's thin crystals in external form, then their stability compared with sodium laurate could be due to a structure different from the bimolecular sheet arrangement widely accepted for soaps of monovalent metals. In his communication Stanley proposed an arrangement of overlapping molecules which might be less easily disrupted. It is noteworthy that such calcium soap fibres seldom show any longitudinal splitting of the type seen, for example, in some sodium soaps.

A comparison of X-ray long spacing values for Stanley's pure calcium stearate (50·3 Å.) and our fibres (47·5 Å.) suggests to us that the two materials may have essentially the same structure. The value for our soap is smaller by an amount roughly in keeping with the probable distribution of chain-length in our raw material. In considering the properties of these fibres we should not, however, overlook the possibility of effects introduced by minor components of the fatty acid mixture employed. Bondi, for example, reported that it was sometimes impossible to produce fibres from pure soaps.

We are continuing and extending these electron microscope investigations on soap fibres in greases, and hope to publish a fuller account in due course. We thank the Directors of "Shell" Research, Ltd., for permission to publish this communication.

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CHEMISTRY

Deviations from the Steady-State in Ion Transfer through Perm-selective Membranes

DURING experiments on polarization phenomena of perm-selective membranes, results were obtained which were inconsistent with the generally accepted idea that during the transfer of ions through such membranes by means of a constant direct current, a steady state is established under given experimental conditions[1].

The observation led to a series of rigidly controlled experiments in which I attempted to determine whether in fact a steady state is obtained.

A constant direct current was passed through a homogeneous cation exchange membrane bounded on both sides by 0·01 *N* silver nitrate solution. Silver electrodes were used, and the current obtained from a constant voltage rectifier (max. output : 400 V.) with less than 0·2 per cent ripple. To eliminate current variations a 44-k.ohm resistance was placed in series with the experimental cell. The potential difference through the system was measured by means of saturated calomel electrodes through 0·01 *N* potassium chloride/0·01 *N* potassium nitrate salt bridges, and a calibrated oscilloscope with d.c. amplifier. Measurements were made at various current densities and the current measured by means of an avometer. The solutions were stirred at 1,100 r.p.m. with glass stirrers.

After allowing some time for constant conditions to be established, I found that with the passage of the direct current, an unharmonic oscillation of the potential difference between the two sides of the membrane was obtained, about a constant average value. The maximum spread of the oscillation varied with the current density and at 30 m.amp./cm.^{2} increased to about 500 mV. (Table 1).

A control experiment in which the membrane was left out gave a maximum value at 30 m.amp./cm.^{2} of 20 mV. (Table 1). Further tests repeated with accumulators as the direct-current source gave similar results.

Table 1

Current density (m.amp./cm.^{2})	Spread of oscillation (mV.)		
	0·01 *N* $AgNO_3$	Control experiment	0·01 *N* $AgNO_3$ in agar–agar gel
1·5	5	5	20
3	10	5	100
6	150	10	1,000
15	300	20	8,000
30	500	20	—

In order to limit all other effects except those caused by current and diffusion, an additional experiment was made in which the solution in the cell was replaced by an agar–agar gel of the same ionic concentration. In this case, the oscillation spread was much wider and gave a value of 8,000 mV. at 15 m.amp./cm.^{3} At higher current densities the overall voltage increased to such an extent that no constant current could be obtained (see Table 1).

Experiments with an anionic exchange membrane in 0·01 *N* potassium chloride gave similar results.

Since, for steady-state conditions, the potential difference between two points in the system must be constant by definition, the experiments seem to indicate that during ion transport through perm-selective membranes by means of a constant direct current, steady-state conditions need not generally appear. I feel that it is necessary, therefore, to examine anew current theories on such transfer processes, and investigations in this direction are now being undertaken.

I am indebted to Mr. Y. Mahler, of the Electronics Laboratory for his co-operation in the planning and setting up of the measurements.

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A New Calcium Magnesiosilicate

A DETAILED investigation is in progress of high-temperature phase equilibria in the system $2CaO,SiO_2 - 3CaO,MgO,2SiO_2$ mainly by special methods of high-temperature microscopy[1,2] and X-ray analysis[3]. A new compound has been located of the approximate molar composition $(2CaO,SiO_2)_{5\cdot 6}(3CaO,MgO,2SiO_2)_{4\cdot 4}$ characterized by the X-ray diffraction pattern given in Table 1. This composition was prepared from crushed quartz (99·95 per cent pure), calcium carbonate (99·9 per cent pure), and magnesium oxide (99·9 per cent pure), by ignition at 1,250° C. in platinum boats in a platinum/rhodium resistance furnace. The fineness of the reaction mixture during ignition is of critical importance to the formation of the new compound. During a heat-treatment of 10 days duration the mixture was ground every two days to pass a 300-mesh sieve. Failure to grind to at least such fineness led to the formation of mainly $\gamma 2CaO,SiO_2$ and $3CaO,MgO,2SiO_2$ with only a small proportion of the new phase.

The X-ray powder measurements were obtained at room temperature in a Guinier-type focusing camera, 114 mm. diameter with copper-$K\alpha$ radiation. Relative intensities (*I*) were estimated visually on an arbitrary scale from 1 (very weak) to 10 (very strong). The density of the new compound is 3·30 and the optical properties are as follows : biaxial positive $2V$ medium (approximately 30°), RI_{Na} $\alpha = 1{\cdot}711$, $\gamma = 1{\cdot}725$.

The phase is formed by solid-state reaction and does not melt congruently.

Sharp, Johnson and Andrews[4] report a new phase, *T*, the presence of which was detected in basic arc

Table 1. ROOM TEMPERATURE X-RAY POWDER MEASUREMENTS FOR A NEW CALCIUM MAGNESIOSILICATE

d	*I*	*d*	*I*	*d*	*I*	*d*	*I*
3·84	1	2·580	2	2·001	3	1·588	1
3·79	1	2·548	2	1·953	1	1·576	1
3·48	2	2·481	2	1·924	5	1·563	3
3·37	2	2·447	2	1·897	2	1·558	3
3·22	2	2·421	2	1·876	2	1·548	2
2·95	2	2·340	2	1·836	2		
2·829	4	2·292	1	1·747	1	1·428	1
2·771	1	2·228	4	1·727	1	1·420	1
2·732	10	2·210	4	1·708	1		
2·718	10	2·172	2	1·687	2	1·363	2
2·668	8	2·110	2	1·669	1	1·336	3
2·648	1	2·080	2	1·649	2	1·297	1
2·631	1	2·070	3	1·621	1	1·243	1*B*
2·594	2	2·020	1	1·606	1		

B, broad line

furnace slags. The lines in Johnson's X-ray patterns (private communication), which he considers to be due to a new phase, seem to accord with the strongest lines found in the pattern reported here. The stable phase assemblages between the new compound and adjoining compounds are being studied. It is already clear, however, that over a wide range of composition between $2CaO,SiO_2$ and $3CaO,MgO,2SiO_2$ the X-ray pattern of the new phase is unaltered, supporting the view that the phase is a singular compound and not a member of a solid-solution series.

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Use of Krypton for Surface Area Measurements

WE have recently discussed[1] some criteria for the applicability of various gases in measuring the surface areas of solids by the Brunauer–Emmett–Teller[2] method. We concluded that the use of krypton in this technique led to valid and useful results and that previous criticism of the use of this gas was unfounded. However, Thomas[3] has again suggested that 'anomalous' Brunauer–Emmett–Teller plots obtained from krypton adsorption measurements render the use of this gas undesirable. He proposes that the occurrence of phase-changes in the adsorbed film, evidenced by discontinuities in the adsorption isotherm, are associated with these 'anomalous' (and presumably suspect) results.

We wish to re-emphasize the fact that an evaluation of monolayer coverage must be based on the behaviour of the adsorbed film when the first monolayer is nearly complete. Two-dimensional condensation or other transitions in the adsorbed film at lower or higher coverages do not appear to affect the validity of the surface areas evaluated from linear Brunauer–Emmett–Teller plots in the monolayer region. McDermot and Lawton[4], for example, were able to evaluate the surface area of a graphite sample from krypton-adsorption data even though they observed two discontinuities in the isotherm at 77° K. The work of Roberts[5] strongly suggests that krypton adsorbed on an evaporated iron film can be considered a two-dimensional liquid at coverages greater than about 80 per cent of the first monolayer. The energetic considerations which we have previously used[1] are therefore applicable, and valid surface area measurements are obtained in these cases.

We submit, therefore, that the non-linear parts of the plots described by Thomas are irrelevant to the purposes of surface area measurement. The application of the self-consistency criterion[1,6]:

$$p/p_0|_{v_m} = \frac{1}{1 + \sqrt{c}}$$

suggests, however, that if his measurements had been carried to somewhat higher pressures, Thomas would have obtained valid v_m values.

It must be remembered that the useful linear range of a Brunauer–Emmett–Teller plot varies, in terms of relative pressure, with the nature of the solid surface, as we have already shown[1].

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Properties of Polyadenylic Acid in Methanol Solution

UNTIL recent years, methanol and ethanol were universally regarded only as precipitants for nucleic acids. However, Geiduschek and Holtzer[1], and later Coates and Jordan[2], showed that at sufficiently low ionic strengths both the above lose their precipitating capacity for deoxyribonucleic acid (DNA) and in fact becomes fairly good solvents, depending upon the concentration of electrolyte.

The above authors have also observed a profound alteration of the over-all macromolecular properties of DNA in the above media, including a drastic fall in intrinsic viscosity and rise in frictional coefficient without any important change in molecular weight. The extensive molecular collapse indicated by the above was largely reversed on replacement of alcohol by dialysis versus water, suggesting that irreversible 'denaturation' in the classical sense had not occurred in this instance.

The present investigation deals with the properties of polyriboadenylic acid (poly A) dissolved in anhydrous methanol. Such criteria as ultra-violet hypochromism and optical rotation have been interpreted as indicating that the alkaline form of poly A in aqueous solution possesses varying degrees of helical content, depending on conditions, and displays in fact many of the characteristics of an intermittently helical system, being rather analogous to ribonucleic acid[3,4].

Solutions of poly A (1 per cent) in water were dialysed exhaustively versus anhydrous methanol according to the procedure of Geiduschek[1]. (The poly A used was prepared by the action of the polynucleotide phosphorylase of *M. lysodeikticus* (supplied by Dr. R. Beere) upon adenosine diphosphate.) After dialysis they were diluted five-fold with methanol. The final water content was less than 1 per cent. No dependence of molecular properties on water content was observed for values of the latter less than 5 per cent.

Table 1

Solvent	Water (0·001 *M* sodium acetate)	Methanol (0·001 *M* sodium acetate)
Absorbancy ($\lambda = 260$ mμ (20° C.))	9,500	13,600
Absorbancy* ($\lambda = 260$ mμ (50° C.))	12,400	13,600
Sedimentation coefficient ($S^{20}_{0,w} \times 10^{13}$)	6·9	50
Intrinsic viscosity	16·8	0·26
Optical rotation ($[\alpha]_d$)	+ 100°	− 25

* Corrected for thermal expansion of solvent.

The results (Table 1) can be summarized briefly: The high degree of dextrorotation ($[\alpha]_d$ = 100–150°) normally characteristic of the alkaline form of poly A in water[3,4] is, in methanol containing 0·001 *M* sodium acetate, replaced by a small negative rotation.

Furthermore, most of the hypochromism displayed by poly A in water disappears in methanol solution, as Table 1 shows. The absorbancy at 260 mμ increases by more than 40 per cent in the latter medium and becomes independent of temperature. Inasmuch as the absorbancy of the monomer, adenosine monophosphate, was found to be the same in water and in methanol there is a definite implication that the result with poly A reflects some modification in the secondary structure or conformation of the polymer.

The macromolecular properties of poly A underwent an equally dramatic change. A profound increase in the sedimentation coefficient and a decrease in intrinsic viscosity occur in methanol solution, as has already been observed in the case of DNA (refs. 1 and 2).

All these effects were reversible on removal of methanol by dialysis versus water.

The effect of methanol upon the over-all *macromolecular* properties of poly A is entirely in harmony with the reported results upon DNA. In both cases a dominant factor in the molecular collapse is undoubtedly the largely ion-paired character of the phosphate groups in alcoholic solution[2]. The electrostatic stress, which is important for aqueous solutions of polyelectrolytes at the low ionic strength (0·001) of these investigations, is thus largely relieved.

Thus the apparent helical content of poly A largely disappears in methanol, as judged by both the hypochromism and optical rotation criteria.

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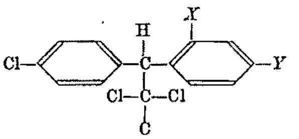
Toxicity of 2-(2-Halogen-4-Chlorophenyl)-2-(4-Chlorophenyl)-1,1,1-Trichloroethanes to Normal and to DDT-Resistant House-Flies

As part of a programme designed to test a free-radical hypothesis of insecticidal action for DDT[1], a series of new DDT compounds substituted by halogen in the ortho-position was synthesized and the toxicity to normal and to DDT-resistant house-flies (*Musca domestica* L.) was determined (Table 1). The results are of unusual significance in regard to : (1) the mechanism of DDT resistance, and (2) the relative toxicity of the positional isomers of DDT.

In the susceptible *S*-strain of the fly, a direct correlation exists between the size of the halogen in the ortho position and the LD_{50} value. A reverse correlation is evident with *in vitro* enzymatic dehydrochlorination. If this detoxication mechanism is indeed the basis for DDT-resistance in the resistant *R*-strain, the toxicity of these compounds to the tolerant strain should be the resultant of these two inverse relationships. Results support this supposition with the maximum insecticidal effectiveness occurring in the 2-(2,4-dichlorophenyl)-2-(4-chlorophenyl)-1,1,1-trichloroethane (III).

Table 1. TOXICITY SPECTRA OF DDT ANALOGUES TO SUSCEPTIBLE AND RESISTANT HOUSEFLIES AND CORRELATION WITH DEHYDROCHLORINASE ACTIVITY

			LD_{50} (μgm./♀ fly)		Relative enzymatic dehydrochlorination (*in vitro*)
Compound	*X*	*Y*	*S*-strain (CSMA)	*R*-strain (DDT-45)	*R*-strain Brei
I	H	Cl	0·1	> 40	+ + + +
II	F	Cl	0·2	14·0	+ +
III	Cl	Cl	0·4	0·8	+
IV	Br	Cl	1·1	2·0	–
V	H	H	3·3	> 40	+ + +
VI	Cl	H	6·2	~ 30	–

LD_{50} values are averages of duplicated tests made on at least two successive generations of 5-day-old flies. Each point on dosage–mortality curve was determined by topical application (in 1 μl. acetone) to 80–100 female flies. Treated flies were held at 75–80° F. and percentage mortality recorded at 24 hr. Enzyme activity was measured on a concentrated homogenate of *R*-strain, assayed by the glass bead procedure (ref. 3).

The toxicity spectrum with the *S*-strain indicates that mono-ortho substitution by a chlorine atom reduces toxicity of the parent compound by a small factor. (Compare I with III and V with VI.) When one observes the much greater difference in toxicity between I and V and between III and VI, it becomes evident that the weak activity of VI is primarily the result of the loss of the para-substituent. Hindrance of 'free rotability' of the benzene rings[2] by a single ortho-chloro-substituent can only be of lesser importance in reducing the insecticidal properties of the molecule. The possibility that samples of VI may contain a small amount of I and thus account for the toxicity of the former compound to *S*-strains may be raised. Barring synergism, the fact that VI is still somewhat toxic to the *R*-strain would appear to preclude this possibility.

Further work is in progress to evaluate these and related compounds against different fly strains, naturally tolerant insects, and species with acquired resistance. Selection experiments are also being conducted to determine if the house fly has the capacity to develop resistance to these new compounds. Data on the methods of synthesis and characterization of these new compounds will be published elsewhere.

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MINERALOGY and GEOLOGY

Infra-red Investigation of the Migration of Lithium Ions into Empty Octahedral Sites in Muscovite and Montmorillonite

In work on the reaction of molten salts with layer-lattice silicates, it was found that molten lithium nitrate removed a large portion of the potassium from muscovite ($< 5\mu$) and lithium was fixed in a not readily exchangeable manner[1]. It was suggested that lithium ions migrated into the vacant octahedral positions of the dioctahedral micas in a manner similar to that postulated previously by Hofmann and Klemen[2] and Greene-Kelly[3] for lithium-saturated montmorillonite on heat treatment. X-ray diffraction measurements of the 060 spacing of muscovite treated with lithium showed no significant increase, whereas an increase might be expected in conversion of a dioctahedral composition to a trioctahedral composition. Attempts to apply the infra-red technique of Serratosa and Bradley[4] for determination of the orientation of OH-bond axes to indicate formation of a trioctahedral composition were also unsuccessful.

Stubican and Roy[5] observed that an increase in the amount of magnesium in octahedral sites in a muscovite-phengite series caused the absorption band with frequency 935 cm.$^{-1}$ to become more poorly defined. They allocated this band to H–O ... X^{3+} by comparison of spectra for minerals with their deuterated synthetic analogues, and showed that it moves from 935 to 910 to 827 cm.$^{-1}$ as the ions change from aluminium to gallium to iron. Serratosa[6] has assigned the 915 cm.$^{-1}$ band in montmorillonite to H–O... Al^{3+} on the basis that this band decreases in intensity at a rate concomitant with those of the stretching frequency (3,600 cm.$^{-1}$) on heating to successively increased temperatures.

The dioctahedral compositions of the muscovite and montmorillonite structures provide each structural OH with two octahedral ion neighbours, predominantly aluminium in these two minerals. The effect of heavier octahedral neighbours or a greater number of neighbours on the OH bending frequency is considerable[5,6].

If lithium ions enter the empty octahedral sites in muscovite and montmorillonite, one would expect that the three octahedral neighbours (2 Al, 1 Li) of the OH would anchor it more firmly and lower its bending frequency. Fig. 1 shows superposed absorption records of the 5–2μ fraction of (*A*) untreated muscovite (Delamica) and (*B*) the same material after treatment with molten lithium nitrate for 480 hr. The absorption feature appearing at 915 cm.$^{-1}$ in the untreated muscovite is absent after the treatment with lithium nitrate. In addition, the features at 828 cm.$^{-1}$ and 798 cm.$^{-1}$ are eliminated. Treatment of montmorillonite (Wyoming bentonite) with molten potassium nitrate at 350° C. did not affect the 915 cm.$^{-1}$ OH bending frequency, whereas treatment with molten lithium nitrate at 300° C. eliminated the band. These results give experimental verification of migration of lithium ions into empty octahedral sites in muscovite and montmorillonite as postulated previously.

A more detailed account of this work will be published elsewhere. We acknowledge helpful discussions with Dr. W. F. Bradley. The work was supported in part by a grant from the National Science Foundation (*NSF G*6063). The assistance of Dr. John Amy and Marcia Dilling, Chemistry Department, in obtaining the infra-red spectra is gratefully acknowledged.

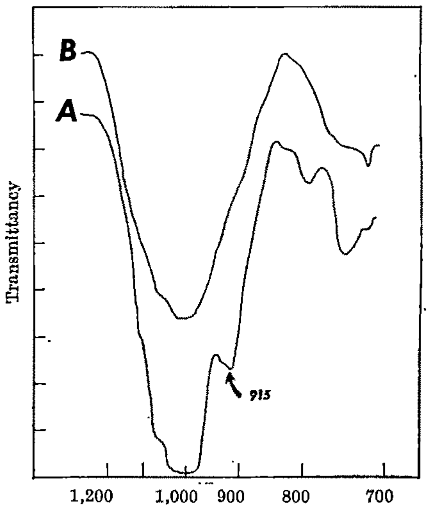

Fig. 1. Superposed infra-red spectra of muscovite (Delamica) (5 – 2μ) (*A*) untreated and (*B*) treated with molten lithium nitrate at 300° C. for 480 hr.

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Mammoths in the Cotswolds

Recent examples of fleeting opportunities provided to archæologists, geologists and others by modern methods of excavation are common knowledge; all too often an opportunity passes before advantage can be taken of it. The following notes illustrate the fruits of sustained observation on one hand, and of good fortune on the other, which we have witnessed in the past year or so.

First, pits worked over a period at Great Chessells, near Bourton-on-the-Water, Glos., in a headwater tributary of the Windrush, were watched by Mrs. Helen E. O'Neil, because she was excavating a Romano-British site progressively destroyed by exploitation. She obtained from the gravels an important collection of mammoth teeth and two molars of woolly rhinoceros[1]. They are comparable with material collected over many years from the lower gravels of the Summertown–Radley terrace of the Upper Thames and its tributaries around Oxford. The association united the deposits of the headwaters of one such tributary, namely, the Windrush, with those of its confluence with the Thames.

Further, material of the same form as these molars of mammoth was recovered recently by one of us

(L. R.) from gravels of the Severn near Worcester[2], and it is known in those of the Avon[3,4].

Within recent months, two notable and unique additions have been made, namely, a comparable molar from the Cheltenham Sands at Charlton Kings[5], and a specimen from the Paxford gravel at Ditchford, between Stretton-on-Fosse and Moreton-in-Marsh, Glos., which again we owe to Mrs. O'Neil.

The molar from Ditchford raises old issues because the succession there was studied years ago by Dr. Mabel Tomlinson[4], who concluded that the gravel lay between two glacial deposits, an older or Campden Tunnel Drift, and a younger or Moreton Drift. The pit was worked extensively for a short time, and in the autumn of 1960 it was filled in ; but the sections confirmed Dr. Tomlinson's conclusions because we saw all three deposits in continuous working-faces[5]. Although irregular surfaces occurred below and above the Paxford gravel, in some places it passed up from the underlying drift with no more than changes of constituents. At the top the distinctive Moreton Drift, with abundant flints, lay on the gravel, which was in some places strongly disturbed, and in others undisturbed. Thus, in spite of striking differences in the constituents of the three deposits, it would be hard to say that they represent events widely separated in time.

The importance of the molar from the gravel at Ditchford thus lies in the fact that it is associated stratigraphically with glacial deposits, which may be traced into the Avon valley and into the head of the Evenlode. Farther down the latter valley, in the Oxford district, is the region of abundant occurrences of teeth and bones of mammoth with an associated fauna, in the lower part of the Summertown–Radley gravels. The upper part, unconformable on the lower, contains remains of hippopotamus and other mammals, and freshwater molluscs[6].

The tributary of the Windrush mentioned above is separated from the upper reaches of the Evenlode by a ridge of Jurassic rocks near Stow-on-the-Wold.

If mammoth teeth found in the various deposits recorded in this communication may be accepted as a common and diagnostic palæontological factor, a sense of contemporaneity is introduced between gravels of the Upper Thames and its tributaries, and of the Severn and Avon, glacial deposits, and the Cheltenham Sand (which in part may be of æolian origin). In fact the molars are known widely, from the Cotswolds to the Lower Severn (for example, Barnwood) and to the Midlands, and they are distinctive: the 'Siberian' form, known also in younger deposits, sometimes occurs. Consequently, the significance of the several deposits and the tooth in the Ditchford pit, close to the divide between the Thames and Severn systems, can be readily determined, and the result is valuable in connexion with the correlation of the superficial deposits in the basins of the two rivers.

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BIOCHEMISTRY

Comparison of the β-Lactoglobulins of Buffalo's Milk and Cow's Milk

Aschaffenberg and Drewry[1,2] demonstrated by paper electrophoresis at pH 8·6 that β-lactoglobulins of cow's milk (sometimes described in the literature as bovine β-lactoglobulin) occurred in two different forms, β-lactoglobulin A and β-lactoglobulin B. The milk of individual animals contained either both or only one of these components, and the formation of these proteins by the mammary gland was genetically controlled. These observations led to the present work on the whey proteins of buffalo's milk, which is widely consumed in India and other eastern countries, to see whether such a possibility existed among this species of animal.

The typing procedure of Aschaffenberg and Drewry[3] was applied to individual samples of milk collected from 70 buffaloes and also to two samples of pooled milk. The animals belonged to mixed breeds with features of one or other of the common Indian buffaloes[4] relatively dominant. Paper electrophoresis experiments were carried out in barbitone buffer of pH 8·6 ($I = 0{\cdot}05$), applying a voltage of 240 V., by both hanging- and horizontal-strip techniques, using Whatman No. 4 filter paper. A sample of whey proteins of cow's milk containing almost equal proportions of β-lactoglobulins A and B was run as a standard for comparison with each experiment. It was found (Fig. 1) that buffalo's milk contained only one type of β-lactoglobulin which migrates to the position corresponding to the β-lactoglobulin B of cow's milk. In view of the fact that the same results were obtained from as many as 70 individual samples, it appears that the genetic control as reflected in the case of cow's milk, does not exist in the buffalo.

The electropherograms also reveal a distinctly different movement of the α-lactalbumin band of the buffalo's milk from that of cow's milk, the former moving ahead of the latter. This offers a possibility of developing a method, which is being explored, of detecting adulteration of one by the other.

The buffalo β-lactoglobulin crystallized in diamond shaped form and the lactalbumin in needles when the

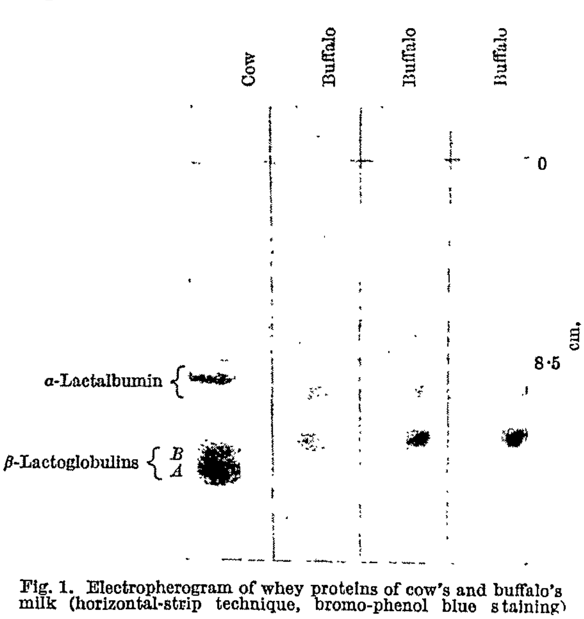

Fig. 1. Electropherogram of whey proteins of cow's and buffalo's milk (horizontal-strip technique, bromo-phenol blue staining)

method of Aschaffenberg and Drewry[3] with minor modifications was employed. Preliminary electrophoretic experiments in a Tiselius apparatus, of crystalline buffalo β-lactoglobulin, indicated its isoelectric point to be approximately 5·25 and the electrophoretic patterns obtained near the isoelectric point with acetate buffers of 0·1 ionic strength were found to be asymmetrical or partially resolved on the descending side and hypersharp on the ascending side. Sedimentation experiments with a Spinco model *E* analytical ultracentrifuge with protein concentrations up to 6·8 per cent and at 20° C. and low temperatures (about 8° C.) gave symmetrical patterns. These results show that there is close similarity between physico-chemical properties of buffalo β-lactoglobulin and β-lactoglobulin *B* of cow's milk[5,6].

Further work is in progress and details will be published elsewhere.

We are indebted to Messrs. Alpine Dairy and Farm, Agarpara, W. Bengal, and particularly to Mr. B. K. Choudhury for supplying the milk and helping in identification of individual animals.

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Intracellular Distribution of Ubiquinone in Rat Liver under Certain Stress Conditions

OBSERVATIONS on the effects of pantothenic acid deficiency on mitochondrial composition in relation to oxidative metabolism in rat liver were reported earlier[1]. In the present communication, observations on intracellular distribution of ubiquinone in the pantothenic acid-deficient rat liver are presented. The effects of vanadium administration and of vitamin A-deficiency have also been studied, since vanadium is known to cause decreased hepatic coenzyme *A* (ref. 2) and vitamin A-deficiency results in increased ubiquinone-level[3].

Dietary deficiencies of pantothenic acid and vitamin A were produced by feeding weanling rats (40–50 gm.), Wistar strain, a 10 per cent purified casein ration deficient in the respective vitamin and replete in all other respects, for a period of 8 weeks. To one group of rats, fed the casein ration with all the vitamins, ammonium vanadate was administered intraperitoneally (0·6 mgm./rat/week), throughout the experimental period. The animals were killed, livers quickly removed and chilled in cracked ice. Livers were homogenized in isotonic (0·25 *M*) sucrose, nuclear and mitochondrial fractions separated in a *PR*-2 International refrigerated centrifuge[4] and microsomes in a Spinco preparative centrifuge[5]. Determinations of coenzyme *A*, ubiquinone and mitochondrial succinoxidase activity were as described before[1]. The supernatant fraction was freeze-dried before saponification in the assay procedure for ubiquinone.

The results (Table 1) show normal rat liver ubiquinone to have an average distribution of 29·1 per cent in the nuclei, 37·8 per cent in the mitochondria, 14·9 per cent in the microsomes and 5·5 per cent in the supernatant fraction. Of the total coenzyme *Q*, 84–93 per cent was recovered in the various cytoplasmic fractions.

Vanadium administration, as also pantothenic acid-deficiency, result in marked and very similar decreases in total coenzyme *A*, mitochondrial ubiquinone and succinoxidase activity. This close parallelism suggests that the reduced hepatic coenzyme *A*-level is the primary cause of the decrease in ubiquinone; the involvement of coenzyme *A* in the biosynthesis of the isoprenoid side-chain of the ubiquinone molecule is known (cf. ref. 1). Vitamin A-deficiency results in elevated levels of ubiquinone in all the cell fractions, being particularly marked in the microsomal and supernatant fractions and is unattended by any change in succinoxidase activity.

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Table 1. INTRACELLULAR DISTRIBUTION OF UBIQUINONE IN RAT LIVER

Group	Ubiquinone					Coenzyme *A* units	Succinoxidase activity (μl.oxygen/hr.)
	Whole liver (μgm.)	Nuclei (μgm.)	Mitochondria (μgm.)	Microsomes (μgm.)	Supernatant (μgm.)		
10 per cent casein	127 ± 7·6	37 ± 4·1	48 ± 2·7	19 ± 3·1	7 ± 1·6	164 ± 11	1,304 ± 32
10 per cent casein + vanadium	97 ± 9·9	33 ± 4·3	33 ± 1·9	13 ± 1·8	6 ± 0·9	89 ± 17	996 ± 51
10 per cent casein − pantothenic acid	103 ± 3·1	33 ± 5·6	36 ± 4·1	17 ± 2·4	6 ± 1·1	96 ± 6	1,031 ± 69
10 per cent casein − vitamin A	187 ± 11·6	46 ± 3·9	61 ± 6·2	34 ± 2·4	17 ± 4·1	—	1,361 ± 98

Results are averages of four independent determinations ± *S.E.*, and are expressed per gm. fresh liver weight.

Extraction of Nucleic Acid-free Lipopolysaccharides from Gram-negative Bacteria

THE extraction of Gram-negative bacteria by the hot phenol method of Westphal[1] yields a lipopolysaccharide along with considerable amounts of nucleic acid. The nucleic acid can be separated from the high molecular weight lipopolysaccharide by high-speed centrifugation, but to reduce the nucleic acid to a level of less than 2 per cent may require four or five sedimentations.

It is known that when the trichloracetic acid method of Boivin is used to extract the somatic antigen, a considerable amount of the antigen may remain in the residue[2]. Working with a *Pseudomonas* species, we have found that the residue left after the dried cells have been treated with trichloracetic acid, can be extracted with phenol to yield a lipopolysaccharide virtually free from nucleic acid.

10 gm. of the acetone-dried cells were mixed for 3 hr. in 100 ml. of 0·25 N trichloracetic acid at a temperature of 2–4° and then centrifuged. The deposit was washed with 100 ml. of distilled water and again centrifuged. This deposit was then extracted with 45 per cent phenol at a temperature of 65–67° and, after cooling, the aqueous phase was separated, dialysed, centrifuged at 4,000 r.p.m. for 10 min. to remove debris, and the supernatant was then freeze dried. The yield of extracted material from different batches was between 300 and 400 mgm. This compares favourably with the yield of lipopolysaccharide obtained after removal of the nucleic acid by high-speed centrifugation.

The lipopolysaccharide obtained by this method contained no detectable amounts of nucleic acid and was highly pyrogenic in rabbits in doses of 0·01 μgm./kgm. body-weight. Preliminary analysis showed the presence of galactose and glucose and 45–50 per cent of a lipid soluble in chloroform.

Similar results have been obtained with *E. coli* 0111 (supplied by Dr. O. Lüderitz) and with *P. vulgaris*. In both cases the modified extraction procedure yielded highly pyrogenic, nucleic acid-free lipopolysaccharides in amounts equivalent to 2–3 per cent of the dry weight of the bacteria.

The results with these three organisms suggest that pre-treatment with trichloracetic acid before phenol extraction may be a general method of obtaining nucleic acid-free lipopolysaccharides without the lengthy procedure of high-speed centrifugation. This suggestion is supported by unpublished observations made by D. A. L. Davies (personal communication) working with different bacteria.

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Enzymatic Reduction of Diphosphopyridine Nucleotide with Urea

In investigating the properties of an enzymatic cycle between ammonia and nitrate[1], we have now observed the enzymatic reduction of diphosphopyridine nucleotide with urea.

The enzyme solution was prepared daily from acetone precipitates of fresh fowl liver according to the procedure already described[1]. To measure the activity, 1 ml. of 0·025 per cent solution of diphosphopyridine nucleotide was mixed with 1 ml. of enzyme solution, 1 ml. of 0·001 M urea solution and 0·5 ml. of 0·2 M *tris* buffer of *p*H 7·5. Then, the increase (+) in absorption ($-\log T$) at 340 mμ was determined by a spectrophotometer at 30° C. after 30 min. As shown in Fig 1, $-\log T$ increased in the presence of urea, and a slight increase was observed in the control which contains no urea. This value must therefore be subtracted from those in tests. In the reaction mixture without enzyme, there was no change in $-\log T$. The increase of $-\log T$ was rapidly reduced when alcohol dehydrogenase and acetaldehyde were added.

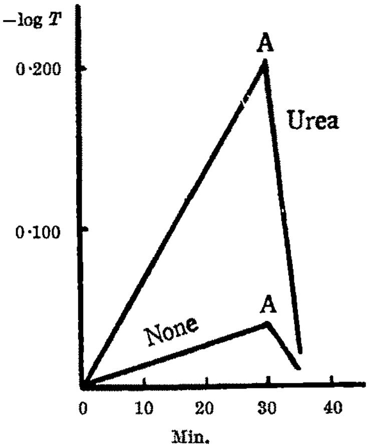

Fig. 1. Reduction of diphosphopyridine nucleotide with urea. At point *A*, alcohol dehydrogenase and acetaldehyde were added

The reduction does not proceed in a mixture containing phosphate buffer. The enzyme exhibits its highest activity at 35° C. and *p*H 7·5 ; it is activated by heating at 35–40° C. for 5 min. but destroyed at 60° C. Diphosphopyridine nucleotide is reduced with biuret or monomethylurea too, although at a slower rate than with urea.

However, it is thought that the enzymatic reduction of diphosphopyridine nucleotide may be attributed to ammonia produced from urea by the action of urease, since the nucleotide is reduced by the same enzyme solution in the presence of ammonium salts. Therefore, the formation of reduced diphosphopyridine was examined comparatively with urea and ammonium sulphate, although distinct formation of ammonia from urea by the enzyme solution employed was not established.

Table 1 shows that triphosphopyridine nucleotide can also be reduced by urea, whereas only the diphosphopyridine nucleotide is reduced with ammonia.

Table 1. REDUCTION OF DI- AND TRI-PHOSPHOPYRIDINE NUCLEOTIDE

Nucleotide	DPN			TPN		
Substrate	Urea	Ammonia	None	Urea	Ammonia	None
$-\log T$	+0·113	+0·140	+0·025	+0·068	+0·009	+0·009

Reduction of diphosphopyridine nucleotide with ammonia accompanies the formation of its oxidation products, hydroxylamine, nitrite and nitrate. On the other hand, as illustrated from Table 2, these substances could not be demonstrated in the case of urea.

Table 2. FORMATION OF HYDROXYLAMINE, NITRITE AND NITRATE IN THE REACTION MIXTURE

Product		Hydroxylamine	Nitrite	Nitrate
Substrate	Urea	0	0	0
	Ammonia	4×10^{-6} M	4×10^{-6} M	1×10^{-5} M

Further work showed that there are some other differences between the reduction of diphosphopyridine nucleotide with urea and that with ammonia, especially in connexion with the re-oxidation of the reduced nucleotide formed by nitrate reductase in the reaction mixture, substrate inhibition, optimum temperature and purification of enzyme.

On the basis of this work, the reduction of diphosphopyridine nucleotide with urea may be an enzymatic electron transfer from urea to the nucleotide, and not from ammonia in association with urease and ammonium dehydrogenase. In other words, the reaction appears to be an enzymatic dehydrogenation of urea which serves as an electron donor. This reaction has not previously been observed so far as we know. However, it should not be called urea dehydrogenase, until the dehydrogenation products of urea are established and the reaction mechanism is explained. Work is in progress to do this.

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A D-Lactic Dehydrogenase from *Leuconostoc mesenteroides*

The properties of the L-lactic dehydrogenases (L-LDH) from muscle[1-3] and yeast[4-6] have been studied extensively. Recently, the presence of lactic dehydrogenases was reported in animal tissues[7], yeast[8-10] and bacteria[11-13], specific for the D-isomer of lactic acid. The present communication describes the isolation, partial purification and some properties of a D-lactic dehydrogenase (D-LDH) from *Leuconostoc mesenteroides*.

L. mesenteroides strain No. 8042 was grown without shaking on a proteose peptone medium, containing 1 per cent glucose. The cells were collected after 24 hr. growth and disintegrated sonically. The sonicate was centrifuged at 105,000*g* for 2 hr. The supernatant extract contains D- and L-LDH activity in a ratio of 20 : 1. This ratio could be increased to more than 200 : 1 by fractionation with ammonium sulphate, using the fraction precipitating between 0·15 and 0·40 saturation. At the same time the specific activity towards D-lactate was increased threefold. In the experiments described here, this partially purified preparation was used.

Dehydrogenase activity was assayed by the reduction of 2,6-dichlorophenol-indophenol (DCPIP) (1×10^{-4} *M*), measured by the decrease of optical density at 620 mμ. The assay was carried out in phosphate buffer (0·25 *M*) at *p*H 6·5, which was found to be the optimum under ærobic conditions. The enzyme preparation contained approx. 5 mgm. protein/ml. The K_m with regard to D-lactate was found to be $1{\cdot}5 \times 10^{-3}$ *M*. In the following experiments a 2×10^{-2} *M* substrate concentration was used.

In contrast to yeast LDH[1,2], neither ferricyanide nor cytochrome c nor molecular oxygen could replace DCPIP as hydrogen acceptor. Added di- or triphosphopyridine nucleotide (DPN, TPN) at a concentration of 2×10^{-3} *M* had no effect on the rate of reduction of DCPIP. Moreover, DPN did not serve as a hydrogen acceptor for D-lactate, nor could DPNH pass on its hydrogen atoms to pyruvate. Flavin adenine dinucleotide or flavin mononucleotide had no effect on the system.

Table 1. Substrates and Inhibitors of D-LDH from *L. mesenteroides*

Substrates	Relative velocity	Inhibitors	I_{50} (*M*)
D-Lactate	1·00	pyruvate	0·05
L-Lactate	0·005	fluoropyruvate	0·001‡
DL-Fluorolactate*	0·15	oxalate	0·0005
DL-Chlorolactate	0·03	copper†	0·0001
DL-α-Hydroxy-butyrate	0·01	mercury†	0·00005
DL-glycerate	0·01		

* In presence of 0·01 *M* hydrogen cyanide; without hydrogen cyanide the enzyme was rapidly inactivated.
† After 15 min. pre-incubation with the enzyme.
‡ Calculated from activity of DL-fluorolactate without hydrogen cyanide.

Table 1 shows the substrate specificity and the effect of inhibitors. It can be seen that the substitution at the β-carbon sharply decreases the activity, in contrast to the yeast enzyme[6] and more resembling the muscle enzyme[1]. Other α- and β-hydroxy acids were not attacked, nor were glycolic acid and the ethyl ester and amide of *d,l*-lactic acid.

The enzyme differs from the flavoprotein yeast LDH in the lack of inhibition by atebrin[4] and *d,l*-mandelic acid[6]. In contrast to muscle L.LDH, *p*-chloromercuribenzoate (2×10^{-4} *M*) and iodoacetate (1×10^{-2} *M*) were without effect. Strong inhibition was caused by oxalate. Fluorolactate inhibited strongly in the absence of hydrocyanic acid, while it acted as a substrate in its presence. Moreover, fluoropyruvate did not inhibit the enzyme. This finding indicates that the product formed as a result of the dehydrogenation of fluorolactate is not identical with the synthetic product. Differences in inhibitory activity between various forms of fluoropyruvate have been described already by Busch and Nair[14]. Zinc, molybdenum, nickel, manganese, cobalt and iron (all at 1×10^{-3} *M*) did not affect enzyme activity, but copper and mercury were strong inhibitors, their effect being reversed not only by cysteine, but also by histidine, both of them at a tenfold excess over the metal ion concentration.

The *Leuconostoc mesenteroides* D-LDH resembles some of the bacterial D-LDH's described[9-11]. However, it is most closely related to that of Molinari and Lara[12] in *Propionibacterium pentosaceum* but differs from the latter in not being inhibited by atebrin and SH-agents. In the enzyme described in this communication the nature of the active site is not clear.

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Stimulation of Active Transport of Sodium from Sodium-rich Frog Muscle by Insulin and Lactate

ACTIVE excretion of sodium from muscle was first shown for rat muscle *in vivo* by Conway and Hingerty[1], and for isolated frog muscle by Desmedt[2] and by Conway *et al.*[3]. The latter workers found that when the sodium-level of muscle fibres was increased to about 60 mM/l. fibre water, by overnight immersion in cold potassium-free Ringer–Conway fluid containing 120 mM/l. sodium, it was necessary to reduce the external sodium in the presence of 10 mM/l. potassium chloride before active extrusion of sodium would occur.

The concept of a critical energy barrier to extrusion of sodium was introduced by Conway[4,5] to explain the necessity of reducing the external sodium, or of increasing the external potassium concentration above its normal level in plasma for excretion of sodium to occur. The energy barrier takes account of the electrical and osmotic gradients against which the sodium is extruded and is expressed by the relation:

$$dG/dn = RT.\ln [\mathrm{Na}]_o/[\mathrm{Na}]_i + E_m F \qquad (1)$$

where dG/dn is the free energy change per equivalent of sodium extruded, E_m the mean membrane potential of the muscle fibres (outside minus inside) in the recovery fluid, measured by the micro-electrode technique[6], and $[\mathrm{Na}]_o$ and $[\mathrm{Na}]_i$ the sodium concentrations in the recovery fluid and fibres respectively. In previous experiments[5], it was found that under the conditions used, sodium could not be extruded against a barrier greater than 2,000 cal., but it could be extruded if the barrier was reduced by lowering Na_o or by increasing K_o, and thereby reducing E_m.

In the presence of 30 units of insulin and 1 mM lactate per litre recovery fluid, I found that with muscles loaded with sodium as described above, the extrusion of sodium would occur, and at the normal plasma potassium-level of 2·5 mM/l. against an external sodium concentration of 120 mM/l. (see Table 1). At the same time about 30 mM potassium was gained by the muscles. It was found that the energy barrier against which sodium was extruded was 2,500 cal. at time of re-immersion in recovery fluid, rising to about 3,000 cal., after 3 hr., as extrusion reached completion. The mean membrane potential was 96 ± 0·9 mV. on re-immersion falling to 90 ± 1·9 mV. towards the end. The energy barrier here was closer to that computed for the intact animal[5], namely, 4,000 cal.

Examining the chloride movement under the conditions of previous experiments[5], I found that during immersion in potassium-free Ringer fluid the muscles gained an average of 20 mM chloride per litre fibre water, and that this chloride remained in the fibres even when the sodium had been extruded in exchange for potassium. However, addition of insulin and lactate to the recovery fluid produced a marked decrease in the fibre chloride.

The effect of some inhibitors on the extrusion of sodium under these conditions is summarized in Table 1.

It would appear from these experiments that the fall of the critical energy-level of extrusion of sodium from isolated muscle is related to a fall in the maximum electro-chemical potential at which the sodium pump can excrete sodium ions. In the light of these observations, and those of Manery *et al.*[7,8] it seems possible that insulin and lactate may play a part in the extrusion of sodium from frog muscle *in vivo*. They may also be the active constituents of plasma, which prevent the loss of potassium from isolated frog muscle[9], and maintain the membrane potential of the muscle at a higher value than found using Ringer fluid as immersion fluid[10]. In this connexion it may be of interest to note that Zierler[11] has reported that insulin increases the membrane potential of isolated muscle of rat.

Table 1. FROG SARTORII SOAKED OVERNIGHT AT 0° C. IN POTASSIUM-FREE RINGER FLUID CONTAINING 120 mM SODIUM, THEN RE-IMMERSED FOR 3 HR. AT ROOM TEMPERATURE (18° C.) IN FLUID CONTAINING 120 mM SODIUM, 2·5 mM POTASSIUM, 1 mM LACTATE AND 30 UNITS OF INSULIN PER LITRE

(Mean value after first immersion—60·6 ± 4·2 mM/l. fibre water)

Inhibitor used	Sodium concentration in muscle (mM/l. fibre water) Control	Inhibited
Ouabain (10^{-6} M/l.)	25·4 ± 1·4	46·1 ± 1·8 (11)
Iodoacetate (2 mM/l.)	23·2 ± 2·5	65·8 ± 3·6 (14)

Companion muscles were used. No. of experiments shown in brackets.

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PHYSIOLOGY

Urocanicacidurea associated with Hepatic Coma

WHILE studying the urinary chromatographic pattern of a patient in hepatic coma the presence of an abnormal spot R_F 0·14 and 0·54 in propanol/ammonia, 7 : 3, and butanol/acetic acid/water, 4 : 1 : 5, respectively, was noted after spraying paper chromatograms with diazotized *p*-sulphanilic acid. This colour reaction suggested that it might be an imidazole derivative, yet chromatography excluded the possibility of it being histamine, histidine or 1-methylimidazole-4-acetic acid (Table 1). As the presence of this unknown compound could not be related to the diet, or therapy, of the patient and appeared to be associated with the disease, an attempt was made to isolate it.

A 24-hr. urine sample was concentrated and acetone-precipitated according to the method of McIsaac and Page[1]. The final concentrate was streaked on Whatman No. 1 paper and chromatographed in butanol/acetic acid/water, 4 : 1 : 5. The corresponding transverse strips of paper with R_F 0·5–0·6 were eluted with ethanol. The eluates were pooled and evaporated to dryness to yield 15 mgm. of crystalline material. Recrystallization from water yielded 10·6 mgm. of chromatographically pure white crystals of urocanic acid (imidazole acrylic acid), m.p. 229° C., mixed m.p. 228–29° C., $\lambda_{\text{max.}}$ 277 mμ (pH 7·4)[2] (found : C, 40·9 ; H, 5·9 ; N, 16·0 per cent ;

Table 1

	Colour of spots on paper with			R_F values in solvent	
	Nin-hydrin	Diazo-*p*-sulphanilic acid	Methyl red	*A*	*B*
Histamine	None	Red	—	0·46	0·20
Histidine	Blue	Red	—	0·15	0·10
Methylimidazole acetic acid	None	None	Pink	0·29	0·21
Imidazole acrylic acid (Urocanic acid)	None	Orange	Pink	0·14	0·54
Isolated compound	None	Orange	Pink	0·14	0·54

R_F values and colour reactions of compounds used. Solvent systems used were: *A*, propan-1-ol/ammonia, 7 : 3 and *B*, *n*-butanol/acetic acid/water, 4 : 1 : 5.

$C_6H_6O_2N_2 . 2H_2O$ requires C, 41·4 ; H, 5·8 ; N, 16·0 per cent).

Urocanic acid, an intermediary breakdown product in the metabolism of histidine to glutamic acid, is not normally excreted in the urine, and has only been observed after animals have been given large doses of histidine[3]. The present finding of an abnormally large amount of urocanic acid in the urine of a patient with hepatic necrosis would indicate that the pathological lesion was associated with a biochemical lesion of the enzyme system urocanase. The failure of the metabolic degradation of urocanic acid in the liver parenchyma might well have caused a deficiency of the ammonia-detoxifying glutamic acid which in part may have been responsible for the high blood ammonia-levels observed in this case.

A detailed case-history of this patient will be reported elsewhere.

We are indebted to Mrs. Pamela Taylor who did the isolation work, and we wish to thank Dr. R. W. Schayer, of the Merck Institute for Therapeutic Research, for supplying us with 1-methylimidazole-4-acetic acid.

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Effect of Chronic High and Low Salt Intakes on Adrenocortical Function

We have presented evidence which suggests that a high salt intake may be related to the development of human essential hypertension[1], and that a prolonged low salt intake is compatible with apparently normal adrenocortical function[2].

Reference[1a,2] was made to observations by others which had been interpreted to indicate that a low salt diet could be harmful by causing hypofunction of the adrenal cortex with the organism thereby rendered less responsive to stress. Such inferences were in sharp contrast to our own experience with more than 125 persons whose daily salt intake was known to have been restricted to levels ranging from 30 to 375 mgm. for periods up to 5 years[3].

In one of the reports[2] adrenocortical function appeared to be within normal limits in 3 individuals who had been maintained on daily intakes of 250–375 mgm. salt for periods of from 1 to almost 5 years. Nevertheless, since no evaluation had been made prior to salt restriction a subtle decline in adrenocortical responsiveness might have remained undetected.

Recently it became possible to re-evaluate adrenocortical activity (as this is indicated by the response to the 48-hr. adrenocorticotrophic hormone infusion test of Jenkins *et al.*[4]) in two of these same individuals when salt intake was resumed after 23 and 35 months of drastic salt restriction, respectively. The two ambulatory patients were White bachelors aged forty-nine and fifty-one years on admission. They had uncomplicated essential hypertension, and were capable of long walks on the Laboratory site daily. All menus were calculated by a dietitian, and the food was prepared and weighed in a diet kitchen. Both men were required to eat all and only the food provided. When salt was added to the regimen it was given as enteric-coated tablets under observation. Continuous 24-hr. urine collections for sodium content indicated compliance with the prescribed diet. Over a period of four months gradual increments of salt were made until during the 3 weeks preceding, and at the time of, the present work each patient was eating 40 gm. of sodium chloride daily. This is a very high salt intake by American standards[5], but these men had no symptoms from it. However, their blood pressures, which had dropped markedly on salt restriction, rose significantly on addition of salt.

Table 1 summarizes the present findings, and compares them with the results of this same test performed at various intervals during the period of salt restriction. It will be seen that the fall in total circulating eosinophiles following the infusion of adrenocorticotrophic hormone on each of days 3 and 4 was similar in these subjects whether they were off or on salt. By contrast, the urinary excretion of 17-ketosteroids and 17-hydroxycorticoids appeared to be somewhat decreased on the high salt intake. This was manifested both by smaller quantities of steroid excreted and by a response which was delayed until after the second infusion of adrenocorticotrophic hormone.

Table 1. 48-hr. Adrenocorticotrophic Hormone Test for Adrenocortical Reserve Function

			N.S. ♂ 51 years		R.B. ♂ 49 years			
Months off (−) or on (+) salt			− 12	+ 4	− 13	− 18	− 25	+ 4
Eosinophiles, mm.[3] (Normal 100–250)	Day 1	Control	181	141	197	413	500	347
	,, 2	,,	166	97	206	460	369	410
	,, 3	Test	19	0	14	25	25	13
	,, 4	,,	3	0	2	38	13	10
Urinary 17-ketosteroids, mgm./24 hr. (normal : ♀ 5–15, ♂ 10–20)	Day 1	Control	13·0	7·9	10·4	10·0	12·7	13·3
	,, 2	,,	14·5	8·9	13·4	8·2	13·6	8·1
	,, 3	Test	21·1	9·7	13·2	14·5	20·5	8·2
	,, 4	,,	23·8	10·1	27·3	28·9	27·6	11·3
Urinary 17-hydroxycorticoids, mgm./24 hr. (normal : ♀ and ♂ 4–11)	Day 1	Control	9·0	8·2	4·7	7·6	7·5	9·1
	,, 2	,,	7·1	3·4	4·0	7·2	8·1	6·3
	,, 3	Test	37·6	5·9	13·1	35·3	32·0	7·7
	,, 4	,,	49·9	27·8	13·9	48·9	50·8	17·1

25 units of adrenocorticotrophic hormone infused over an 8-hr. period on each of days 3 and 4.

It would be premature to interpret the present studies as indicating that a high salt diet can impair adrenocortical function. The prompt, marked fall in total circulating eosinophiles when these patients were on the high salt intake is highly suggestive of a normally functioning adrenal cortex. Furthermore, evaluation of adrenocortical steroid production by means of urinary steroid excretion must always be suspect. Nevertheless, the results clearly indicate that by this method of estimating adrenocortical responsiveness, a high salt diet is not beneficial and a low salt diet is not harmful.

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Hypothalamic Control of Lactogenic Function in the Cat

While it is generally accepted that the hypothalamus controls the systemic release of anterior pituitary prolactin, it still remains to be established where in the hypothalamus the regulatory centres are located and how they mediate their influence.

The present investigation involves the use of a comparatively simple and effective technique which has facilitated systematic study of this problem without the use of drugs, hormones, or pituitary transplantation. It is the result of an incidental observation made by one of us (A. B. R.) that transverse section of the anterior tuber cinereum in the cat resulted in mammary growth and lactation.

A total of ten animals was used, four of which served as controls. In the six experimental cats, the diencephalic floor was aseptically exposed through the transbuccal route under barbiturate anæsthesia and the tuber cinereum sectioned transversely just behind the optic chiasm, so as not to impair the hypophyseal blood supply by injuring the median eminence or portal vessels. In three control animals the similarly exposed tuber cinereum was perforated in the midline by a stab with a large bore (No. 18) hypodermic needle, while in the fourth cat the hypothalamic section was carried out posteriorly, behind the median eminence in the premamillary region. Each operation included the removal of a mammary gland for histological examination and comparison with subsequent mammary biopsy specimens.

Because milk secretion can be induced with prolactin only in cats with fully developed ductal and lobulo-alveolar systems, positive findings can only be obtained in post-partum cats or in cats primed with ovarian hormones. The investigation reported here was confined to post-partum cats the mammary glands of which were allowed to regress over a period of up to two months.

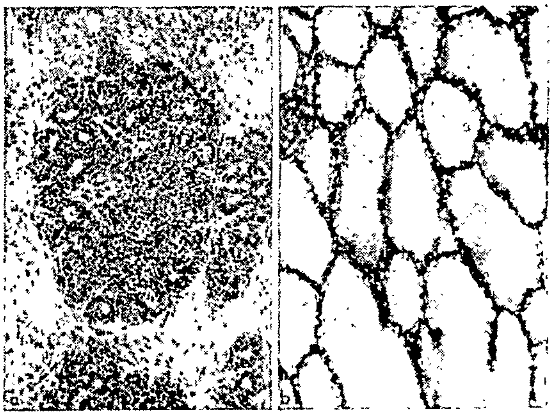

Fig. 1. Photomicrographs of mammary glands showing (*a*) preoperative resting state and (*b*) active secretory state of mammary gland in the same cat 14 days after anterior tuberal section

At the time of the operation, all the mammary glands were flat to inspection and no underlying mammary tissue could be palpated. Within three to twelve days after the tuber cinereum transection all six experimental cats, but none of the controls, showed the onset of mammary growth, which within another few days culminated in mammary hypertrophy and milk secretion indistinguishable from that seen during normal lactation (see Fig. 1).

The diabetes insipidus which often ensued at about the same time bore a variable relationship to these changes: mammary hypertrophy was observed both before and after the onset of the 'normal interphase' which accompanies the release of neurohypophyseal hormones from the degenerating posterior lobe. After a few weeks, a progressive decline in the mammary hypertrophy ensued in all but the one cat from which milk was actively withdrawn under administration of oxytocin. In this case, the involutional process was interrupted by a brief period of renewed growth.

The initiation of the observed mammary changes is known to require stimulation by prolactin. Since in these experiments the discharge of this hormone from the adenohypophysis resulted from the separation of the median eminence and hypophysis from their neural connexions with the anterior hypothalamus, the findings are explained tentatively as due to the removal of anterior hypothalamic inhibition, with or without additional facilitation through the intact posterior and lateral hypothalamic-hypophyseal pathways.

It is of interest to note that in the rabbit a centre inhibiting release of prolactin appears to be located in hypothalamic regions caudal rather than rostral to the hypophysis[1], and in an area of the hypothalamus which appears closely related to the centre controlling œstrus behaviour activated by œstrogen[2]. Since in the cat a corresponding centre controlling œstrus behaviour has been described in the anterior hypothalamus[2], which from this work appears to be the centre inhibiting release of prolactin, it is possible that these lactation-inhibiting regions of the hypothalamus may be topologically associated with the regional hypothalamic control centres sub-serving more complex and purposive patterns of behaviour such as mating and the care of the young. The latter is supported by observations that administration of prolactin to females of some species evokes maternal behaviour[3].

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Release of 5-Hydroxytryptamine from Rat Mast Cells *in vitro*

Histamine is released from mast cells following the injection into animals of histamine liberators. Coincident with the release of histamine other substances have been detected in the plasma capable of causing a slow contraction of isolated smooth muscle[1-4]. Paton[4] was able to demonstrate the presence of a 'slow-contracting' substance in addition to histamine in the plasma of cats and dogs following an intravenous injection of the potent histamine release agent, compound 48/80. Preliminary experiments have shown that a similar substance appears in plasma collected from rats 5 min. after an intravenous injection of compound 48/80 (1 mgm./kgm.). The origin of the slow-contracting substance in rat plasma was investigated by means of an *in vitro* system consisting of a solution of compound 48/80, rat plasma, mast cells from the peritoneal cavity of the rat, and an isolated rat uterus preparation.

Mast cells were obtained from the rat by a method previously described[5]. The rat uterus preparation was set up according to the method of Sollero *et al.*[6]. Compound 48/80 was dissolved in physiological saline solution to a strength of 10 μgm./ml. Blood was collected from the jugular vein of rats into a syringe containing heparin (5 units/ml. blood). The blood was transferred to a centrifuge tube and plasma free of platelets obtained by centrifugation. All glassware, including syringes and the organ bath, were siliconed with 'Dri-Film' (General Electric Co.) before use.

A mixture was prepared consisting of 0·1 ml. each of the solution of compound 48/80, fresh heparinized plasma and mast cell suspension (containing approximately 1 million mast cells per ml.). When added to the organ bath the mixture was found to cause a contraction of the rat uterus. A similar contraction was produced in the absence of plasma when 0·2 ml. of a mixture consisting of 0·1 ml. vol. of compound 48/80 solution and mast cell suspension was added. 0·1 ml. of the solution of compound 48/80 alone, or when mixed with an equal volume of plasma and added to the bath, failed to stimulate the rat uterus. Similarly intact mast cells alone or with an equal volume of plasma did not cause a contraction of the isolated uterus. Prior treatment of the isolated uterus for 5 min. with dibenamine (1 μgm./ml.), 2-brom-D-lysergic acid diethylamide, known as BOL 148 (1 μgm./ml.), 1-methyl-D-lysergic acid butanolamide, known as UML 491 (0·2 μgm./ml.), or excess 48/80 (1 mgm./ml.) was found to prevent a contraction of the uterus on the addition to the bath of the mixture containing compound 48/80 and mast cells. These substances are known to inhibit the action of 5-hydroxytryptamine but are not specific. No response of the treated uterus was obtained on the addition to the bath of plasma obtained from rats 5 min. after an intravenous injection of compound 48/80.

This work suggests that a substance capable of causing a contraction of the isolated rat uterus is released from mast cells disrupted by compound 48/80. Histamine is found in mast cells in a concentration of 40 μgm. per million cells, but it does not cause contraction of the rat uterus. The 5-hydroxytryptamine concentration in rat mast cells is approximately 1/20 the concentration of histamine[7]. 5-Hydroxytryptamine was added to the bath in amounts equivalent to the calculated 5-hydroxytryptamine content of 0·1 ml. of the mast cell suspensions, and the resulting uterine contraction was similar to that produced by the mixture containing 0·1 ml. of mast cells and 0·1 ml. of compound 48/80.

The smooth muscle stimulant was released also from mast cells disrupted by a number of procedures shown previously to cause the release of histamine from mast cells[5,8-10] including (*a*) mechanical disruption ; (*b*) distilled water ; (*c*) an extract obtained from rat splenic tissue ; (*d*) dextran, when added to serum obtained from rats following adrenalectomy ; (*e*) addition of human red cells to fresh rat antiserum at 37° C. and *p*H 7. In experiments (*d*) and (*e*) with rat serum it was necessary first to decrease 5-hydroxytryptamine content of the serum by an intraperitoneal injection of reserpine (5 mgm./kgm.) at least 8 hr. before collection of the blood.

It may be concluded that 5-hydroxytryptamine or a similar substance is released together with histamine when rat mast cells are disrupted *in vitro*. It seems likely that this substance is the 'slow-contracting' substance found in rat plasma following the intravenous injection of compound 48/80.

Compound 48/80 used in these experiments was kindly supplied by Burroughs Wellcome (Australia), Ltd., reserpine by Parke Davis and Company, and BOL 148 and UML 491 by Sandoz Australia Pty., Ltd.

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Interaction of Acetylcholine, Calcium and Depolarization in the Contraction of Smooth Muscle

Smooth muscle which has been depolarized by external application of potassium still contracts in response to acetylcholine[1]. This response requires calcium (ref. 2, and personal communication from D. H. Jenkinson). Calcium ions alone cause the depolarized smooth muscle to contract. If an isolated rat uterus is immersed at room temperature in calcium-free isotonic potassium sulphate–Ringer (22·0 gm. K_2SO_4, 0·36 gm. $KHCO_3$, 1·0 gm. glucose/litre) it contracts briefly and then relaxes completely. At this stage the application of very low concentrations of calcium (0·1 m*M*) causes the development of a reversible tension. The magnitude of the tension increases with concentration of calcium up to about 1·2 m*M*. Higher concentrations of calcium produce diminishing responses in tension. The maximum tensions developed by calcium are less than the maximum of which the preparation is capable, and they can be increased by acetylcholine. The effect of 10^{-5} acetylcholine added to 2·4 m*M* calcium is shown in Fig. 1.

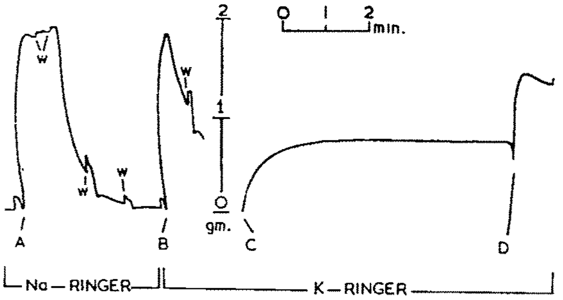

Fig. 1. Effect of acetylcholine superimposed on a maximal calcium contraction in depolarized rat uterus at 24° C. Record of isometric tension: *A*, 10^{-5} (gm./ml.) acetylcholine in sodium–Ringer solution ; *B*, depolarization by calcium-free potassium sulphate–Ringer solution ; contraction followed by complete relaxation ; *C*, 2·4 m*M* calcium chloride ; *D*, 2·4 m*M* calcium chloride + 10^{-5} acetylcholine. Between *B* and *C*, 105 min. *W*, washing

The findings that the effects of acetylcholine in depolarized smooth muscle require calcium ions, that calcium ions contract the depolarized muscle and that acetylcholine increases the uptake of calcium ions by smooth muscle[2] make it tempting to assume that the contractions produced by acetylcholine are due to an increase in uptake of calcium; but the above experiment showing that tensions due to acetylcholine can exceed those obtainable by calcium ions alone, makes it difficult to accept this simple explanation. Nevertheless, it is conceivable that acetylcholine may affect the movement of calcium at a functional site in a manner which cannot be reproduced by the external application of calcium ions.

It has been shown for heart[3] and striated muscle[4] that depolarization contractions produced by potassium ions require calcium. This also applies to smooth muscle. When rat uterus is immersed in calcium-free sodium–Ringer and depolarization contractions are elicited intermittently by immersion of the preparation in isotonic potassium sulphate–Ringer solution, the responses decline progressively and eventually disappear. At the same time the acetylcholine contractions in sodium–Ringer solution also diminish; but their decline is slower, so that a stage is reached in which depolarization contractions are abolished while considerable acetylcholine contractions are still obtainable (Fig. 2). The effects of lack of calcium are readily reversible on addition of calcium. This experiment provides new evidence that acetylcholine can cause contractions of smooth muscle which are not mediated by depolarization.

It has been previously shown that maximal doses of acetylcholine produce less tension in depolarized than in polarized smooth muscle[1]. This raises the question whether acetylcholine acts by the same mechanism in the two cases. In both instances the effects are antagonized by small concentrations of atropine, which seems to suggest that the receptors involved are similar. Part of the increased tension developed in polarized preparations is probably due to the occurrence of conducted impulses which are abolished after depolarization. Spike discharges in smooth muscle are increased in number by acetylcholine[5], suggesting that conducted impulses may be increased.

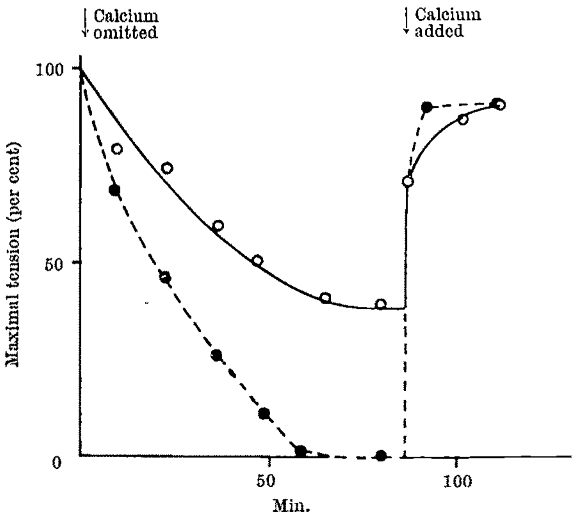

Fig. 2. Differential decline of isometric tensions produced by 10^{-4} acetylcholine (○) in sodium–Ringer solution and depolarization (immersion in isotonic potassium–Ringer solution, ●) after omission of calcium. Each point is the mean of two responses obtained from the two horns of a rat uterus, the lumens of which were opened by a longitudinal section giving sheets of approximately 1 mm. thickness. Temperature, 23° C.

It appears, however, that an additional factor is involved in the greater development of tension of polarized preparations, as may be inferred from the record shown in Fig. 3. A rat uterus horn was immersed first in calcium-containing Tyrode solution and then successively in the following calcium-free solutions : potassium–Ringer, sodium–Ringer and again potassium–Ringer. Taking into account the progressive decline due to lack of calcium, it is seen that

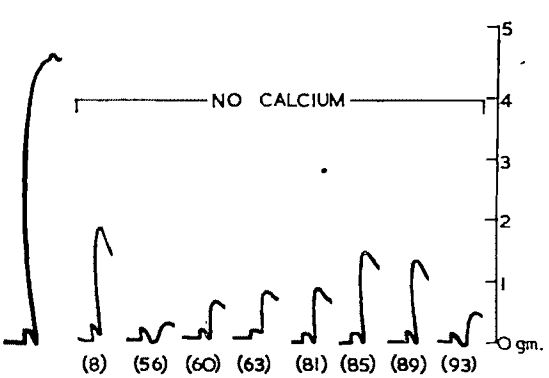

Fig. 3. Effects of acetylcholine (5 × 10^{-5}, gm./ml.) on rat uterus in calcium-free solutions during successive periods of immersion in sodium chloride–Ringer solution and potassium sulphate–Ringer solution at 23° C. Numbers within brackets denote minutes after removal of calcium chloride from the bath. Note : (1) overall decline of responses due to lack of calcium ; (2) increased responses after change from potassium–Ringer to sodium–Ringer solution ; (3) initial potentiation of response after change from sodium–Ringer to potassium–Ringer solution

not only are the acetylcholine responses increased when the preparation is changed over to sodium–Ringer, but there also is a further potentiation of the first few acetylcholine responses after re-introduction of potassium–Ringer solution. The persistence and slow decay of this effect suggests that immersion in sodium–Ringer solution has produced some persistent, slowly decaying alteration in the smooth muscle cell which improves the response to acetylcholine.

To summarize : (1) Calcium ions in low concentrations cause development of tension in the depolarized rat uterus muscle. The tensions produced are submaximal but can be further increased by the application of acetylcholine. (2) Contractions induced by depolarization and by acetylcholine are both dependent on calcium. However, depolarization responses decline more rapidly in the absence of calcium than acetylcholine responses, so that a stage can be reached when acetylcholine is effective but depolarization by isotonic potassium sulphate is ineffective. (3) Acetylcholine causes greater development of tension in polarized than in depolarized smooth muscle preparations. This difference is probably due partly to conducted impulses in the polarized muscle and partly to some other factor, so far unexplained.

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Influence of Changes of Blood Volume on the Concentration of Aldosterone in Peripheral Plasma of Intact Unanæsthetized Dogs

Investigations by F. C. Bartter *et al.* and by G. Farrell and his collaborators[1] on the regulation mechanism of aldosterone secretion have indicated a close relationship between blood volume and the production-rate of the hormone aldosterone. The effect of changes of fluid volume has been studied by these authors, measuring the aldosterone secreted in the urine or in the adrenal venous blood. None of these methods tells anything definite about variations of the concentration of hormone in the peripheral blood. As the action of the hormone in the organism is supposed to be closely dependent on the concentration in peripheral blood, it is of great interest to follow these concentrations directly, and information about the latency of variations may prove to be useful for the elucidation of the nature of regulation of the level of the hormone.

The double-isotope derivative method for determination of cortisol described by Bojesen[2] has been refined to the low level of aldosterone in peripheral plasma[3]. Using this method, we have examined the effect of bleeding, blood transfusion, infusion of a dog Ringer's solution, and infusion of dog Ringer's solution made up with 5 per cent dextran, on the concentration of aldosterone (extractable with chloroform) in the peripheral plasma of dogs. Docile dogs were selected for the experiments, which lasted about 1½ hr., during which the animals were lying on a table. Being accustomed to this, the animals were not agitated during the experiments. They had been fed with a diet low in salt (pork meat, potatoes, distilled water) for more than a week before the experiments were carried out. In all cases, a sample of blood, about 30 ml., was taken from the saphenous vein or the femoral artery just before the treatment to be studied was given. This was finished within 2–5 min. from zero time. Blood samples were then collected at intervals of about 20 min. during 1 hr. In the bleeding experiments, the blood was collected from the femoral artery into a sterilized transfusion bottle, using citrate as an anticoagulant. The blood was given to the same dog through the saphenous vein 3–4 days later, in the transfusion experiment. The dog Ringer's solution employed has been described previously[4] and is a solution imitating an ultrafiltrate of the plasma of normal dogs. The dextran used was 'Dextran 150' (Pharmacia, Copenhagen). As it was found in preliminary experiments that the dog Ringer–dextran solution, when infused, had the same depressing effect on the aldosterone-level as had the blood transfusion, every blood sample was simultaneously compensated for by injection of an amount of Ringer–dextran solution equal to the amount of plasma taken from the animal. Besides the experiments mentioned, in which the fluid volumes taken from or given to the dogs were 200–250 ml., control experiments were carried out, in which no change of the blood volume of the animal was induced, the samples being compensated for by dog Ringer–dextran solution as mentioned.

Table 1. Response of Plasma Aldosterone to Induced Changes in the Blood Volume by Different Means

The figures in parentheses indicate the interval of time between the induced change in blood volume and the collection of the blood sample. With the amount of plasma analysed (15 ml.), 5 ngm. per 100 ml. is the lower limit of sensitivity of the method

Type of experiment	Dog	Aldosterone in plasma ngm. per 100 ml. (time, min.)			
		1	2	3	4
Bleeding	*C*, ♀, 22 kgm.	33 (0)	162 (22)	156 (39)	236 (72)
	M, ♀, 18 kgm.	7 (0)	29 (13)	31 (30)	23 (62)
	J, ♀, 17 kgm.	31 (0)	41 (16)	51 (38)	59 (64)
Blood transfusion	*C*	40 (0)	26 (15)	14 (30)	10 (50)
	M	40 (0)	16 (22)	23 (42)	13 (67)
	A, ♀, 15 kgm.	51 (0)	14 (30)	10 (43)	8 (60)
Dog Ringer's solution	*M*	45 (0)	19 (20)	11 (50)	17 (65)
	M	46 (0)	44 (25)	16 (50)	25 (65)
	M	28 (0)	18 (22)	12 (45)	17 (65)
Dog Ringer–dextran	*M*	100 (0)	39 (22)	16 (37)	20 (58)
	J	55 (0)	14 (14)	12 (29)	5 (45)
Control	*J*	5 (0)	5 (21)	13 (56)	8 (82)
	J	17 (0)	20 (18)	17 (33)	19 (58)

The results (Table 1) are as might be expected from the previous work by others[1]. The very marked difference between the level of aldosterone in the first and the second blood samples except in the control experiments clearly shows that the blood volume is directly related to the level of aldosterone by means of a very rapid mechanism. As the rates of elimination and production will both influence the level in the peripheral plasma, the results do not lead to a final conclusion concerning the latencies of the regulation of the rate of production. In the case of bleeding, a comparison with the results obtained in similar experiments with the collection of adrenal venous blood suggest, however, that decreased elimination is not a main factor. In the case of transfusion and infusion experiments with dog Ringer–dextran, the steep slope of the plasma curve indicates that if the rate of elimination were a main factor, the increase of rate required is too great to be likely. Therefore our results suggest that the mechanism by which aldosterone secretion is regulated operates with a latency of a few minutes in relation to changes in the blood volume.

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Œstrogen Antagonisms: 19-Norprogesterone and 18,19-Dinortestosterone as Œstrone Antagonists

CLEAVAGE of the angular methyl group at carbon-10 of the testosterone molecule results in a compound that is much more potent than the parent as an œstrogen antagonist (unpublished work) and myotropic agent[1], but not as an androgen[1]. Since 19-norprogesterone has been known as a potent progestational agent for some years[2] and 18,19-dinortestosterone has recently been synthesized by Dr. W. F. Johns, of the Division of Chemical Research, G. D. Searle and Co., these two materials have been examined as antagonists of œstrone-induced vaginal smear changes in spayed mice.

The assay will be described in detail elsewhere (unpublished work). Spayed, adult, female mice were selected from a colony and injected with a total dose of 2 μgm. of œstrone, given in four equal, daily, subcutaneous injections over a 4-day period. Vaginal smears were taken on the afternoon of day 5 and morning of day 6, and were scored as positive if no leucocytes were present[3]. The standard dose of œstrone produced 80–100 per cent positive responses in various tests with 10–15 mice in a group. Comparisons between and among compounds were made at the dose-level that was estimated to produce a 50 per cent depression in this response.

Table 1. EFFECTS OF PROGESTERONE, 19-NORPROGESTERONE AND 18,19-DINORTESTOSTERONE AS ANTAGONISTS OF THE VAGINAL SMEAR EFFECTS OF 2 μGM. OF ŒSTRONE

Dose: œstrone 2 μgm. + other steroid	Progesterone: No.	Progesterone: No. positive	Progesterone: Per cent positive	19-Norprogesterone: No.	19-Norprogesterone: No. positive	19-Norprogesterone: Per cent positive	18,19-Dinortestosterone: No.	18,19-Dinortestosterone: No. positive	18,19-Dinortestosterone: Per cent positive
0	33	30	91	29	28	97	14	14	100
1				13	12	92			
3				13	11	85			
10	28	21	75	27	13	48			
30	35	30	86	26	8	31	14	12	86
100	36	19	53	14	1	7	14	14	100
300	25	12	47	14	0	0	15	15	100
1,000	17	0	0				14	14	100

Approximate ED_{50}: progesterone, 180 μgm.; 19-norprogesterone, 14 μgm.; 18,19-dinortestosterone, >1,000 μgm.

19-Norprogesterone is some thirteen times more potent than progesterone as an antagonist of the vaginal smear effects of œstrone (Table 1). 18,19-Dinortestosterone had no effect on the proportion of positive responses at doses up to 1,000 μgm. (This dose of 18,19-dinortestosterone produced slight but significant inhibition of the uterine growth stimulated in immature mice by 0·3 μgm. of œstrone.)

The high potency of 19-norprogesterone correlated well with previous results in which the 19-nor-configuration was more potent than the natural form (unpublished work). The absence of activity of 18,19-dinortestosterone is of particular interest; cleavage of the angular methyl group at carbon 10 increases the potency of testosterone (80–170 per cent, unpublished work) as an œstrone antagonist, whereas with the further cleavage of the methyl group at carbon 13 activity is largely lost.

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RADIOBIOLOGY

Lead-210 in Some Human and Animal Tissues

IN two recent publications[1,2] measurements have been reported of the levels of lead-210 (radium-*D*) in the bones of non-occupationally exposed American subjects. In the present communication similar findings are reported for some human and animal bone and liver specimens from Great Britain.

Six samples of bone, obtained from subjects who had died of malignant disease, were analysed for both total lead and lead-210. The specimens were dry ashed at 470–500° C., using lead-free concentrated nitric acid as an ashing aid. After solution of the ash in lead-free hydrochloric acid, lead was extracted with diethyl-ammonium diethyl-dithiocarbamate at *p*H 0·1 and then estimated colorimetrically using dithizone, lead-210 being estimated afterwards by low-background beta counting of the daughter bismuth-210, which was separated from lead with cupferron (to be published). The results are given in Table 1. It will be seen that the mean stable lead concentration was 6·3 μgm./gm. bone, in good agreement with the value of 6·6 μgm./gm. found by

Table 1

Tissue	Stable lead (μgm./gm. wet bone)	Tead-210 (picocurie/gm. wet bone)
Human vertebra	6·8	0·026
Human vertebra	4·4	0·029
Human vertebra	8·9	0·025
Human vertebra	8·7	0·034
Human vertebra	4·0	0·022
Human tibia	4·5	0·021
Mean	6·3	0·026
Ox bone (shaft)	3·8	0·26
Ox bone (shaft)	2·4	0·24
Mean	3·1	0·25

Tipton[3]. The mean lead-210 concentration of 0·026 picocurie/gm. of bone is comparable with the values found by Hursh[1] at Rochester, and Holtzman[2] at Argonne, of 0·015 and 0·040 picocurie/gm. respectively.

At the same time, measurements have been carried out to determine the nature of the α-radioactivity previously reported[4] in the liver of seven non-occupationally exposed individuals at levels of the order of 0·34 picocurie/gm. ash, equivalent to about 0·003 picocurie/gm. tissue. Using an improved version of our sensitive α-spectrometer (ref. 5, and to be published), α-spectra have been obtained of the ashed liver of two other unexposed individuals (both malignant cases, as were also the earlier subjects). These show at least 80 per cent of the activity in a single spectral line at approximately 5·3 MeV., corresponding to polonium-210. One of these spectra is shown in Fig. 1. Since it is to be expected that most of the polonium present in the original sample would be volatilized during ashing, the subsequent observation of polonium-210 in the ash is presumably indicative of the presence of its parent lead-210. Although these identifications have so far been made for two individuals only, it seems reasonable to suppose that some 80 per cent or more of the total α-activity previously reported for the larger group of liver samples (that is, approximately 0·0025 picocurie/gm. of wet tissue) was also due to the presence of lead-210.

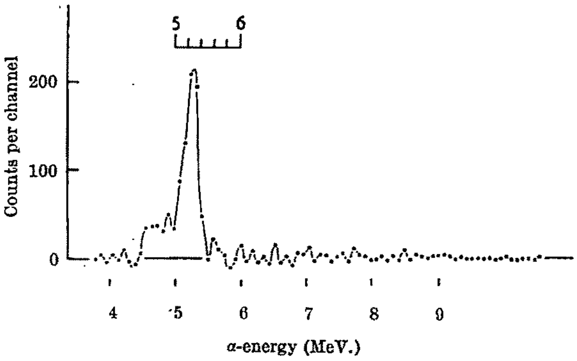

Fig. 1. α-Particle spectrum of a sample of human liver ash

We have also measured the total lead and lead-210 contents of two samples of ox bone. The results, included in Table 1, indicate that the lead-210 contents are some ten times higher than for humans, although the total lead contents of ox bone are lower by a factor of two. By means of α-spectroscopy, supported by observations of the rate of growth of total α-activity after ashing, the lead-210 content of a sample of ox liver was found to be approximately 0·05 picocurie/gm. of tissue, some twenty times greater than the value reported above for human beings. It appears that the relatively high levels of lead-210 in the ox may be attributable to the previously reported occurrence of this nuclide on foliage[6].

We wish to acknowledge the support and encouragement of Prof. W. V. Mayneord in this work, and our thanks are also due to Dr. W. Anderson and Mr. J. O. Crookall for helpful discussion. We are indebted to Dr. J. P. M. Bensted of this Department, and to Dr. N. F. C. Gowing, director of the Department of Pathology, Royal Marsden Hospital, for providing post-mortem material. Financial support for one of us (Z. S. J.) was provided by the International Atomic Energy Agency.

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Incorporation of Phosphorus-32 into Adenosine Triphosphate of Adrenal Chromaffin Granules

THE discovery by Hillarp and his colleagues[1] of the presence of large amounts of adenosine triphosphate (ATP) in the adrenal medulla has led to speculations on its role in the storage and retention of catechol amines in this tissue. It has been shown that the amines are stored in cell organelles, the chromaffin granules, which are distinct from the mitochondria ; they can be separated from the latter by centrifugation over a density gradient[2]. This has made it possible to establish that ATP occurs in a high concentration in the sites of storage of the catechol amines, in stoichiometric amounts in relation to the amines present.

It is known from work on many different types of cells that the mitochondrial ATP is rapidly turned over ; but it is not known if the same holds for the ATP in the chromaffin granules. Either the main function of the ATP is to act here as the anion that holds the catechol bases in the storage position, or it serves as an energy donor in maintaining the high concentration of amine in this site against a diffusion gradient. Thus, some insight into the function of the ATP might be gained by a study of the incorporation of phosphorus-32 into the ATP of chromaffin granules, as compared to that in the mitochondria.

Animals received 0·5–1·0 mc.^{32}P/kgm. body-weight 0·5–2 hr. before death. The rats were killed by decapitation and the adrenals were rapidly removed, adherent fat was dissected away and the glands were dropped into 0·3 *M* sucrose at 0°. The dog was anæsthetized with pentobarbitone before death and the cats were killed by cervical fracture. The mitochondria and chromaffin granules were separated by density-gradient centrifugation and deproteinized with trichloroacetic acid, which was then removed with ether. ATP in the granules was measured by the firefly method[3] and its phosphorus-32 activity was obtained after chemical hydrolysis. Good agreement was found between chemical and firefly estimations, a result which suggests that the predominant nucleotide was ATP. ATP in the mitochondria either was determined chemically as inorganic phosphate liberated by 15 min. hydrolysis in *N* hydrochloric acid at 100°, or the nucleotides were separated on 'Dowex' columns[4], the phosphorus-32 was counted and the concentration determined by the firefly method.

Table 1. INCORPORATION OF PHOSPHORUS-32 INTO ADRENAL MITOCHONDRIA AND CHROMAFFIN GRANULES

Species	Time after injection (hr.)	Specific activity*	
		Mitochondria	Granules
Cat	0·5	285	< 5
	2·0	465	—
Rat	1·0	65	< 5
	1·0	78	< 5
	1·0	66	13·5

* Specific activity as counts/min./μgm. phosphorus.

It will be observed (Table 1) that in both the cat and the rat the specific activity of granular ATP was very much less than that of the mitochondrial ATP. With these two species the adrenal cortex was not separated from the medulla so that the mitochondrial fraction represented material from two different parts of the gland. Since these could have different turnovers of ATP, an experiment was performed with a dog, with which species separation of the two regions of the gland could be achieved before isolation of the mitochondria. Although Spiro and Ball[5] have reported that the respiratory enzymes in the cortex of bovine adrenals are several times more active than those in the medulla, at 1 hr. after administration of phosphorus-32 to the dog, the specific activity of the ATP (separated on 'Dowex') of the cortex was only 50 per cent greater than that of the medullary mitochondria. Therefore, it seemed unlikely that the large differences in specific activity of ATP between cat and rat mitochondria and granules was due to ATP of high specific activity from the cortical mitochondria exaggerating a very low activity from the medullary mitochondria.

The presence of ATP in different cell compartments with different turnovers has been discussed by Chance[6], with reference to mitochondria and cytoplasm, and a distinction between nuclear and cytoplasmic ATP metabolism has also been observed (Ord, M. G., and Stocken, L. A., unpublished work). The low uptake of phosphorus-32 in the granule fraction could be readily understood if we assume that the ATP is functioning in the granules as a relatively inert binding anion for the catechol amines, rather than as an energy donor concerned with maintenance of their concentration. It is hoped to obtain further evidence on this point by a study of the uptake of phosphorus-32 into ATP during the restoration of catechol amines after depletion of the granules.

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PATHOLOGY

Production of Pyrogenic Substances by Extracts of Delayed Hypersensitive Cells mixed with Antigen *in vitro*

MANY published experiments on the passive transfer by cells of hypersensitivity undoubtedly indicate that the mechanism of delayed (tuberculin) hypersensitivity is based on a reaction of hypersensitive cells with antigen. In previous work I have demonstrated that, on incubation of delayed hypersensitive cells with antigen *in vitro*, substances are formed imitating manifestations of delayed hypersensitivity, namely, fever and inflammation of the skin[1]. Simultaneously with the formation of pyrogenic substances, the cells are desensitized, that is, they lose the ability to transfer passive hypersensitivity[2].

The question whether a similar reaction (formation of pyrogenic substances) could be evoked when mixing and incubating cell-free extracts of hypersensitive cells with specific antigen has now been investigated.

Sensitization of the animals (guinea pigs, rabbits), preparation of cell suspensions from spleen and lymph nodes, antigen used (2 μgm. purified protein derivate or 0·5 L_f purified diphtheria toxoid), and the method of evaluation of the fever reactions have been described in detail elsewhere[1]. A positive reaction is manifested by a short monophasic fever beginning usually within 15 min. and culminating in 30 min. after injection of the substance tested to normal, non-hypersensitive animals.

Cell-free extracts were prepared either by repeated (8–10 times) freezing and thawing or by ultrasonic radiation; active substances are released even on 24-hr. storage of hypersensitive cells in Hanks's solution at + 4° C. In the preparation of the extracts the decrease of the cell count was followed, and also the loss of staining characters as viable cells, as well as the increase of protein substances in the supernatant fluid.

A typical experiment is reported in Table 1. Neither the cell-free extract of hypersensitive cells nor the antigen is found to be pyrogenic. The mixture of these substances is pyrogenic, irrespective of the incubation period; pyrogenicity is developed even when the antigen and the cell-free extract are mixed and injected immediately to the test animals. It is evident from Table 1 that not even a 10-fold quantity of antigen or 5-fold amount of cell-free extract is capable of evoking pyrogenic reaction by itself. The same results were obtained in a number of experiments with rabbits and when mixing extracts

Table 1. PYROGENIC ACTIVITY OF CELL EXTRACTS OF GUINEA PIGS SENSITIZED BY B.C.G. VACCINE MIXED WITH TUBERCULIN *in vitro*

Substance tested	*In vitro* incubation period	Temperature changes in recipients (deg. C.)
Cell extract, 1 ml.	—	0 0·2 0·2
,, ,, 5 ml.	—	−0·1 0·2 0·1
Purified protein derivate		
1γ	—	0 0 0·1
10γ	—	0 0·1 0·2
Cell extract, 1 ml. + 1γ purified protein derivate	20 hr.	0·6 0·8 1·1
,, ,, ,,	2 hr.	0·8 1 1·3
,, ,, ,,	30 min.	0·7 0·9 1·1 1·4
,, ,, ,,	none	0·8 0·9 1·2 1·3
Diphtheria toxoid, 0·5 L_f	—	0 0·1 0
Cell extract + diphtheria toxoid, 0·5 L_f		0 0 0 0·1
	various	0·1 −0·2 −0·2 0

of diphtheria toxoid hypersensitive cells with the specific antigen. Extracts of normal cells mixed with antigen or extracts of hypersensitive cells mixed with the non-specific antigen yield completely negative results.

The effective substance (factor giving rise to pyrogenicity on mixing with antigen) can be stored in the frozen state. Its physical and biochemical properties and further biological effects are under investigation.

A few observations can be found in the literature concerning the possibility of passive transfer of delayed hypersensitivity by cell-free extracts in laboratory animals[3] and especially in man (so-called 'transfer factor')[4]. The appearance of pyrogenicity (as a manifestation of delayed systemic hypersensitivity)[5] in the reaction of hypersensitive cell extracts with antigen *in vitro* is in agreement with the experiments reported.

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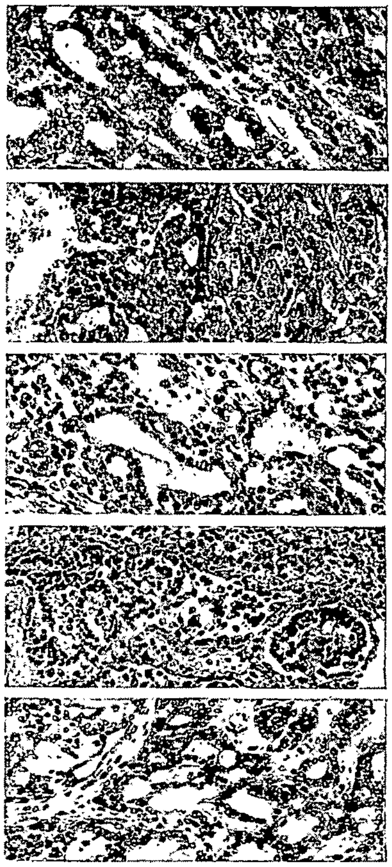
Induction of Mammary Carcinoma in Hypophysectomized Rats treated with 3-Methylcholanthrene, Œstradiol-17β, Progesterone and Growth Hormone

THE rapid induction of mammary carcinomas in rats treated by intra-gastric administration of 3-methylcholanthrene has been described by Huggins, Briziarelli and Sutton[1]. It was afterwards shown by Huggins, Grand and Brillantes[2] that the physiological state of the mammary tree was critical in the development of such tumours, and they were unable to induce mammary carcinomas in hypophysectomized rats. The present report describes the induction of mammary carcinomas in hypophysectomized rats which were treated intra-gastrically with 3-methylcholanthrene and in which partial replacement therapy was attempted with œstradiol-17β, progesterone, and growth hormone.



Figs. 1–5. Photomicrographs of tumours from rats 1–5 respectively. (Hæmotoxylin and eosin. × 135)

The pituitaries of female Sprague–Dawley rats were removed when they were 50 days old, when they weighed between 150 and 170 gm. Treatment was begun 13 days after hypophysectomy according to the following scheme.

(1) Œstradiol-17β, 1 μgm. and progesterone, 4 mgm. in 0·2 ml. sesame oil subcutaneously daily for 6 days every week.

(2) 3-Methylcholanthrene, 10 mgm. dissolved in 1 ml. sesame oil, intra-gastrically, once weekly.

Table 1

		Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
Age at hypophysectomy	(days)	50	50	50	50	50	50
Age at first injection	(days)	64	64	64	64	64	64
Weight at operation	(gm.)	150	172	150	166	158	166
Weight at death	(gm.)	354	353	320	296	363	251
Weights of organs at death:							
Heart	(mgm.)	935·5	1,070	867	1,274	1,350	1,615
Kidneys (right and left)	(mgm.)	2,105	2,515	2,140	1,855	2,872	2,008
Spleen	(mgm.)	427·5	625	378	565	640	824
Adrenals (right and left)	(mgm.)	28	23	28	22	34	28
Ovaries (right and left)	(mgm.)	16	43	42	110	165	82
Uterus	(mgm.)	462	509	534	620	632	654
Total dose of œstradiol	(μgm.)	121	142	160	164	189	214
Total dose of progesterone	(mgm.)	484	568	640	644	669	694
Total dose of methylcholanthrene	(mgm.)	210	240	270	280	320	360
Total dose of growth hormone	(mgm.)	161	224	282	298	398	498
Duration of treatment	(days)	141	165	186	191	220	249
Incubation period	(days)	102	147	122	170	184	220
Site of tumours		5 R., 3 L.	1 R.	2 L., 3 L.	2 L.	3 R., 4 L.	4 L.
Size of tumours at death	(mm.)	15 × 15 × 10 10 × 10 × 5	15 × 15 × 10	6 × 6 × 4 3 × 3 × 2	15 × 15 × 3	15 × 15 × 15 40 × 40 × 30	10 × 7 × 3
Nature of tumours		Adeno-carcinoma	Adeno-carcinoma	Adeno-carcinoma	Necrotic adeno-carcinoma	Adenocarcinoma Sarcoma	Sub-epidermal basal cell tumour of skin

(3) Bovine growth hormone, 0·5 mgm. in 1 ml. saline daily for 6 days every week, rising to 1 mgm. then 2, then 3 and finally 4 mgm./rat/day after intervals of 48, 31, 35 and 36 days respectively.

Mammary gland tumours developed in five rats out of nine. Histologically each tumour had the structure of an adenocarcinoma with some areas of comparative anaplasia. Nuclei were small, and regular in size and shape. One tumour, in rat No. 4, was almost entirely necrotic. In addition to its mammary gland tumour, rat No. 5 developed a spindle-celled sarcoma which grew rapidly; and rat No. 6 developed a sub-epidermal basal cell tumour of skin but no mammary tumour. Relevant data concerning these rats are given in Table 1 and illustrations of the mammary tumours in Figs. 1–5.

It is evident that partial replacement therapy with œstradiol, progesterone and growth hormone, as in the present experiments, permits hypophysectomized rats to develop mammary carcinomas following the administration of methylcholanthrene. This is in keeping with experiments of Nandi and Bern[3] who found that the development of hyperplastic mammary nodules to carcinomas in mice, required the presence of steroids together with either growth hormone or prolactin from the pituitary; and of Kim and Furth[4] who found that growth of mammary carcinomas in rats, stopped by hypophysectomy, could be restarted by the implantation of pituitary tumours known to secrete mammotrophin.

I am grateful to Dr. Charles Huggins and to Dr. Dwight Ingle for permission and encouragement in carrying out these experiments, and to the Endocrinology Study Section of the National Institutes of Health, Bethesda, Maryland, for the gift of growth hormone. The work was supported by a grant (*C*–2213(*C*6)) from the U.S. Public Health Service.

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HÆMATOLOGY

Identity of the α-Chains of the Minor Human Hæmoglobin Components A_2 and A_2' with the α-Chains of Human Hæmoglobin *A* and *F*

The occurrence of a minor hæmoglobin component (A_2' or B_2), which is related to the well-known hæmoglobin fraction A_2, has recently been described[1,2]. This variant, found in families of a Negro community of James Island, is characterized by a very low mobility at an alkaline *p*H. The electrophoretic pattern (at *p*H 8·8) of the hæmoglobin of a person heterozygous for this abnormality, shows a major hæmoglobin fraction (A_0), the fast moving fraction (A_1), the slow moving hæmoglobin A_2, and the hæmoglobin A_2' with a still lower mobility. The hæmoglobin A_2 and hæmoglobin-A_2' are present in equal amounts (0·8–1·2 per cent), the sum of both being equal to the percentage of hæmoglobin A_2 present in the blood of a normal individual. The hæmoglobin A_2' has also been demonstrated in cases with sickle cell trait[2], indicating that the two abnormalities ('double A_2 spot' and 'the presence of the abnormal hæmoglobin *S*') are controlled by a different pair of alleles. In some cases of thalassæmia trait both hæmoglobin A_2 fractions were found to be increased by a factor of two[1,2], the 'double A_2 spot' being contributed by one parent and the thalassæmia trait by the other parent.

The abnormal hæmoglobin A_2' is not rare in the Negro population of the State of Georgia. In the course of a search for hæmoglobin abnormalities of some 300 cases, using the starch gel electrophoretic technique[3,4], the double A_2 spot abnormality was found in members of at least eight families. In all cases the relative amounts of hæmoglobin A_2 as well as of hæmoglobin A_2' were about 1 per cent each (estimated with CMC chromatography[5]). In two cord blood hæmoglobin samples minor amounts (0·2–0·5 per cent) of these two fractions were demonstrable. The occurrence of hæmoglobin A_2' in cases with known hæmoglobin abnormalities (including thalassæmia) has not yet been recorded in our investigations.

Since a close relationship between the hæmoglobin A_2 and the hæmoglobin A_2' seems likely, a study of possible identical structural abnormalities was undertaken. In this communication we report part of the results of this work, namely, some hybridization experiments carried out in order to demonstrate the possible identity of the α-chains of the two minor hæmoglobin components with the α-chains of normal hæmoglobin *A* and hæmoglobin *F*. Large quantities of hæmoglobin A_2 and hæmoglobin A_2' were prepared by CMC-chromatography[5,6] using a 30 cm. × 3 cm. column and 0·01 *M* phosphate buffers of *p*H values ranging from 7·3 to 7·9 as eluting solvents. The dilute hæmoglobin solutions obtained after elution were concentrated on a small CMC column[6] and finally dialysed against distilled water. Small amounts of these components (10–20 mgm.) were mixed with equal amounts of Bart's hæmoglobin, prepared from the blood of cases described before[7]. The mixtures were dialysed at 4° C. against 0·1 *M* sodium acetate buffer (*p*H 5·0) for 36 hr. followed by dialysis for 36 hr. with a 0·01 *M* phosphate buffer of *p*H 6·8. Chromatographic separations of these mixtures before and after hybridization were carried out as described before[4–6]. The results obtained are presented in Fig. 1. In both control experiments (Bart's hæmoglobin + hæmoglobin A_2 and Bart's hæmoglobin + hæmoglobin A_2') the two major fractions are present while minute amounts of hæmoglobin *A* (and also of hæmoglobin A_2 in the mixture of Bart's hæmoglobin + hæmoglobin A_2') as impurities were demonstrable. After hybridization, however, considerable amounts of a hæmoglobin fraction were found, which showed the same mobility in CMC-chromatography as hæmoglobin *F*. The fœtal character of this component was proved by its high resistance towards alkali[6] and by its ultra-violet spectral absorption. It seems, therefore, that fœtal hæmoglobin is formed when hæmoglobin A_2 as well as hæmoglobin A_2' is hybridized with Bart's hæmoglobin. Since both hæmoglobin A_2 and hæmoglobin A_2' are composed of two different polypeptide chains (α- and δ-chains) with chromatographic properties (as tested on 'Amberlite *IRC*' 50 (ref. 4) similar to the α- and the β-chains of normal human hæmoglobin *A*,

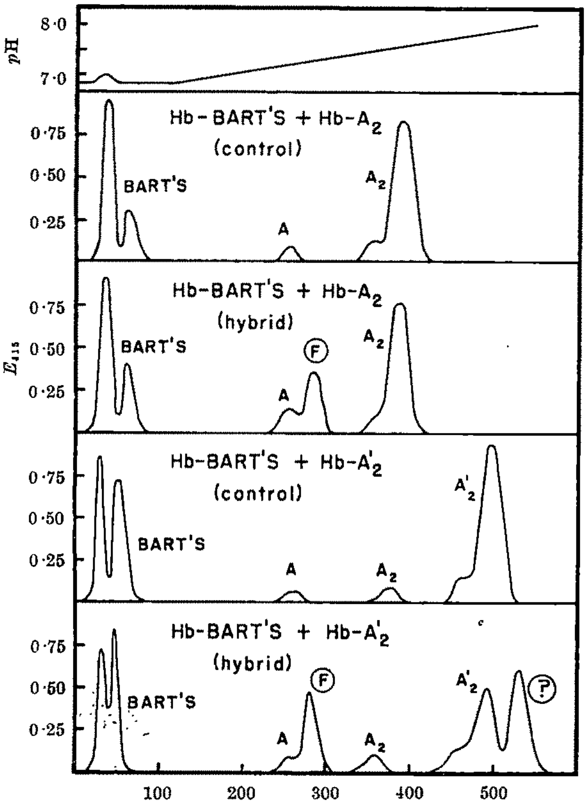

Fig. 1. Chromatographic separation on carboxymethyl-cellulose of mixtures of hæmoglobin A_2 (Hb-A_2) or hæmoglobin A_2' (Hb-A_2') and of Bart's hæmoglobin before and after hybrid formation

and since Bart's hæmoglobin consists solely of the γ-chains of hæmoglobin-*F* the following equations seem probable :

$$\alpha_2^A\delta_2 + \gamma_4^F = \alpha_2^A\delta_2 + \gamma_4^F + \alpha_2^A\gamma_2^F + \delta_4 \qquad (1)$$

$$\alpha_2^A\delta_2' + \gamma_4^F = \alpha_2^A\delta_2' + \gamma_4^F + \alpha_2^A\gamma_2^F + \delta_4' \qquad (2)$$

It thus seems probable that the α-chains of hæmoglobin A_2 and hæmoglobin A_2' are identical with the α-chain found in hæmoglobin *F* and hæmoglobin *A* [8] since the α-chains of hæmoglobin A_2 and hæmoglobin A_2' produced fœtal hæmoglobin after transfer of chain components with Bart's hæmoglobin by the hybridization technique. The components δ_4 and δ_4' formed during hybrid formation were not definitely recorded in our chromatographic experiments. It may be that both fractions possess chromatographic mobilities similar to that of hæmoglobin *H* ($\beta_4{}^A$), which is indistinguishable from Bart's hæmoglobin under our experimental conditions. It is also possible that the δ_4 component is eluted at the same *p*H as is hæmoglobin A_2 and the δ_4' component at an elution *p*H value slightly higher than found for hæmoglobin A_2' (Fig. 1). Further work, however, was not possible because of lack of material.

Our results support the hypothesis that possible structural differences between both minor hæmoglobin components and hæmoglobin *A* and between the two minor hæmoglobin fractions itself must lie in the δ-chains of the proteins.

Full extension of our results of differences between these two components and normal human hæmoglobin *A* will be given elsewhere. This work was supported in part by Public Health Service Research Grant No. *H*–5168 from the National Heart Institute, Public Health Service.

Note added in proof. Since this article was submitted, we have found a homozygous Hb-A_2' or (Hb-B_2) carrier with 2·1 per cent Hb-A_2' and no Hb-A_2.

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CYTOLOGY

Aggregation of Protein Particles in Tumour Cells

IN the cytoplasm of the cells of some tumours (Ehrlich ascites tumour, Yoshida ascites tumour, Walker carcinoma, hepatoma of the rat and human malignant lymphogranuloma) a form of dispersion of the ribosomes has been seen[1–3] at the electron microscope described[4–6] as 'scattered' or 'unorganized ergastoplasm'. A similar condition also occurs[3,7] during the previtellogenesis of the oocytes in some animal species (*Haliotis lamellosa*, *Paracentrotus lividus*, *Sphaerechinus granularis*, *Arbacia lixula*). While in tumour cells 'scattered ergastoplasm' persists, it is seen[8] that in the oocytes the ribosomes, after having increased and probably having undergone a transformation, aggregate during vitellogenesis to make yolk globules (Figs. 1 and 4).

It was supposed therefore that in tumour cells there might also occasionally be found formations deriving from the aggregation of protein particles. I have now shown this to be the case.

Fragments of Walker tumour of the Wistar albino rat were put for 5 hr. at room temperature (about 22° C.) in an aqueous solution of deoxyribonuclease ('Crystalline' of Light and Co., 10 mgm./ml., non-activated). At the same time controls were kept on fragments not treated with enzyme, and on fragments kept for the same time at room temperature in distilled water only. In each case the fragments were fixed in a 1 per cent solution of osmic acid (buffered by Palade's method). For observation under the optical microscope, the fragments were embedded in paraffin ; for observation in the electron microscope they were placed in a mixture of butyl and methyl methacrylate.

Preparations and observations were made at the Centre for Electron Microscopy at the University of Padua with a Siemens Elmiskop I*a* apparatus. Under the optical microscope cells notably swollen through absorption of water were observed in the preparations treated with enzyme ; in the cytoplasm of those cells of the outer layers of the sections some very evident globules of various sizes (Figs. 2, 3) of a similar character to those of the yolk of some oocytes (Fig. 1) appeared. These globules have not hitherto been found either in control preparations or, at least

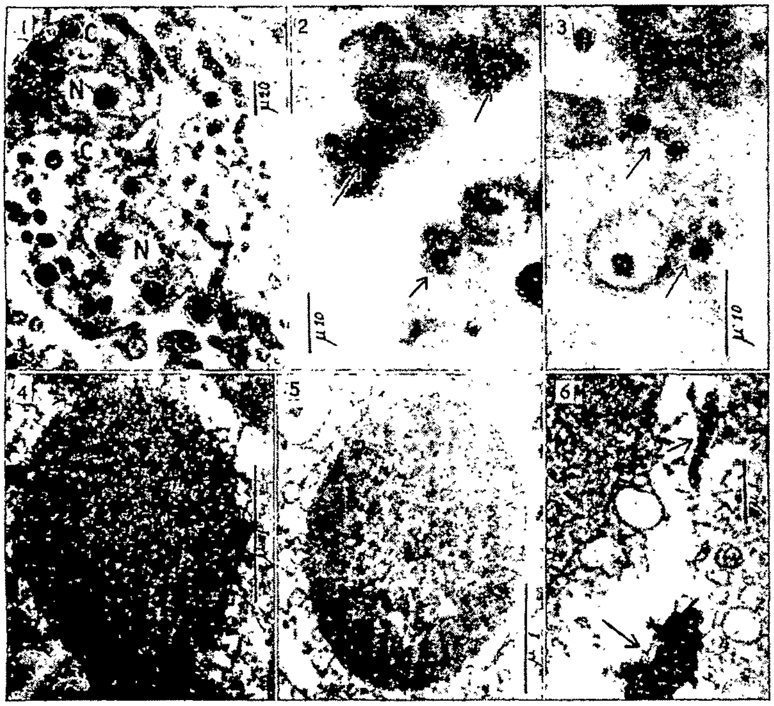

Fig. 1. Two oocytes of *Aplysia depilans*; *C*, cytoplasm with yolk globules; *N*, nuclei with nucleoli. Figs. 2 and 3. Walker tumour cells with globular formations (arrows) in a preparation treated with deoxyribonuclease. Fig. 4. Yolk globule of oocyte of *Paracentrotus lividus* derived from aggregation of granulations. Fig. 5. Globular formation of Walker tumour cell, treated with deoxyribonuclease, derived, like the yolk globule of Fig. 4, from an aggregation of granulations. Fig. 6. Portion of a Walker tumour cell kept for control in distilled water. Note the accumulations of granulations with completely irregular aspects (arrows). Figs. 1, 2 and 3, under the optical microscope; Figs. 4, 5 and 6, in the electron microscope

so far as can be seen with the optical microscope, in the cells of the more internal parts of the sections of the preparations treated with enzyme.

In the electron microscope some globular formations (Fig. 5) manifestly derived, as in the oocytes (Fig. 4), from an aggregation of granulations (ribosomes) were always seen in the cytoplasm of the cells of the tumour undergoing enzyme action. These formations, corresponding evidently to the globules seen under the optical microscope, are very variable in number and size in each cell. In the cells of the control preparation, kept in distilled water, other accumulations of granulations, but with completely irregular aspects occurred (Fig. 6).

These results must be supported by other experiments, made also in a proper isotonic and isohydrogenionic medium. It has to be established if the deoxyribonuclease, too, constitutes the only substance which can determine the process of aggregation, or rather what, like other substances, is suitable for creating the favourable conditions for this process to take place. The same result, in any event, appears to be of notable importance in connexion with: (*a*) the existence of an enormous number of unorganized ribosomes; (*b*) the possibility of obtaining their aggregation artificially as normally in the oocytes; (*c*) a new orientation for the understanding of very intense intracytoplasmatic proteic syntheses; (*d*) ascertaining of the real alteration of the tumour cells. The hypothesis[3,7] that the cellular structure, which reacts directly to the action of carcinogenic agents is the nucleolus, would seem to be more and more consistent. In fact, this, by producing the ribonucleoproteic granulations (karyoribosomes) in large quantities, would seem to provoke the transformation of the normal cell into a tumour cell. The principle would lie in a modification of the ribonucleoproteins which, through the presence of the so-called infective ribonucleic acid[9,10], would be induced to self-duplication without control.

All the alterations which, among others, include modifications in the chromosomes, hypertrophy of the Golgi bodies, and noticeable variations in the number of mitochondria[6], would derive from the incapacity of the cell to organize the ribonucleic granulations (cytoribosomes), which flow from the nucleus into the cytoplasm in enormous quantities. The uncontrolled cellular division of tumours would be but a consequence of the uncontrolled divisions of the ribosomes in the nucleus.

Finally, it should be pointed out that the result of artificial aggregation (above), from the theoretical side, is still valuable even if its realization is very rare and difficult. In this connexion the rarity with which very numerous embryos have been found in malignant tumours of the testes[11] and the ovary[12] should be emphasized; these embryos deriving probably from a kind of vitellogenesis (through aggregation of proteic particles as in the normal oocytes) within the germinative cells[7].

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Distribution of Deoxyribonucleic Acid in the Cell Nucleus

THE problem discussed in this communication is the state of intermitotic chromosomes, and since deoxyribonucleic acid (DNA) is characteristic of the chromosomes it is the distribution and localization of the structure carrying DNA in the nucleus which are to be examined.

This question of distribution of DNA is of importance for the cytophotometric method of Pollister and Ris[1] measuring the relative DNA content of a Feulgen-stained nucleus. This method assumes a more or less regular distribution of DNA in the nucleus. Indeed, different investigations concerning the state of the intermitotic chromosomes have shown that they are in a very extended and hydrated state and differ in this way from the condensed and dehydrated mitotic chromosomes. According to a model first designed by Pollister[2] the swollen chromosomes nearly fill the entire nucleus. Investigations of the effects of different dehydrating agents on the nuclear structure, as we see them in the phase-contrast microscope, have confirmed this idea[3].

Recently, another interpretation of the distribution of DNA was proposed: according to Iversen's observations[4], the DNA-carrying structure is distributed in a peripheral zone of the nucleus and the remaining observed Feulgen-stained masses would be the 'nucleolus associated chromatin'. According to Iversen's illustrations, the remaining interior of the nucleus is empty and free from DNA. Similar findings have been reported by Ludford[5].

My investigations with paraffin sections fixed in various ways (non-buffered formol, Carnoy's, Stieve's, Zenker's solution, Susa mixture) have not confirmed Iverson's findings unless the preparations were dehydrated very rapidly in alcohol or were fixed in absolute methyl alcohol or formalin at pH 4·0. In normal dehydrated preparations the Feulgen-stained masses were not only on the peripheral membrane or adjacent to the nucleoli but also distributed throughout the remaining nuclear compartment. It is known that most fixing agents precipitate the proteins, especially the nucleoproteins, the intensity of the precipitation being subordinated to the pH, the destruction of the lipoids and many other factors[6]. This relationship of dehydration and the milieu can be followed with the phase-contrast microscope[3].

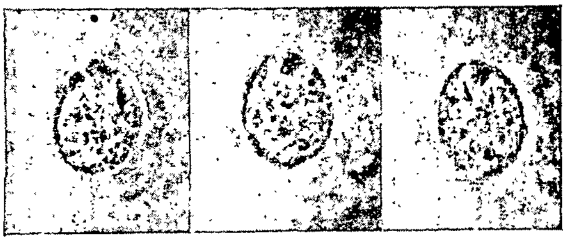
Fig. 1. Feulgen squash method, phase contrast. Nucleus from the epithelium of the colon of a female rat photographed at three different levels. Small and large chromatin granules can be seen in the nucleus

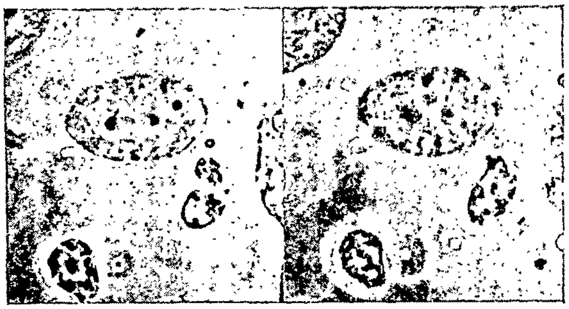
Fig. 2. Carmine acetic acid squash method, phase contrast. Nucleus from the epithelium of the small intestine of a female rat photographed at two different levels. The whole nuclear compartment is interspersed with granules. Five chromocentres are demonstrated

Squash methods, for example those described by Darlington and La Cour[7], have advantages over the paraffin section method for demonstration of the nuclear structure. With these squash methods the nuclei to be observed should not be flattened, that is, they should move freely with the current existing in the fluid between slide and cover-slip. By moving the fine adjustment one can convince oneself that, using the Feulgen-staining method[8] or the carmine acetic acid stain[9], the DNA-containing material is more or less equally distributed throughout the whole nucleus (Figs. 1 and 2). Although much chromatin accumulates directly on the inner face of the nuclear membrane, it is also often distributed without any evident order in the interior of the nucleus especially when the nucleolus also is situated in the interior. Extended research on the nuclei of different tissues has demonstrated that the distribution of the DNA-containing chromatin in the nucleus is specific with every tissue and that this specificity is lost in cancerous cells as a result of dedifferentiation[10].

These observations illustrate the fact that the DNA-carrying chromosomes are interspersed rather evenly in the nuclear compartment.

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Differences in Mechanism of Staining Reaction for Mast Cells of Some Lower Vertebrates

I HAVE examined the differences in staining reactions in the mesenterial and myocardial mast cells of the viviparous Cyprinodonts *Lebistes reticulatus*, *Xiphophorus helleri* and *Xiphophorus maculatus*[1], the mesenterial mast cells of the carp *Cyprinus carpio* and in the cutaneous mast cells in precancerous skin (carcinoma planocellulare) of the lizard *Lacerta agilis*[1]. Just as in the mast cells of the mouse during the course of experiments on mucopolysaccharide reactivity after peracetic acid oxidation (Greenspan's mixture, 25° C., half an hour)[2], a change was observed in the cytoplasm of the mast cells. When the peracetic acid oxidation was followed by digestion with mucopolysaccharide-hydrolysing enzymes, various staining reactions were obtained by the methods used for mucopolysaccharides.

To permit a satisfactory comparison with the results obtained by Fullmer[3] with the mast cells in the skin, the tongue and other organs of adult *BALB/c* strain mice, I used the corresponding histological technique. Immediately after death by decapitation, excised pieces 2 mm. thick were put into aqueous neutral calcium acetate formalin at 17° C. for a period of 24 hr. (Lillie[2]). After washing and dehydration, these pieces were embedded in paraffin and sectioned at a thickness of 6μ.

The mast cells were stained in the control sections with aldehyde fuchsin, the Hale stain, the Rinehart stain and azure *A* (0·5 per cent azure *A* in aqueous 0·2 *M* phosphate buffer *p*H 4, at 25° C., half an hour). These mast cells reacted excellently, either with or without previous oxidation of tissue sections with peracetic acid. In general, the peracetic acid appeared to intensify the subsequent staining reactions. Just as in the mouse[3], if the sections were oxidized with peracetic acid and then digested with lysozyme (5 mgm. of lysozyme, Worthington ; 30 ml. of 0·2 *M* phosphate buffer, *p*H 5·3 ; 37° C. ; 24–48 hr.) or with β-glucuronidase (15 mgm. of β-glucuronidase, Nutritional Biochemicals Corp. ; 50 ml. 0·1 *M* acetate buffer, *p*H 4·5 ; 37° C. ; 24–48 hr.) only a few mast cells were affected by aldehyde fuchsin, whereas none was affected by the Hale stain. The few mast cells which reacted with the aldehyde fuchsin were only slightly stained. With azure *A*, however, the granules and the cytoplasm of the mast cells remained strongly reactive. The staining reaction appeared to be only slightly, if at all, reduced. In some cases testicular hyaluronidase (25 mgm. of testicular hyaluronidase, Nutritional Biochemicals Corp. ; 50 ml. 0·2 *M* phosphate buffer, *p*H 6 ; 37° C. ; 24–48 hr.) prevented the subsequent Hale staining reaction. If the sections were not oxidized with peracetic acid (Greenspan's mixture, 25° C., half an hour)[2] prior to the digestion step, no effect on any staining reaction was observed. By boiling for half an hour in alkaline buffer at *p*H 9, the lysozyme was inactivated, which confirms previous work by Fullmer[3]. The mast cells were found to react strongly with all stains after incubation in the usual manner with lysozyme treated in this way.

My results, like those of Fullmer[3], using mast cells of the mouse, are fully compatible with the view that after the peracetic acid oxidation both the lysozyme and the glucuronidase enzymatically remove from the mast cells a certain component which is stainable with the Hale stain and the aldehyde fuchsin, and there remains a component inducing a strong metachromasia with azure *A*. Since the inhibitors, which are not otherwise likely to alter the enzyme, prevent the effect on the staining reactions, Fullmer[3], and rightly in my opinion, maintains that the insufficient staining with the Hale stain and the aldehyde fuchsin in this case represents an uptake of basic protein by acid mucopolysaccharides, rendering these groups inaccessible to the stains. Several authors have described this type of blockage. On the basis of the reactions mentioned above, one can conclude that although, in all the three histochemical methods used, mucopolysaccharides are stained, the staining reactions of granules and other cytoplasmic components of mast cells are not equivalent at all, and probably do not form the same substance with the stain. These results are in agreement with those of Gomori[4], who had noted differences in the degree of staining of various mucopolysaccharides using various methods, and also with the fact that the periodic acid oxidation of tissue sections has been found to induce the staining of certain normally unreactive substances with aldehyde fuchsin, and to alter the susceptibility of oxytaban fibres to lysozyme, hyaluronidase and elastase.

Furthermore, I used for the staining of the mast cells of the lower vertebrates mentioned two new techniques, namely, the night-blue method[5] and tartrazine counter-staining according to the periodic acid – Schiff procedure[6].

The night-blue method is essentially the same as that reported by Hallberg[7], although for the staining of human and mammalian mastocytes by Winkelmann[5] some minor modifications were made. The mast cells of the lizard and the teleost species mentioned above showed a positive orthochromatic night-blue stain in the granules. The nuclei of mast cells were found not to stain, nor did the cytoplasm. After alcohol fixation the stain disappeared completely.

After applying tartrazine counterstaining, following the periodic acid – Schiff procedure, the granules of the mast cells of the lizard, the carp and the viviparous toothcarps stained in a different way from the other granules giving a positive periodic acid - Schiff reaction. As tartrazine, an azo derivative of 1-phenyl-5-pyrazolone-3-carboxylic acid, we used the tartrazine *O* (*C.I.* 640, from G. T. Gurr, London, S.W.6). With the tartrazine counterstaining the colour of the granules of the mast cells of the lizard, the carp and the viviparous toothcarps turned from fuchsia-red to brick-orange, whereas the other substances giving a positive periodic acid – Schiff reaction remained completely unchanged.

The night-blue method and the tartrazine counterstaining described appear to be excellent techniques also for the mast cells of the lizard, the carp and the viviparous toothcarps. The clarity of the two staining methods greatly facilitates quantitative routine estimation of the mastocyte content of tissues in lower vertebrates.

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BIOLOGY

Perception of Distance in Animal Echo-Location

In a recent article[1] Nordmark fails to explain two features of the bat's ultrasonic transmissions ; one is the frequency sweep used by most species of bat employing echo-location as a means of orientation, and the other is the apparent difference between Vespertilionidae and Rhinolophidae which in fact has been shown to be a similarity[2]. As with all hypotheses related to the behaviour of animals none can be proved beyond doubt, nor can we expect any to be complete; we may, however, assume reasonable efficiency in the use of the information contained in echoes received by the bat as it relies on such information for its very livelihood.

The means, suggested by Nordmark, whereby the bat locates objects in space by listening to the note produced by two pulses arriving at the ear in quick succession is only a second-order effect (the response of the ear to the fundamental of the wave envelope is very small compared with that from the higher frequency wave), and it is unlikely that it is used directly. The bat would find no benefit from the frequency-sweep within the pulse, first observed by Griffin[3], if it used this method. Analysis of some of Griffin's records on an instantaneous spectrum analyser show the frequency modulation sweep to be nearly linear. Such a frequency sweep can provide very much more information than the simple pulse method although it is much more difficult to explain adequately[4]; and since Rhinolophidae also employ a frequency-sweep at the end of the tone transmission, it seems reasonable to believe this to be an important feature of the bat's echo-location system.

I have recently carried out some experiments in connexion with a blind guidance device which have served to confirm my beliefs. Both a pulse and a frequency-modulation echo-location system were tried on several subjects who had no knowledge whatsoever of the systems being tested or of the results to expect, so that they could only judge by their direct observations. Both systems used the same ultrasonic transducers which were capable of operating over a frequency band of 20 kc./s. centred on 50 kc./s. The transmitted energy was sufficient to render background noise insignificant at the ranges used in the tests. The pulse system employed pulses of 50 kc./s. tone lasting from between 0·1 msec. to 15 msec. which could be chosen in 5 steps and the signals presented to the ear were pulses of 3 kc./s. tone approximately. The maximum distance to an object was 15 ft., and therefore the transmission and echo overlapped in some of the tests. A 9-in. diameter metal sphere was detected at up to 10 ft. but the distance could not be determined except by the amplitude of the echo, and this with very little certainty. In some tests the transmission could not be heard by the observer; in those where it could the intensity was comparable with the largest echo experienced. Nevertheless there was little difference in the poor ability of the subject to locate an object as a result of this.

The frequency-modulation system on the other hand used a transmission which varied linearly from 60 kc./s. to 40 kc./s. at a rate which produced a difference frequency of 3 kc./s. between transmission and an echo from 15 ft. Thus the subject experienced signals covering a frequency-range of 0–3 kc./s. depending on the distance to the object. Using this system there was no difficulty in determining the distance to an object, depending only on the ability of the subject to estimate the frequency; for example, an object at 10 ft. would produce an echo at the subject's ear corresponding to an interrupted 2 kc./s. tone. With such a system even fine wires of only 1 mm. diameter could be detected with reasonable ease. This was quite impossible with the pulse system.

The conclusion drawn from these tests was that the effect described by Nordmark is very weak compared with what can possibly be obtained from the frequency-modulation sweep. Pye has shown how such a system is possible physiologically in the bat and therefore more likely to be used[2].

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THE theory of range perception in animal echo-location, recently advanced by Nordmark[1], was of great interest as I had just published a theory for bats which also depends on a frequency analysis[2].

Dr. Nordmark refers to the work of Thurlow and Small[3], who describe a time-difference tone which is heard by man when pairs of sound pulses are repeated at rates greater than 25/s. The frequency of this tone is inversely proportional to the shorter pulse-separation time, and Thurlow and Small obtained some evidence that it is a central effect, produced as a measure of the interval between two sets of sensory nerve impulses. This phenomenon thus appears to provide evidence for a central timing mechanism, originally proposed for bats by Hartridge[4] and which I had found difficult to visualize.

However, any central mechanism must be limited for very short time-intervals by the refractory period of the sensory nerve. In support of this, Thurlow and Small found that for two pulses of equal intensity the minimum interval which produced a time-difference tone was about 1 msec.; shorter intervals were only effective if a small pulse preceded a larger one[5], an improbable situation in echo-location. If the second pulse were decreased in intensity (or the first one increased) this period was progressively lengthened. Grinnell and Griffin[6] have shown that the bat's auditory system can resolve pairs of equal pulses down to a separation of 1 msec., corresponding to a very intense echo returning from 16·5 cm. But a bat feeding on flying insects, which may be taking evasive action[7], must surely be able to obtain information down to the last few centimetres.

I have therefore suggested that at close range, when the echo overlaps with the emitted pulse, the bat could obtain information from beat-notes between the two signals. The refractory period is then not involved, since different receptor cells will be excited within the cochlea. The frequency-modulated pulses of Vespertilionids will give range and directional information, while the very constant-frequency pulses of Rhinolophids will measure the Doppler shift and so indicate the relative velocity.

Möhres[8] states that accuracy of perception extends to about 50 cm. for Vespertilionids, and to at least 6·4 m. for Rhinolophids—approximately the ranges expected for beat-note operation. But Vespertilionids in flight appear to have some appreciation of objects at greater distances. Possibly location is initially achieved by the time-difference apparatus, while at close range the beat-notes are used. The latter could be much more accurate as the beat-frequency may change by 40 kc./s. over a range of 50 cm. This depends however on an equal rate of frequency change in every pulse, which has not yet been established.

The long, unmodulated pulses and low repetition-rates (less than 10/s.) of the Rhinolophids do not appear to lend themselves to time-difference measurement. Möhres has rejected such mechanisms for these bats and suggested that they obtain information from the direction and loudness of the echoes. The beat-note mechanism makes this possible by avoiding masking. The rapid vibration of the ears, by producing additional Doppler shifts, can endow them with greater directionality than their size suggests, and also allows operation under stationary conditions.

An electronic model which produces various bat-like sounds and allows one to hear the beat-notes shows that the human ear can achieve considerable precision in this form of echo-location. Preliminary tests show that time-difference tones are weak for the echoes of small objects, although this need not be so in bats. It seems that frequency modulation gives little, if any, improvement. Rectification (used for the detection of beat-notes) does appear to help, presumably by allowing the ear to use the pulse envelope component as well as the carrier. No time-difference tones can be produced with artificial Rhinolophid pulses.

The complex pulses of other echo-locating animals will not form useful beat-notes. Direct echo-delay measurement, by a process such as that indicated by the time-difference tone, appears to be the only possible mechanism for these cases at present. It is suggested that the interception of flying prey by insectivorous bats is related to the characteristically simple frequency structure of their pulses, which are thus potentially more informative. But as Dr. Nordmark states, it is not certain which properties of the echoes are actually used. Further investigation is necessary before these theories can be evaluated and some understanding of the true mechanisms can be achieved.

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THE frequency sweep used by some bats is unlikely to be essential for distance perception by these animals, and is possibly no more than an accidental consequence of their method of sound production. When the cricothyroid muscles of the larynx of *Eptesicus* bats are denervated the modulation is altogether absent and the frequency becomes constant at about 10 kc. Yet such bats land normally on walls and avoid obstacles of moderate size. A slight decline in performance can be attributed to the greater wave-length used and the reduced vigour of the bats as a result of the surgical operation[1].

The success of these animals even under such drastically altered conditions points to the danger of attaching too much importance to specific properties of the sounds used in estimating distance. If distance is indicated by the pitch of a tone some other facts are also more difficult to explain if one assumes that the pitch is a function both of distance and definite properties of the sounds, as Kay and Pye do, rather than only of distance, as I do. If the frequency-sweep were utilized in the way these authors suggest, each animal would have to work out its own scale for measuring distance and maintain its particular sweep characteristics under all conditions. The critical variable is the rate of change of frequency, which would have to be constant. As this is a function of the frequency-range and the duration of the sweep, each of these factors must be held constant or changed to a degree which compensates exactly any change in the other factor. Such precise and complicated mechanisms for events of a few milliseconds' duration are unknown in biology. Novick[2] and Griffin[1] give many examples of the variability of the frequency-range, rate of change and duration of the sweep. Griffin's records for the bat *Eptesicus*, for example, show clearly that the decreased pulse duration as the bat approaches an obstacle results in an increased rate of change of frequency.

The typical decrease in pulse duration will also preclude the overlap between outgoing pulse and returning echo, which a frequency-modulation method of echo-location presupposes, until the last 17 cm. or so. Pye does not take this progressive decrease into consideration and it is indeed inexplicable if an overlap is to the bat's advantage. He might possibly argue that it is only during the last 17 cm., where the time-difference mechanism may fail, that the frequency sweep acquires its importance. We have to bear in mind, though, that this distance will be covered in about 1/20 of a sec., which is the reaction time between the hearing of an echo and the turning of a bat's flight according to Griffin. The interception of flying prey can be accomplished also by insectiverous bats using a different type of pulse and therefore likely, as Pye has pointed out, to use a time-difference mechanism.

The alleged advantages of the frequency-modulation method cannot be substantiated by experiments which use instruments capable of a high degree of precision for sound production and a non-linear device instead of the ear to generate the difference tones. Nor can the failure to hear a time-difference tone under certain conditions prove anything as long as we are not given the experimental details.

As Thurlow and Small had no reason to measure the thresholds for the sweep-tone under the extreme conditions—particularly the high intensity—which would correspond to animal echo-location, we cannot use their results to decide whether their pitch phenomenon is the whole answer to the problem of distance perception or not. I have recently been able to verify though, that the sweep-tone can be heard: (*a*) when the pulse-rate is irregular; (*b*) when pulses corresponding to those of other animals are present; (*c*) when the signal/echo ratio is much greater than in earlier experiments; (*d*) when the pulse-rate is no more than 2–3 per sec. and the (long) pulses overlap, corresponding to the situation for the Rhinolophidae.

Pye has raised an important point when he asks how short the interval between pulse and echo can be without abolishing the time-difference tone. The basis of this pitch phenomenon can scarcely be that suggested by Kay, and is likely to be found in the synchronized response of the nerve to pulsed stimuli. As synchrony can be maintained up to 4,000 c.p.s., the shortest interval may be 0·25 instead of 1 msec.[3]. As Dijkgraaf[4] has shown that a bat can react to a worm held about 5 cm. from the animal's mouth, the tentative limit of just over 4 cm. for the time-difference mechanism is low enough in this case.

The maximum distance recorded at which an object was detected, 6·4 m. for a Rhinolophid bat, is more difficult to explain. A pitch of 22 c.p.s. may be slightly low to serve as a reliable sign. It is important to remember, though, that echo-locating animals probably react to change and rate of change of pitch rather than absolute pitch. Under certain conditions no sharp distinction can be made between change of pitch and change of timbre, and it may be that a slight modification of the timbre is the earliest sign in many situations. If we assume that the Rhinolophidae judge the distance by the loudness of the echo we will instead be faced with new difficulties. How, for example, can these bats judge the loudness of the echo when the interval between outgoing pulses may be as short as a few milliseconds[3] ?

It should be added that the frequency of these pulses is not quite constant and therefore unlikely to be used for estimating distance according to the Doppler principle.

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Insecticide Resistance in Mosquitoes

A DETAILED study of the nature and mode of inheritance of dieldrin-resistance in a strain of *Anopheles gambiae* from the Western Sokoto region of Northern Nigeria, and of DDT-resistance in a strain of *A. sundaicus* from Indonesia has already been made and recorded[1-3]. Dieldrin-resistance in *A. gambiae* was shown to depend on a single genetic factor semi-dominant in its manifestation and imparting cross-resistance to other cyclodiene chlorinated hydrocarbons and to γ-BHC but not to DDT. DDT-resistance in *A. sundaicus* also shows a monofactorial type of inheritance, but here the factor is recessive in nature and imparts cross-resistance to DDT-analogues but not to dieldrin or to γ-BHC.

Two other dieldrin-resistant strains of *A. gambiae* have now been studied, one from Kano in Northern Nigeria[4], and one from Bobo Dioulasso in French West Africa, and an identical type of resistance to that found in the strain from Western Sokoto demonstrated. In addition, dieldrin-resistance has been studied in three other species of mosquitoes, *A. albimanus*, *A. quadrimaculatus* and *Culex fatigans*, and a single semi-dominant factor has been shown to operate in all of them. The resemblance of the resistance in the two anopheline species to that previously investigated in *A. gambiae* is enhanced by the fact that the discriminating dosages of dieldrin separating susceptibles from hybrids, and hybrids from resistants, are almost identical in both larvæ and adult tests. In the case of *C. fatigans* the discriminating dosages applicable to the adults are somewhat higher than in the anophelines. In other words, the susceptible and hybrid adults are more tolerant to the insecticide than are the adult anophelines.

Three strains of *A. quadrimaculatus*, one from Maryland, one from Florida, and one from South Carolina, United States, have been used in these investigations. From the first two, dieldrin-resistant strains have been selected which are susceptible to DDT. Attempts to select DDT-resistance from the original mixed populations have failed. The South Carolina strain is susceptible to both insecticides.

The dieldrin-resistant strain of *C. fatigans* originates from Ceylon. Attempts to select DDT-resistance from it have so far failed. The susceptible strain of this species, used for crossing with this resistant strain, is one from Malaya. Both strains are much more tolerant to DDT in the adult stage than any susceptible anophelines. In fact they appear to be as tolerant as the DDT-resistant strains of anophelines so far investigated. However, the larvæ are more susceptible to DDT than those of susceptible strains of anophelines. It would thus appear that *C. fatigans* shows a natural high tolerance to DDT in the adult stage, and that this is a characteristic of the species and does not stem from any specific factors for resistance.

The dieldrin-resistant strain of *A. albimanus* was selected from a mixed population of this species from El Salvador. Like the other dieldrin-resistant species it is susceptible to DDT. The susceptible strain of the same species used for inheritance studies originated from Panama. Selection of the original mixed El Salvador population with DDT resulted in the production of what was apparently a true DDT-resistant strain of this species. Testing this selected strain on dieldrin revealed the presence of some dieldrin-resistant individuals, and selection of it with this insecticide produced a strain which would tolerate very high dosages of both DDT and dieldrin on consecutive exposures, that is, a strain showing double resistance.

From the strain showing DDT-resistance and some dieldrin-resistant individuals has now been isolated a strain resistant to DDT and completely susceptible to dieldrin by selection from the offspring of individual females.

This isolation of three strains of the same species, one resistant to DDT but susceptible to dieldrin, one resistant to dieldrin but susceptible to DDT, and a third resistant to both insecticides, is in itself a proof that two separate genetic factors are involved. Further confirmation has come from the crossing of the DDT-resistant strain with the susceptible Panama strain and studying the mode of inheritance of DDT-resistance. A pattern of inheritance has been demonstrated almost identical with that shown in *A. sundaicus*, namely, a monofactorial type of inheritance dependent on a virtually recessive factor. It remains now to cross the DDT-resistant strain with the dieldrin-resistant to show whether the factors occur on the same chromosome (and, if so, how far apart) or on separate chromosomes, and this work is in process.

Finally, DDT-resistance has also been studied in a third anopheline species, *A. stephensi*. By comparing a resistant strain from Iraq with a susceptible strain from India and crossing them, DDT-resistance in this species has been shown to impart cross-resistance to DDT-analogues but continued susceptibility to dieldrin and γ-BHC. The inheritance pattern differs slightly from that shown by *A. sundaicus* and *A. albimanus* in that the single factor involved is semi-dominant when 24-hr. mortalities from exposures to the insecticide are considered. When, however, immediate knockdown counts after removal from the insecticide are made, the factor appears to be more recessive than dominant.

DDT-resistance in *A. stephensi* is of a comparatively low order, some 10–20 times only, and exact discrimination between the genotypes is impossible. The establishment of the monofactorial character of the inheritance of resistance in this species has been confirmed finally by a process of repeatedly back-crossing the heterozygote with the susceptible parent and exposing the offspring to a dosage of DDT which is known to eliminate susceptibles completely. The survivors have then been re-crossed with the susceptible strain and the process of selection repeated. The mortality at each stage of selection has remained more or less at the same level. It has not shown the consistent rise expected where more than one genetic factor is involved.

These investigations, then, have amply confirmed that resistance in mosquitoes is of two independent types, DDT-resistance and dieldrin-resistance. Further, they have shown that resistance is dependent on a single genetic factor, semi-dominant in the case of dieldrin-resistance and recessive or nearly so in the case of DDT-resistance, the remarkable feature being that these characteristics apply to different species in widely separated parts of the world. That the two factors can exist together in the same population of the same species is now definitely established, and should further stimulate the search for new and efficient insecticides outside the chlorinated hydrocarbon group of organic chemicals.

The details of these experiments are being prepared for separate publication.

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Parental Care in a Rare Spider *Pellenes nigrociliatus* (L. Koch) var. *bilunulata* Simon

In May 1959, on a strongly sunlit slope of a roadside scarp adjacent to a pine wood in Toruń (northern Poland), about 20 specimens of snail shells (*Helicella candicans* (L.Rfv.) were found each of which was suspended high above the ground (up to 18 cm.) on plants (mostly grass) by means of a 'rope' twisted together from many spider-silk threads 1–3 cm. long. The entrance to the shell was closed by a 'curtain' made from thick spider's fabric. From time to time a horizontal slit (Fig. 1) opened in the curtain, and a pair of white-haired spider's pedipalps appeared in it (Fig. 2). It was, however, difficult to make the inhabitants of the shells come out of them, though they attacked fairly vigorously a needle brought close to the opening in the curtain.

The spiders were compelled to leave the shells by putting them on a warmed base. The spider inhabiting the shell (Fig. 3) proved to be a rarity. Dr. Jacques Denis (Anzin-Nord, France) identified it as *Pellenes nigrociliatus* (L. Koch) var. *bilunulata* Simon. According to him, until now this form has been known only as one specimen found in France (Tarn).

During June and July 1959, in the course of several trips, I found, on the same scarp, 20 more suspended shells of the same species. All specimens of spiders found were females. They were taken to the laboratory and placed in small glass terraria, with empty snail shells on the floor—the same which abounded on the scarp. Expelled from their shells, in the same way as in the first case, they were left in the terraria. Soon it was noticed that some of the spiders were attaching threads to the empty shells. Then, in absence of plants, they attached the threads to the wall of the terrarium. After suspending the shell they entered and closed the entrance with a thick curtain of spider's web. As it appeared later, they made nests in the shells. During June–July, about 10 eggs enveloped in a very thick, silky, unusually ductile fabric were found in the particular shells. The spiders hatched underwent their first moult in the shell, and only then began to leave the shells; but for a long time they would return to them.

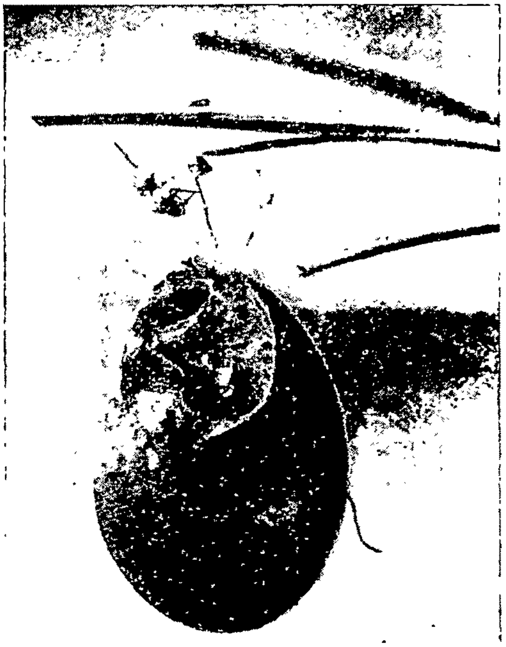
Fig. 1. Entrance to spider's 'nest' (× c. 4·7)

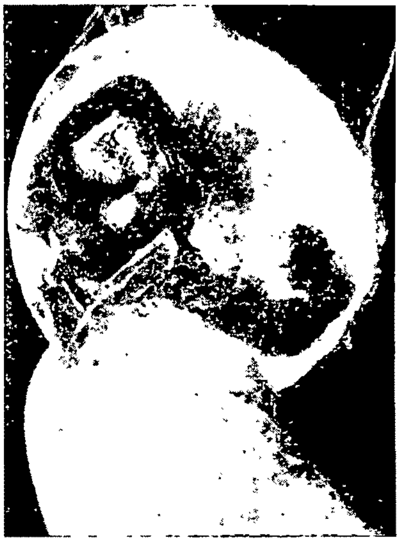
Fig. 2. The spider appearing in the 'nest' entrance (× c. 18)

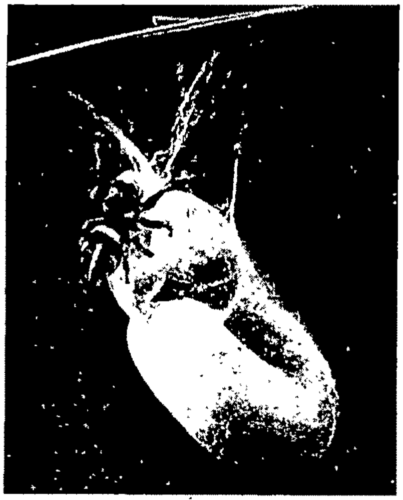

Fig. 3. The spider on the shell (× 4)

In 1959 no male was found on the scarp. During early April 1960 the scarp was searched. At that time only the earliest plants were growing and grass was still scarce. One male spider, similar to the females found before, was seen on the ground. There were, however, no suspended shells. In May the search was repeated, and 18 suspended shells with a female in each of them and eggs in some were seen.

The spiders were taken to the laboratory, where systematic observations have been conducted on the behaviour of the adults and of the young.

I am grateful to my assistant, W. Kokociński, for the photographs.

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Flowering Behaviour of Vanilla

THE vines of *Vanilla planifolia* Andrews will climb to heights of 60 ft. or more, if provided with a sufficiently tall support plant. The vine must therefore be trained to a height convenient for pollination and harvesting in the commercial production of vanilla. The prevention of the natural tendency to climb is thought to enhance the production of flowers[1]. It is believed that flowering will not occur as long as the vine can climb upward[2-7]. Childers[3] suggests that "bending of vine appears to be an important factor in causing it to flower and fruit beyond the bend, which may be due to an accumulation of carbohydrates and possibly other flower-forming materials in this region of the vine".

Observations on the flowering and fruiting of *V. planifolia* have recently been made in an abandoned vanillery in western Puerto Rico, which had not been under cultivation for at least 15 years. Since the plants in this area were growing on both short and tall supports, the plants were of both a rambling and a climbing habit of growth. Both types were inspected for flowering. Five plants of the low-growing type gave a total of 65 inflorescences on horizontal and descending branches, while no evidence of flowering was observed on the few short ascending stems present. Ten vigorous, climbing plants were selected at random, and counts were made of flower spikes on ascending and descending stems. Ascending, or climbing stems had a total of 82 inflorescences, while descending stems had a total of 29 flower spikes. One plant with 18 flower spikes consisted entirely of two main branches which had grown to a height of 60 ft. and were still climbing. The majority of inflorescences occurring on ascending vines were 30–50 ft. above the ground, and were on stems that were free of bends for at least 10 ft. in either direction. The absence of flowers on the lower 30 ft. of the climbing plants suggests that a considerable amount of growth may be necessary before flowering occurs, and that flowers should not be expected to occur on the short basal stems of rambling plants.

The inflorescences on ascending vines were capable of bearing fruit, and on one plant with 27 pods all occurred on climbing stems.

These observations indicate that *Vanilla* will flower and set fruit, whether climbing or not, and that a change in the direction of growth is not necessary for flower formation.

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Striate Mosaic: a New Disease of Sugar Cane

THE term 'striate mosaic' is proposed for a new disease of sugar cane found in the varieties Pindar and Q.57 in the Lower Burdekin district in north Queensland. The most obvious features are the short, fine striations which can just be separated by the naked eye. They are a lighter colour than the normal leaf blade and show first on the youngest exposed leaf and reach their best development as the leaf expands, but are difficult or impossible to find on mature and older leaves. They occur less in the region of the large vascular bundles than in the tissue between and so in the aggregate often give a marked striping effect on the younger leaves, or even a yellowing to the entire top. Severe stunting is usually associated with the striations but striations without obvious stunting have been found in Pindar.

The disease is sett-transmitted and heat treatment of the setts in water at 50° C. for 3 hr. or in air at 54° C. for 16 hr. (as used in the control of ratoon stunting disease[1]) failed to free them from the disease. Inoculations into healthy setts did not produce the disease but the striations alone or with the stunting may be transmitted by the Sein inoculation technique[2]. It spreads very slowly in the field, and the use of disease-free planting material should prevent it ever causing serious losses.

It would appear that viruses, one causing the striations and one the stunting, are responsible for the disease.

A detailed report is in course of preparation.

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Moisture Conditions for testing Germination

The International Standards for Seed Testing[1] detail the requirements for assessing the viability of many species, but provide only general directions for the moisture regime to be used, namely, "For most kinds of seeds, blotters or other paper substrata should not be so wet that by pressing, a film of water forms round the finger". In soil physics terminology, these substrata would be at an indefinite suction approaching zero (that is, free water). Furthermore, the suction is not necessarily constant throughout the test, namely, "subsequent watering if any should be left to the analyst".

We have adapted simple experimental equipment[2] to determine the influence of small changes of suction at or about zero suction. Representative replicated results for three species under identical illumination, temperature, and relative humidity environments are listed in Table 1. Seeds were selected for uniformity and dusted with tetrachloro-*p*-benzoquinone to prevent fungal growth. Germination was taken as being the emergence of the radicle, instead of the "emergence and development from the seed embryo of the essential structures which for the kind of seed are indicative of its ability to develop into a normal plant under favourable conditions"[1]. If this latter definition had been used, the differences between suctions would have been more marked, since the faster germinating seeds also showed faster development; for example, after 18 hr., oats at 0 cm. suction had 1 cm. green shoots, while at 10 cm. water suction there was only an emerged radicle, these two conditions not being differentiated in Table 1.

For each of the species described in the table, and over this limited suction-range, the total number germinated after 10 days was constant. Hence the total of the four replicates at each time has been expressed as a percentage of the total final count for that species.

Table 1. Cumulative Germination expressed as Percentage of the Number of Viable Seeds obtained after 10 days of Treatment

Species	Suction cm. of water	Days of treatment 1	1½	2	2½	3	5
Avena sativa L. var. Klein (oats)	0	0	0	30	51	80	98
	2	0	0	2	15	57	93
	4	0	0	2	3	33	92
	6, 8 and 10	0	0	0	2	21	82
Medicago sativa L. (lucerne)	0	90	93	96	97	98	99
	2	70	83	96	97	98	99
	4	67	83	96	97	98	99
	6, 8 and 10	61	77	96	97	98	99
Lolium perenne L. Mansfield strain (perennial rye-grass)	0	0	0	24	39	69	95
	2	0	0	35	51	84	97
	4	0	0	37	53	91	99
	6, 8 and 10	0	0	39	65	93	99

Both oats and lucerne show a significant decrease in germination-rate on increasing the suction from 0 to 6 cm. water, while perennial rye grass showed an increase in rate over the same range.

These germination results were compared with those from the New South Wales State Department of Agriculture Seed Testing Laboratory using the same seed sample and the procedure of the International Rules[1]. The final count numbers at 10 days were identical, but the rates for 'standard' treatments varied more than those of all the suction regimes listed in Table 1. This variation can therefore be attributed to inconstancy in the moisture regime of the substrata, which under the existing standards is outside the control of the tester. In those laboratories where seed testing is infrequent, it is likely that variability will be greater than in routine testing, particularly when attempting to obtain comparable moisture conditions for seeds differing in size and shape.

It is suggested that small variations in moisture regime have been the cause of inconsistencies attributed to other factors; for example, Ahring *et al.*[3] investigated temperature differentials encountered in seed germination experiments, but as the moisture regime was not specifically controlled (it would require some form of tensiometer to detect small differences within the range 0–6 cm. water) all the detected responses cannot be attributed to a temperature factor.

Isely[4] recommends placing the Petri dishes of substrate within plastic freezer bags containing wet filter papers to saturate the atmosphere. However, this would not easily maintain any particular suction and would only minimize loss of vapour. If seeds were to be germinated on tension plates, a suction for optimal germination could be specified for each species, hence eliminating the use of various types of substrata.

Acknowledgement is made to Miss Amy Meyers of the New South Wales State Department of Agriculture Seed Testing Service for her help.

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ENTOMOLOGY

Termination of Diapause in *Acheta* by Urea

The rate of termination of diapause in eggs of *Acheta commodus* (Walk) has a negative temperature coefficient within the range $-16 \cdot 5$ to $+5°$ C.[1]. It has been reported by Jacobsen and Christensen[2] that the initial stages of denaturation of β-lactoglobulin by urea has a negative temperature coefficient. Accordingly, experiments have been carried out with certain denaturants, primarily urea, for evidence of any effect on diapause. The attempt was made to incorporate the chemicals into the eggs by making use of the capacity of Orthopteran eggs to take up water during their development. In *Acheta* the uptake of water occurs slightly later than the onset of diapause[3].

(1) Eggs, up to one day old, were placed on blotting paper disks previously moistened with solutions of the chemicals under test. They were then incubated, in sealed tubes, at 27 (± 0·2)° C.

Definite responses were obtained with urea. Table 1 shows that urea solutions at the strengths normally used for denaturation of protein were extremely toxic but at a concentration of 0·08 *M*, a highly significant increase occurred in the proportion of eggs that completed their development without evidence of diapause.

Table 1. PERCENTAGE OF EGGS DEVELOPING WITHOUT EVIDENCE OF DIAPAUSE AFTER EXPOSURE DURING THE PRE-DIAPAUSE STAGES TO SOLUTIONS OF UREA AT THE CONCENTRATIONS INDICATED

Molar concentration	0	0·02	0·04	0·08	6·0
Percentage hatch	6·7	5·3	8·3	31·7	0
Percentage mortality	5·3	0	4·0	32·0	100

(2) The capacity of urea to increase the rate of termination of diapause was revealed in the following experiment. Pre-diapause eggs were treated with urea at a concentration, 0·02 *M*, insufficient to eliminate diapause in eggs held at 27° C. When held at this temperature, 95 per cent of the eggs, both treated and controls, entered diapause.

Incubation was continued at 27° C. until the completion of hatching. The median duration of exposure (period for 50 per cent hatch) was 35 days for the eggs treated with urea, in comparison with 54 days for the control.

Evidence to be described elsewhere shows urea to be absorbed into the egg.

The low concentration of urea in the above experiments would seem to make it unlikely that denaturation takes place. Nevertheless, relatively mild agents can cause reactions of this type, such as sulphydryl – disulphide exchange[4], or disturbance of the protein hydration structure (ref. 5 and personal communication). Although no reactions to concentrations as low as those reported here have been described, the possibility of steric effects must be considered.

A more detailed report of these and other experiments will be published elsewhere.

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The Prothoracic Gland and Colour Change in Locusts

MANY locust species are characterized by large variations in colour, morphometrics and behaviour, variations which are related, under natural conditions, to the density of the population[1]. When numbers are high the nymphs, or hoppers, live in dense bands, and these gregarious hoppers are orange or yellow and black in colour. When populations are low the hoppers do not group together, and such solitary locusts are fawn or green, with only small amounts of darker pattern. In the laboratory, gregarious locusts may be produced by rearing the hoppers in crowds, and solitary locusts by rearing each individual in a separate cage, out of visual and tactile contact with others[2]. The behaviour of locusts changes rapidly, according to the number of contacts that hoppers make with each other[3]. Changes in colour and in morphometrics require more continuous contact (or lack of contact) with other locusts[4,5]. It has been suggested that the colour changes are controlled, at least in part, by hormones, and that the hormonal balance of solitaries differs from that of gregarious individuals[5,6].

During an endocrinological investigation of two species of locusts, *Locusta migratoria migratorioides* (R. and F.) and *Schistocerca gregaria* (Forsk.), we observed that the prothoracic gland (ventral gland) is shaped differently and significantly larger in solitary than in gregarious individuals (Fig. 1). The corpus allatum was also slightly larger in solitary individuals, while there was no apparent difference in the size of the corpus cardiacum. The larger size of the prothoracic gland in solitary individuals suggested the experiment of extirpating part of this glandular tissue, leaving *in situ* an amount approximately equal to that found in gregarious animals. The results of preliminary experiments of this kind, performed on nine pairs of *Schistocerca* hoppers, have substantiated the validity of this approach to the problem of colour change in locust phases. The hoppers were reared in isolation from hatching and throughout the course of the experiment. Soon after the moult to the fourth instar, hoppers were matched in pairs of the same sex and coloration. In one of each pair, three-quarters of the prothoracic gland on one side was then removed. The other animal of the pair was subjected to a blank operation and served as a control. Before the operation the animals were green in colour, with varying small amounts of blackish-brown pattern. After the moult, to the fifth instar, the hoppers that had been deprived of a portion of the prothoracic gland had developed extensive areas of black pigmentation, similar to that of gregarious hoppers, while the background colour had changed from green to yellow or creamy-pink, similar to that found in gregarious hoppers, or in those which are becoming gregarious[4]. The

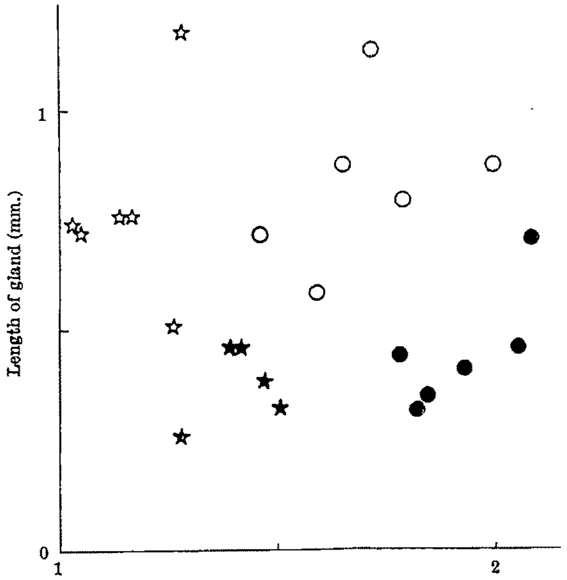

Fig. 1. The size (greatest antero-posterior measurement) of the prothoracic gland in solitary and gregarious adults of *Schistocerca gregaria* killed within 6 hr. of fledging. Open symbols, solitary animals ; solid black symbols, gregarious animals ; stars, males ; circles, females

control hoppers remained green in colour with only small amounts of black pattern.

These results seem to provide evidence that secretions of the prothoracic gland play a part in governing the metabolism of the pigments and, in balance with other hormones, in controlling changes in colour from the solitary to the gregarious phase in locusts. Further work is in progress relating to the possible role of hormone balance in other aspects of locust biology, especially behaviour.

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Experimental Hybridization of *Aedes* (*Stegomyia*) *aegypti* (L.) with *A.* (*S.*) *simpsoni* (Theobald)

Hybridization between *Aedes* (*Stegomyia*) *aegypti* (L.) and the morphologically very distinct[1] *A.* (*S.*) *albopictus* Skuse has been a source of keen controversy since the initial work of Toumanoff[2] and Hoang Tich Try[3]. These results and those of Downs and Baker[4] and Bonnet[5] have been fully discussed by Kitzmiller[6] and Mattingly[1]. Offspring were only obtained in one direction in each case, though the successful direction varied with the strains used. Inheritance was completely maternal in the F_1 and subsequent generations with the exception of two single individuals of paternal resemblance. Suggested explanations involve sperm-entry induced parthenogenesis[4], cytoplasmic inheritance[7] and chromosomal mechanisms[1]. Recently, Woodhill[8] has repeated the crosses on a much larger scale with stringent precautions to avoid contamination. From nearly 50,000 eggs only a single adult was obtained, intermediate in characters between the two parents, indicating a certain degree of chromosomal compatibility.

A. (*S.*) *simpsoni* (Theobald) is morphologically much closer to *A. aegypti* than is *A. albopictus*, widespread in the Ethiopian region, frequently occurring together with *A. aegypti* and an important vector of yellow fever; it is nevertheless of distinct habits, breeding almost exclusively in plant axils while *A. aegypti* does so only rarely. *A. simpsoni* used in the present work were the F_1 from wild-laid eggs collected at Ganda, Kenya, by members of the Medical Department, to whom I am indebted. The adults reared in London were hand-mated[9] (normal mating does not occur under usual laboratory conditions); 8 out of 10 mated females oviposited normally and 7 of the egg batches were fully fertile. The *A. aegypti* used were a heterogeneous F_2 from larvæ collected at Port Swettenham, Malaya, through the kindness of Dr. R. H. Wharton of Kuala Lumpur. Mostly var. *queenslandensis*[10], they were largely heterozygous for the genes for white abdomen[11] and white-spot[12] and mated naturally in ½-pint paper cups.

In making the crosses the females were first given a blood meal and then hand-mated[9] with males of the other species. The mated females were confined in a 3 × 1 in. tube over a moist pad on which they oviposited. Hatching was effected by adding deoxygenated water to these tubes.

18 out of 19 female *A. simpsoni* mated with male *A. aegypti* laid egg batches, from 4 of which 9 larvæ hatched. 7 out of 17 female *A. aegypti* of the reciprocal mating laid egg batches, none of which hatched. 3 of the larvæ died before reaching the 4th instar; the remainder, from 3 different matings, were reared to give 2 identical adult males and 4 identical adult females in perfect condition. A male and female are illustrated in Fig. 1 together with examples of the parental species.

These hybrids have some characters typical of one parent and some of the other, with a few intermediate between the two. Characters typical of the maternal parent *A. simpsoni* include the spines at the base of the thoracic tufts of the 4th-stage larvæ, absence of any scales on the clypeus, the anterior scutal spot large and broad-scaled and the supra-alar spot large.

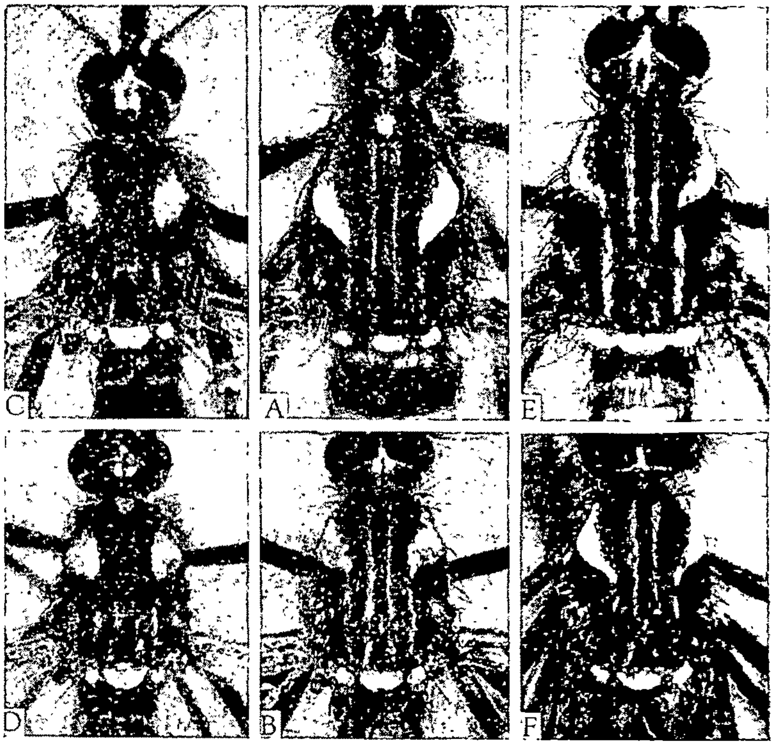

Fig. 1. A hybrid between the mosquitoes *Aedes* (*Stegomyia*) *aegypti* (L.) and *A.* (*S.*) *simpsoni* (Theobald). The thoraces of *A*, ♀ hybrid; *B*, ♂ hybrid; *C*, ♀ and *D*, ♂ maternal species *A. simpsoni*; *E*, ♀ and *F*, ♂ paternal species *A. aegypti*

Characters typical of *A. aegypti* include the median paired yellow lines on the scutum, the front femora with anterior pale stripes on the basal halves and the mid femora with anterior pale stripes and no median spots. Intermediate characters include the larval pecten-spines which have large basal denticles as in *A. aegypti* but are bilaterally symmetrical; the anterolateral scutal spots are a compromise between ovoid in *A. simpsoni* and crescentic in *A. aegypti* (Fig. 1), the 4th hind tarsal, pale on the basal two-thirds in *A. aegypti* and entirely dark in *A. simpsoni*, is pale on the basal one-third.

These hybrids represent about 1 per cent of the eggs laid in the particular cross, compared with not more than about 0·06 per cent in the case of Woodhill's hybrid[8]. This seems reasonable in view of the closer affinity between the species in the present instance, although it is possible that hand-mating may result in better insemination than natural mating. The uniformity among the 4 female and 2 male hybrids suggests that chromosomal combination, though rare, is constant in its effect. The absence of offspring in the reciprocal cross must remain inconclusive until more successful matings have been made. The results support Woodhill's[8] suggestion that the earlier results with *A. albopictus* might be due to contamination by stray eggs. True hybridization when it occurs between *Stegomyia* species probably results in a blending inheritance with a frequency of occurrence indicative of the extent of evolutionary divergence within the subgenus.

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VIROLOGY

Use of a 'Mixed Hæmadsorption' Method in Virus-infected Tissue Cultures

THE principle of mixed agglutination[1] has been used by Högman[2] to indicate the presence of blood-group antigens in tissue culture. In this system the cultured cells are sensitized with antibodies directed towards antigens of the cell surface. Thereby the cells acquire capacity to adsorb homologous red cells. Vogel and Shelokov[3] have shown that certain viruses can be located in tissue cultures by their capacity to adsorb sensitive erythrocytes, the so-called hæmadsorption test. Two pre-requisites for this system are: (1) that virus antigen be available on the surface of the infected tissue cells; (2) that this antigen fix the erythrocytes.

This communication outlines some attempts to apply a similar system to indicate virus antigens present on tissue culture cell surfaces, but which do not fix red cells spontaneously. Initially cells infected with vaccinia virus were chosen as an experimental model because viral antigen located on the cell surface is assumed to exist (spontaneous hæmadsorption with chicken erythrocytes). The sheep red cells used in the present experiments are not spontaneously adsorbed on vaccinia-infected cells unless treated as described here.

Table 1. 'MIXED HÆMADSORPTION' IN VACCINIA VIRUS-INFECTED TISSUE CULTURES TREATED WITH DIFFERENT CONCENTRATIONS OF ANTI-VACCINIA RAT SERUM.

Positive hæmadsorption graded as + + +, + + or +; – indicates no hæmadsorption

	Indicator cells	Dilution of rat serum added to the tubes					
		Anti-vaccinia serum					Normal rat serum
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-2}
Vaccinia virus-infected tubes	Positive cells	+ + +	+ +	+	(+)	–	–
	Negative cells	–					
Normal tube	Positive cells	–					

Positive cells, tanned sheep erythrocytes coated with 100 γ/ml. rat globulin and rabbit anti-rat serum 1/1,000. Negative cells, as above except anti-rat serum substituted for normal rabbit serum diluted /1,000.

Viral antibodies obtained from either rats or rabbits were fixed to virus-infected tissue culture cells and detected by the addition of sheep red cells coated with antiserum to either rat or rabbit sera, depending on the source of the viral antibodies.

The vaccinia virus was grown in monolayer cultures of monkey kidney tissue. Tubes with moderate virus degeneration were washed three times with the culture medium used (usually 0·5 per cent lactalbumin hydrolysate buffered with Hanks solution) and drained. Then 0·2 ml. of rabbit or rat antivaccinia serum was added in different dilutions to the virus cultures (Table 1). The tubes were kept at room temperature for 1 hr. in a slightly inclined position (about 10°) and were then carefully washed three times. Following this, the indicating system, sheep red cells coated with the specific anti-γ-globulin serum on their surface, were added to the tubes. It was established that if antibodies were fixed to the tissue culture, then, the red cells would agglutinate around the tissue cells as shown in Fig. 1.

The specific species antiserum, prepared in horse (anti-rabbit serum) or rabbit (anti-rat serum), can be fixed to the sheep red cells in two ways. One method consists of coating tanned sheep cells[4] with, for example, rat γ-globulin 50–100 γ/ml. Rabbit anti-rat serum was then allowed to combine with the coated sheep cells in a dilution of the serum (1/1,000) that produced agglutinates of moderate size.

The other method differs only in that the sheep cells were not tanned, but were sensitized with rabbit immune (anti-sheep) serum, and then allowed to combine with anti-rabbit sera, of which a suitable dilution was also chosen.

The result of a representative experiment with vaccinia virus-infected cultures is shown in Table 1. It can be seen that anti-viral serum when added to vaccinia-infected cultures in dilutions up to 1/10,000 will absorb 'positive' red cells. (The HI-titre of this serum measured in the conventional way against

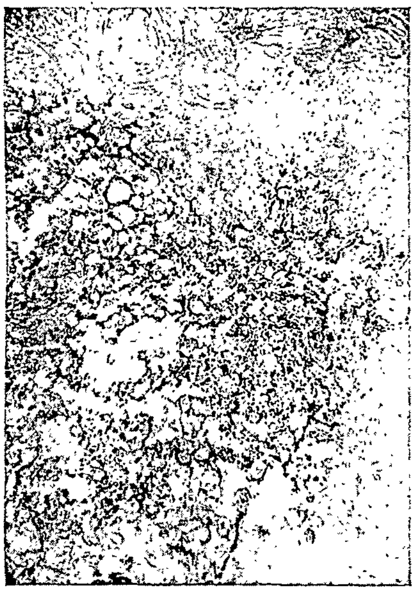

Fig. 1. Photomicrograph showing positive 'mixed hæmadsorption' on a vaccinia virus degeneration focus. Tissue culture from the experiment summarized in Table 1

4 units of vaccinia hæmagglutinin was 1/512, and the 50 per cent plaque neutralizing titre was about 1/3,000). The specificity of the system is indicated by the negative outcome of the controls given in Table 1.

Experiments have shown that essentially the same results are obtained in the vaccinia virus system when the indicating cells are prepared according to the second method, that is, sheep erythrocytes covered with, for example, rabbit anti-sheep serum and followed by anti-rabbit serum.

This latter system has also been tried with success in tissue cultures infected with measles or canine distemper virus as shown in Table 2.

The hæmadsorption on virus-infected foci was massive, and clearly visible macroscopically. When viewed microscopically, it was found that the red cells clump on the edges of the infected cells. In addition, when a very strong hæmadsorption occurred, the red cells covered the entire cell surface, presumably because the binding was strong enough to prevent the red cells from being washed away.

Attempts have been made to use this method of detection for other virus – tissue culture systems, such as those of polio and herpes simplex, but the results have been negative to date. This may be due to a difference in the release of the viruses from the infected cells as compared with that of the vaccinia and distemper viruses.

Table 2.* 'MIXED HÆMADSORPTION' IN TISSUE CULTURES INFECTED WITH CANINE DISTEMPER VIRUS. HÆMADSORPTION GRADED AS IN TABLE 1

	Indicator cells	Rabbit serum added to tubes: Anti-distemper serum dilution 1/5	
		Anti-distemper serum dilution 1/5	Normal serum dilution 1/5
Distemper virus infected tubes	Positive cells	+ + +	–
	Negative cells	–	–
Normal tubes	Positive cells	–	–
	Negative cells	–	–

Positive cells, washed sheep red cells coated with rabbit amboceptor (anti-sheep erythrocyte serum produced in rabbit) diluted 1/1,000 and with horse anti-rabbit serum diluted 1/500. Negative cells, as above, but anti-rabbit serum substituted for normal horse serum 1/500.

* This experiment was performed in co-operation with Dr. Erling Norrby.

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New Herbaceous Test-Plants for Study of the Infectious Degeneration of Grape Vines

RECENT progress achieved in the transmission of the virus diseases of the grape vine to herbaceous test-plants, induces us to disclose further results[1].

In a series of inoculations made on 1,026 plants, of 48 different species, using various techniques, positive results were obtained in the following tests: (1) by mechanical inoculation of sap, (*a*) on *Impatiens holstani* (*I. holstii* × *I. sultani*) (one plant out of 48 of which 17 were in artificial light and 31 in greenhouse; experiments made during various periods of the year) with leaves of the clone of grape vine Alfa *T.A.*, (*b*) on alfalfa (two plants out of six) with roots of the clone 34*C*/59; (2) by chip-budding from young shoots, (*a*) on *I. holstani* (one plant out of 18) with the clone 101*P*, (*b*) on *Lycopersicum esculentum* (4 plants out of 30) with the clone 16*C*/59, (*c*) on the hybrid *L. esculentum* × *L. pimpinellifolium* (7 plants out of 13), with the clone 1*C*/59; (3) by dodder, on *Ammi majus* (10 plants out of 19) in five experiments made from February to June, with the clones of grape vine 3*CA*/59, *V*1*F*22*V*1, and 101*P*.

For the transmission, material was used from 32 different clones of diseased grape vines. Results were positive with only seven of them, coming from three different districts and showing at the same time malformation of the shoots and of the leaves, mosaic and yellow mosaic. These are the only symptoms noticed so far in Italy on the 'infectious degeneration' of the grape vine.

The following are the symptoms observed on the herbaceous test plants: *I. holstani*—severe malformation and twisting of the leaves, accompanied by mosaic and yellow streaks (after 4 months, by sap inoculation and after 3 months, by chip-budding); Alfalfa—mild mosaic or yellowish spots with sharp or indefinite edges on the leaves (after about 30 days); *L. esculentum* and *L. esculentum* × *L. pimpinellifolium*—vein-clearing and leaf malformation (after 30–50 days); *A. majus*—malformation and twisting of the leaves with light green or necrotic patterns along the margin (after 10–30 days).

We deduced that viruses were transmitted from the vine to herbaceous plants since: (*a*) with *I. holstani*, although the percentage of infections is very slow, we had the same symptoms using two different methods of transmission and working during different periods of the year; (*b*) with *A. majus* the percentage of infection is quite high, and, moreover, we had the transmission, only by dodder, on the same species (after 17 days); (*c*) the same clone of grape vine gave positive results on *A. majus* and *I. holstani*; (*d*) transmission made in different periods of the year gave the same symptoms; (*e*) on *L. esculentum* × *L. pimpinellifolium* the symptoms were identical both in the inoculation from the vine and from diseased *I. holstani*.

From *I. holstani* the isolate has so far been transmitted to tobacco (varieties White Burley and Samsun) and to *Petunia*, by sap and by *Myzodes persicae*; to *Nicotiana glutinosa, Datura stramonium, Vigna sinensis* and *I. holstani* by sap. The percentage infection in the transmission from these species to the same species or to the other species that gave positive results in the inoculation from *I. holstani*, is higher than in the transmission from *I. holstani*.

We are trying to transmit the isolates from the herbaceous plants to grape vine. For this work we use symptomless grape vines, selected during three years and belonging to varieties that appeared to be very receptive to the 'infectious degeneration' in previous experiments on transmission by grafting from vine to vine.

Other work in progress is the identification of the isolates.

No rod-shaped virus particles were seen in a series of observations, using the electron microscope, with exudates obtained by Johnson's method and with drops prepared with Brandes's dipping method both with diseased grape vines (leaves, shoots and roots) and with infected herbaceous plants.

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GENETICS

Gene Action in the X-chromosome of the Mouse (*Mus musculus* L.)

Ohno and Hauschka[1] showed that in female mice one chromosome of mammary carcinoma cells and of normal diploid cells of the ovary, mammary gland and liver was heteropyknotic. They interpreted this chromosome as an X-chromosome and suggested that the so-called sex chromatin was composed of one heteropyknotic X-chromosome. They left open the question whether the heteropyknosis was shown by the paternal X-chromosome only, or the chromosome from either parent indifferently.

The present communication suggests that the evidence of mouse genetics indicates: (1) that the heteropyknotic X-chromosome can be either paternal or maternal in origin, in different cells of the same animal; (2) that it is genetically inactivated.

The evidence has two main parts. First, the normal phenotype of XO females in the mouse[2] shows that only one active X-chromosome is necessary for normal development, including sexual development. The second piece of evidence concerns the mosaic phenotype of female mice heterozygous for some sex-linked mutants. All sex-linked mutants so far known affecting coat colour cause a 'mottled' or 'dappled' phenotype, with patches of normal and mutant colour, in females heterozygous for them. At least six mutations to genes of this type have been reported, under the names mottled[3,4], brindled[3], tortoiseshell[5], dappled[6], and $26K$ [2]. They have been thought to be allelic with one another, but since no fertile males can be obtained from any except, in rare cases, brindled, direct tests of allelism have usually not been possible. In addition, a similar phenotype, described as 'variegated', is seen in females heterozygous for coat colour mutants translocated on to the X-chromosome[7,8].

It is here suggested that this mosaic phenotype is due to the inactivation of one or other X-chromosome early in embryonic development. If this is true, pigment cells descended from cells in which the chromosome carrying the mutant gene was inactivated will give rise to a normal-coloured patch and those in which the chromosome carrying the normal gene was inactivated will give rise to a mutant-coloured patch. There may be patches of intermediate colour due to cell-mingling in development. The stripes of the coat of female mice heterozygous for the gene tabby, *Ta*, which affects hair structure, would have a similar type of origin. Falconer[9] reported that the black regions of the coat of heterozygotes had a hair structure resembling that of the *Ta* hemizygotes and homozygotes, while the agouti regions had a normal structure.

Thus this hypothesis predicts that for all sex-linked genes of the mouse in which the phenotype is due to localized gene action the heterozygote will have a mosaic appearance, and that there will be a similar effect when autosomal genes are translocated to the X-chromosome. When the phenotype is not due to localized gene action various types of result are possible. Unless the gene action is restricted to the descendants of a very small number of cells at the time of inactivation, these original cells will, except in very rare instances, include both types. Therefore, the phenotype may be intermediate between the normal and hemizygote types, or the presence of any normal cells may be enough to ensure a normal phenotype, or the observed expression may vary as the proportion of normal and mutant cells varies, leading to incomplete penetrance in heterozygotes. The gene bent-tail, *Bn* [10], may fit into this category, having 95 per cent penetrance and variable expression in heterozygotes. Jimpy, *jp*, is recessive, suggesting that the presence of some normal cells is enough to ensure a normal phenotype, but Phillips[11] reported one anomalous female which showed the jimpy phenotype. Since it showed the heterozygous phenotype for *Ta* this animal cannot be interpreted as an XO female; it is possible that it represents an example of the rare instance when by chance all the cells responsible for the jimpy phenotype had the normal gene inactivated.

The genetic evidence does not indicate at what stage of embryonic development the inactivation of one X-chromosome occurs. In embryos of the cat, monkey and man sex-chromatin is first found in nuclei of the late blastocyst stage[12,13]. Inactivation of one X at a similar stage of the mouse embryo would be compatible with the observations. Since an XO female is normally fertile it is not necessary to postulate that both X-chromosomes remain functional until the formation of the gonads.

The sex-chromatin is thought to be formed from one X-chromosome also in the rat, *Rattus norvegicus*[14], and in the opossum, *Didelphis virginiana*[15]. If this should prove to be the case in all mammals, then all female mammals heterozygous for sex-linked mutant genes would be expected to show the same phenomena as those in the mouse. The coat of the tortoiseshell cat, being a mosaic of the black and yellow colours of the two homozygous types, fulfils this expectation.

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Genetic Basis for Graft-against-Host Immunological Reactions between Two Inbred Lines of Chickens

IT has been established that the enlargement of the embryonic spleen which follows the injection of adult chicken blood into chick embryos is due, at least in part, to a proliferation of cells derived from the injected blood[1,2]. Cock and Simonsen[3] have shown that virtually no splenic enlargement occurs when the blood-donor and embryonic recipients are members of the same inbred line of chickens. The phenomenon of splenic enlargement seems to be fundamentally immunological in nature, and due to donor cells proliferating in response to those host antigens which differ from any in the donor.

It should be possible, by injecting blood from adult birds from one parental line into F_2-generation and back-cross embryos between two inbred lines, to analyse the antigenic difference of the other parental line. Assuming that the antigens of the parental lines are dominantly determined and that they segregate in crosses between the lines in a Mendelian fashion, then a proportion of F_2-generation embryos, and of embryos of the back-cross to the parent of the blood-donating line, will be expected to lack those genes which determine antigens occurring exclusively in the non-blood-donating line. The proportion of embryos which lack these genes will be $(\frac{1}{4})^n$ in the F_2-generation and $(\frac{1}{2})^n$ in the back-cross, where n is the number of pairs of genes involved. Since splenomegaly will occur only when the recipient embryo possesses antigens foreign to the donor cells, these are also the proportions of embryos in the respective crosses which will show no splenic enlargement. All F_1 hybrids and embryos of the back-cross to the parent of the non-blood-donating line will receive the genes which determine antigens peculiar to the non-blood-donating line and all these embryos will therefore show splenic enlargement. Thus, an estimate of the value of n can be obtained by observing the proportion of F_2 and back-cross embryos which show no splenic enlargement. The genetic basis for this method is essentially similar to that used in analysing histo-compatibility differences between inbred strains of mice using tumour transplantation[4], and skin transplantation[5].

The method outlined above has been used to observe antigenic differences between the Reaseheath C- and I-inbred lines of White Leghorns[6]. Both lines have been brother-sister mated annually for more than twenty generations. Chick embryos were injected intravenously at 15 days of incubation with 0·1 ml. of citrated blood from I-line cocks, and killed 4 days later and their spleens weighed. The embryos injected were: C-line embryos, I-line embryos, the F_2-generation ($CI \times CI$ and $CI \times IC$), and the back-crosses ($C \times CI$ and $C \times IC$, $I \times CI$ and $I \times IC$). In designating the crosses, the male parent is stated first. Two I-line cocks were used as blood-donors to the F_2 and back-cross embryos and experiments with each donor were performed twice. Only a small number of C embryos were available for injection, but the marked splenic enlargements obtained indicate that C tissues are antigenic to I cells. A small number of I-line embryos injected with I-blood showed no splenomegaly. So far, we have had no F_1-hybrids to test, but Cock and Simonsen[3] have obtained splenic enlargement after injecting I-blood into newly hatched $C \times I$ chicks. The patterns of spleen weights obtained after injecting I-blood into F_2 and back-cross embryos were similar in each of the four experiments, and the results have been pooled in Fig. 1. The proportions of spleens in the different crosses showing no enlargement are shown in Table 1, and these results are compared with the theoretical frequencies expected for 1, 2 and 3 pairs of dominant genes determining antigens peculiar to the C-line. The proportions best fit the expectancy for one pair of genes, and the results suggest, therefore, that the C-line carries one antigen (capable of stimulating splenic enlargement) which is absent from the I-line. The results also fit the expectancy if the C-line possesses one dominantly determined antigen and one recessively determined antigen foreign to the I-cells. In this case, the proportion of unenlarged spleens in the F_2-generation would be 18·75 per cent (the proportion falls in a series $(\frac{3}{4})^{n_1} \times (\frac{1}{4})^n$, where n_1 is the number of pairs of recessive genes and n is the number of pairs of dominant genes). The proportion of unenlarged spleens in the back-crosses would remain unchanged. However, until we have other evidence for the

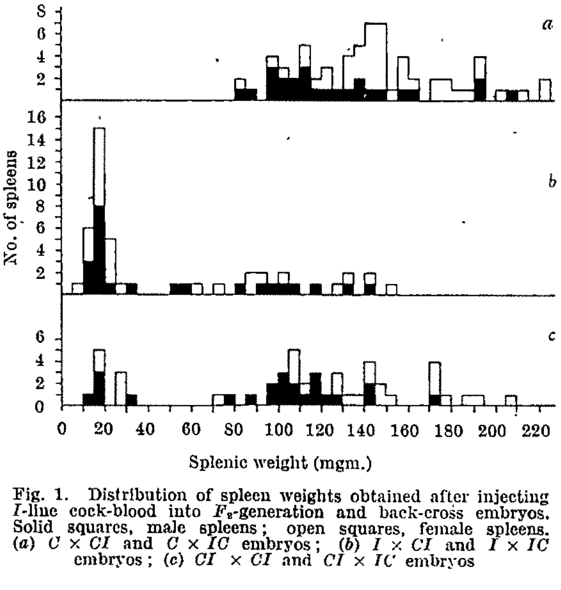

Fig. 1. Distribution of spleen weights obtained after injecting I-line cock-blood into F_2-generation and back-cross embryos. Solid squares, male spleens; open squares, female spleens. (*a*) $C \times CI$ and $C \times IC$ embryos; (*b*) $I \times CI$ and $I \times IC$ embryos; (*c*) $CI \times CI$ and $CI \times IC$ embryos

Table 1

Pairs of genes	Percentage of unenlarged spleens in F_1	Percentage of unenlarged spleens in F_2	Percentage of unenlarged spleens in back-crosses	
	CI	*CI* × *CI* *CI* × *IC*	*I* × *CI* *I* × *IC*	*C* × *CI* *C* × *IC*
Experimental results	no data	20·4 } $\chi^2 = 0·55$	59·2 } $\chi^2 = 1·65$	0
1	0	25 } N.S.	50 } N.S.	0
2	0	6·25	25	0
3	0	1·56	12·5	0

Comparison between experimental segregation results and theoretical results expected for 1, 2 and 3 pairs of genes determining antigens present in *C*-line birds, but absent from *I*-line birds. The experimental data do not differ significantly from the values expected for one pair of genes.

Table 2. DIFFERENCES IN MEAN WEIGHTS OF ENLARGED SPLEENS FROM MALE AND FEMALE EMBRYOS

Cross	No. of embryos	Mean splenic weight (mgm.)		Values of *t* (from logarithm of spleen weights)
		Male	Female	
C × *CI* and *C* × *IC*	68	128·3	151·6	2·97 $P < 0·01$
I × *CI* and *I* × *IC*	20	98·2	105·7	0·55 $P > 0·5$
CI × *CI* and *CI* × *IC*	39	114·1	145·5	3·33 $P < 0·01$

occurrence of recessively determined antigens in the chicken, we are inclined to favour the former interpretation of the segregation results.

The spleens of uninjected control embryos ranged in weight from about 10 to 20 mgm. The slight enlargements (up to 35 mgm.) of some spleens which have been included in the 'no enlargement' groups in Table 1 may be attributable to the presence of additional weak antigens in the *C*-line. On the other hand, the slight antigenic diversity which exists within the *I*-line[3] must also contribute to these small enlargements.

The different patterns of distribution of the enlarged spleens in the three crosses suggest that the homozygotic or heterozygotic state of the gene determining the *C* antigen influences the spleen weight. That is, a gene dosage effect appears to be involved, with embryos homozygous for the *C* gene providing a stronger antigenic stimulus to *I* cells (and therefore a larger spleen) than embryos heterozygous for the gene. However, the weight of the spleen may probably not be taken as directly indicative of the homozygosity or heterozygosity of the embryo concerned, since there is evidence in two of the crosses that the sex of the recipient influences the spleen weight. The mean weights of the enlarged female spleens from *C* × *CI* and *C* × *IC* and from *CI* × *CI* and *CI* × *IC* embryos are significantly greater than the mean weights of enlarged male spleens (Fig. 1 and Table 2). The reason for this sex difference is not known; a similar effect has been observed by J. B. Solomon (personal communication). In addition to the sex and gene dosage effects, the variability in weight of the enlarged spleens will also be increased by residual antigenic diversity within both the *C*- and *I*-lines.

These results will be reported in more detail elsewhere, together with results obtained by injecting *C*-line blood into the *C*- and *I*-crosses. Similar analyses of differences between the *C*- and *W*-inbred lines will also be described.

We wish to thank Dr. P. A. Gorer, who kindly commented on the manuscript. Our thanks are also due to Miss R. Ford for technical assistance and to the Agricultural Research Council for a grant which supported this work.

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MISCELLANY

Extra-Terrestrial Radio Transmissions

WE are witness again to a surge of interest in, and speculation about, extra-terrestrial radio transmissions which had an earlier flare in the late 'twenties[1]. The favourable change in climate for the expression of such ideas since the turn of the century has been of particular interest to me. In 1899, Nikola Tesla established an experimental station at Colorado Springs, where he would be free to pursue certain electrical investigations, unrestrained by the limits imposed by his New York City laboratory. In an article by him[2], published after his return to New York in the following year, he presented a rather thrilling account of signals received while alone one night in the experimental station:

"The changes I noted were taking place periodically, and with such a clear suggestion of number and order that they were not traceable to any cause then known to me. I was familiar, of course, with such electrical disturbances as are produced by the sun, Aurora Borealis and earth currents, and I was as sure as I could be of any fact that these variations were due to none of these causes. . . . It was some time afterward when the thought flashed upon my mind that the disturbances I had observed might be due to intelligent control. . . . The feeling is constantly growing on me that I had been the first to hear the greeting of one planet to another. . . . I announced to the Red Cross Society, when it asked me to indicate one of the great possible achievements of the next hundred years, that it would probably be the confirmation and interpretation of this planetary challenge to us."

I have acquired the original of Tesla's statement to the Red Cross Society, dated Christmas (January 7), 1900, which I believe is the first expression of the possibility of radio communication with other planetary communities. The announcement was harshly criticized. This was particularly unfortunate, since, because of such attacks, Tesla never produced detailed information concerning the results of his intended investigations and the chance reception of signals.

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Minneapolis 14, Minnesota.

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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, April 24

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. J. A. Steers and others : "Coastal Cliffs".

BRITISH NUCLEAR ENERGY CONFERENCE (at the Federation of British Industries, 21 Tothill Street, London, S.W.1), at 5.30 p.m.—Mr. A. M. Weinberg (Oak Ridge National Laboratory): "The Development of Fluid Fuel Reactors".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. G. W. Green: "Development of City Distribution Systems".

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. G. Gee, F.R.S.: "Polymer Science in the University" (Jubilee Memorial Lecture).

Tuesday, April 25

INSTITUTION OF AGRICULTURAL ENGINEERS (at the Piccadilly Hotel, London, W.1), at 10.30 a.m.—Annual General Meeting. 11.15 a.m.—Conference. 7 p.m.—Dinner.

BRITISH BIOPHYSICAL SOCIETY (in the Physics Department, Birkbeck College, Malet Street, London, W.C.1), at 11 a.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. D. M. Mackay : "Simulation of Intelligence".

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. S. Lodge: "The Rheology of Concentrated Polymer Solutions".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. A. H. Pople: "Instrumentation in the Iron and Steel Industry".

Wednesday, April 26

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Sir John Cockcroft, O.M., F.R.S.: "The Training of Scientists for Industry" (Trueman Wood Lecture).

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 3 p.m.—Annual General Meeting. Prof. S. E. Hollingworth: "The Climatic Factor in the Geological Record" (Presidential Address).

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. H. Franklin and Mr. J. Rhodes: "Data Transmission".

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—Annual General Meeting. Mr. R. L. Coet: "The Movement of Brake Bank, South of Ramsgate".

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium on "Electronic Counting Techniques".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Annual Report of the Council.

Wednesday, April 26—Friday, April 28

BRITISH INSTITUTE OF RADIOLOGY (at the Central Hall, Westminster, London, S.W.1)—Twenty-second Annual Congress. Exhibition of Equipment and Materials used in Medical Radiology and Radiotherapy.

Thursday, April 27

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Meeting for the election of Foreign Members, followed by Mr. D. Lowe: "The National Vegetable Research Station".

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (joint meeting with the ROYAL COMMONWEALTH SOCIETY, at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—The Right Hon. The Earl De La Warr, P.C., G.B.E.: "The Work of the Commonwealth Societies".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Prof. F. Lynen (Munich): "Biological Pathways to the Carbon Skeleton in Natural Products".* (Further lectures on May 1 and 2.)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. R. F. Woolmer: "Medical Electronics" (Fifty-second Kelvin Lecture).

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Mr. H. J. Bunker: "Recent Microbiological Problems in Industry".

Friday, April 28

BRITISH PSYCHOLOGICAL SOCIETY, OCCUPATIONAL PSYCHOLOGY SECTION (in the Department of Psychology, Birkbeck College, Malet Street, London, W.C.1), at 1 p.m.—Miss Lisle Klein: "Some Implications of Rationalized Production".

WORSHIPFUL SOCIETY OF APOTHECARIES OF LONDON, FACULTY OF THE HISTORY OF MEDICINE AND PHARMACY (at the Apothecaries' Hall, Black Friars Lane, Queen Victoria Street, London, E.C.4) at 4.30 p.m.—Second Annual General Meeting. 5 p.m.—Sir Geoffrey Keynes: "Timothy Bright" (Gideon de Laune Lecture).

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. Edwin H. Land (U.S.A.): "Colour in the Natural Image".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned :

LECTURER or ASSISTANT LECTURER IN AGRICULTURAL BOTANY—The Registrar, The University, Leeds 2 (April 28).

ASSISTANT LECTURER, Grade B, IN PHYSICS, to teach in the first instance up to General Certificate of Education "A" level, and Part 1 B.Sc. General Degree—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (April 29).

LECTURER (with a special interest in crop husbandry) IN THE DEPARTMENT OF AGRICULTURE—The Secretary, The University, Edinburgh (April 29).

LECTURER or ASSISTANT LECTURER IN BACTERIOLOGY—The Registrar, The University, Leeds 2 (May 1).

LECTURER (preferably, though not necessarily, with some industrial experience and interested in teaching and research in one or more of the following fields: electrical machines; electrical power; computers, or electronics) IN ELECTRICAL ENGINEERING—The Registrar, The University, Nottingham (May 5).

READER IN APPLIED MATHEMATICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (May 5).

ASSISTANT LECTURER (preferably with an interest in mycology) IN BOTANY—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (May 8).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Liverpool 3 (May 10).

LECTURER (with a special interest in mycology, plant pathology or cognate subjects) IN BOTANY—The Secretary, The Queen's University of Belfast, Belfast 7, Northern Ireland (May 10).

PROFESSOR OF ORGANIC CHEMISTRY—The Secretary and Bursar, University College, Dublin (May 15).

ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (May 20).

CHAIR OF ZOOLOGY—The Registrar, University College of Wales, Aberystwyth (May 20).

FRUIT RESEARCH OFFICER (PHYSIOLOGIST) (honours graduate in science or agricultural science, or equivalent qualifications, with relevant postgraduate research experience, preferably in plant physiology or biochemistry) at the new Headquarters Laboratories of the Division of Food Preservation, C.S.I.R.O., North Ryde, Sydney, Australia, to participate in a programme of physiological and biochemical investigations to obtain a better understanding of the ripening of fruit and its behaviour in storage—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 300/298 (May 20).

LECTURER IN ZOOLOGY at Fourah Bay College (University College of Sierra Leone), to specialize in marine work—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 20).

SENIOR FELLOW and a SENIOR RESEARCH FELLOW (preferably with experience in elementary particle physics) IN THE DEPARTMENT OF THEORETICAL PHYSICS, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 26).

LECTURER IN BIOCHEMISTRY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 31).

SENIOR LECTURER IN PSYCHOLOGY; and a SENIOR DEMONSTRATOR IN PSYCHOLOGY, in the Department of Psychology, School of General Studies, Australian National University, Canberra, A.C.T., Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, June 24).

ASSISTANT PROFESSOR (with a Ph.D. or equivalent, and subsequent academic, industrial or research experience) OF METALLURGY—Prof. F. A. Forward, Department of Metallurgy, University of British Columbia, Vancouver, B.C., Canada (June 30).

SENIOR LECTURER (preferably with a degree in pharmacology, physiology or biochemistry) in PHARMACOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

MIDDLEMASS HUNT CHAIR OF TROPICAL HYGIENE—The Registrar, The University, Liverpool (July 1).

ASSISTANT PROFESSOR (preferably with a science degree in zoology or a medical qualification) IN MICROANATOMY, to teach medical and science students and conduct research in embryology; and an ASSISTANT PROFESSOR IN GROSS ANATOMY, at Dalhousie University, Halifax, Nova Scotia, Canada—Prof. R. L. de C. H. Saunders, Canada House, Trafalgar Square, London.

LABORATORY TECHNICIAN (with a good background of hæmatological and allied laboratory technique, and preferably one "A" level science subject) IN THE RADIOISOTOPE DEPARTMENT, Victoria General Hospital, Halifax, Nova Scotia, Canada—The Nova Scotia Civil Service Commission, P.O. Box 943, Provincial Administration Building, Halifax, Nova Scotia.

LECTURER IN PHYSICS—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

LECTURER (with a degree in chemistry or pharmacy) IN PHARMACEUTICAL CHEMISTRY; and a LECTURER (with a degree in either botany or pharmacy) IN PHARMACOGNOSY at Kumasi College of Technology, Ghana—The Assistant Registrar, Kumasi College of Technology, 29 Tavistock Square, London, W.C.1.

LECTURER (with an M.D. or Ph.D. degree) IN THE DEPARTMENT OF PHYSIOLOGY—Prof. M. Beznak, University of Ottawa, Ottawa, Canada.

RESEARCH ASSISTANT IN THE DEPARTMENT OF CHEMISTRY, for the study of high pressure solubility of gases in salt solutions—Dr. Norman O. Smith, Department of Chemistry, Fordham University, New York 58, N.Y., U.S.A.

RESEARCH ASSOCIATE (physics graduate, preferably with some knowledge of geology) IN THE SUB-DEPARTMENT OF GEOPHYSICS of the Department of Geology, to work on some aspect of rock magnetism —The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15.

SENIOR LECTURER (qualified to direct research) IN PHYSICS—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Engelhard Industries, Ltd. Some Industrial Uses of Platinum Metals. Pp. 16. (London: Engelhard Industries, Ltd., 1961.) [251

Department of Scientific and Industrial Research. Fuel Research 1917–1959: a Review of the Work of the Fuel Research Organization of D.S.I.R. Pp. viii+120+4 plates. (London: H.M. Stationery Office, 1960.) 15*s*. net. [251

Planning, Vol. 27, No. 448 (23 January, 1961): Parent's Views on Education. Pp. 1–32. (London: Political and Economic Planning, 1961.) 3*s*. 6*d*. [251

Ministry of Education. Selected Statistics relating to Local Education Authorities in England and Wales, Educational Year 1958–1959. Pp. ii+25. (London: H.M. Stationery Office, 1960.) 3*s*. net. [251

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Bulletin of the British Museum (Natural History). Entomology. Vol. 10, No. 3: A Revision of Two Species Complexes in the Pyraustinae (Lepidoptera, Pyralidae), 1: *Cotachena histricalis* Walker. 2: *Syngamia floridalis* Zeller. By Paul E. S. Whalley. Pp. 96–111+plates 3 and 4. 7*s*. 6*d*. Vol. 10, No. 4: Microlepidoptera from the Soloman Islands: Additional Records and Descriptions of Microlepidoptera collected in the Soloman Islands by the Rennell Island Expedition, 1953–54. By J. D. Bradley. Pp. iii–168+plates 5–19. 30*s*. Vol. 10, No. 5: A Revision of the Genus *Ducetia* Stål (Orthoptera: Tettigoniidae). By D. R. Ragge. Pp. 169–208. 12*s*. Geology. Vol. 5, No. 2: Post-Ypresian Plant Remains from the Isle of Wight and the Selsey Peninsula, Sussex. By Marjorie E. J. Chandler. Pp. 13–41+plates 4–11. 20*s*. (London: British Museum (Natural History), 1961.) [251

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United States Department of Agriculture. Farmers' Bulletin No. 2151: The Japanese Beetle: How to Control It. Pp. 16 (1 plate). (Washington, D.C.: Government Printing Office, 1960.) 15 cents.

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PHYSICS IN GRAMMAR SCHOOLS

PHYSICS D[illegible] [illegible]

THE recommendations of the Physics Panel of the Science Masters' Association and the Association of Women Science Teachers for new physics syllabuses for grammar schools in Great Britain[1] have three main points of departure from the usual run of school-teaching at the present time. A good deal of material which, on reconsideration, did not appear really fundamental has been discarded. Twentieth-century physics, including some of the most recent developments, has been included both in the Ordinary Level and the Advanced Level recommendations for the General Certificate of Education examinations. There has also been a determined effort to trace the development of the basic concepts as they are understood to-day—matter and energy, particles and waves—and to present the subject to the pupil as a unified study that probes the interchanges and interactions of these concepts from various points of view. They are, as yet, only recommendations; it will take some time before they crystallize into examination syllabuses, and probably still longer for them to establish themselves firmly in the curriculum—assuming that both of these consequences ensue. The Associations are, of course, hopeful that this will happen. If it does not, the chances of establishing an effective system of science teaching, within the next twenty years, seem remote. The recommendations are a 'breakthrough' in the original sense of the term, which means starting an attacking movement by finding an opening in the opposing side's defence—after, in this case, a long spell of safety play on the blind side. This metaphor is appropriate here, because the most enterprising breakthrough will lead nowhere unless it is supported by the rest of the team. Now is the time for all progressive teachers to come to the aid of the Panel.

The opposing side in this case is the aggregate of all the well-known difficulties that beset the teaching of science in schools; the reasons for believing that they can be outflanked will be given shortly. In addition, it must be frankly stated, there is a certain distrust in some university quarters of the capacity of the schools to manage anything except teach pupils to pass examinations. Views that have been expressed in this sense cover the whole range from deploring the efficiency of school instruction that anticipates most of the first two years of a university course, to writing off as a total loss most of what has been inadequately done at school and having to start again from scratch. To the university lecturers who may well say that the suggested new syllabus is adding to the factual knowledge milling around unco-ordinated in the undergraduate mind, and that they would prefer, instead of a nodding acquaintance with nucleons, a clear capacity to distinguish between an ampere and a volt, one would counsel patience. The recommendations are on their side all the way. For the whole object is to present physics in the middle school as part of the general education of all pupils, in a way that can be grasped; and to continue with advanced work for the future specialist in the same spirit, concentrating on the matter that is fundamental and can be readily understood. As Mr. J. L. Lewis has pointed out[2], the great beauty about studying radioactivity experimentally is the immense scientific content that can be extracted with very simple mathematics; and this is the sort of bias that the new syllabus has. There is a good chance that pupils may be really educated while they are learning, as well as passing an examination at the end of the process.

A good chance: But is it the best chance ? It has been said that, in aim, the recommendations are to some extent at variance with those of the Ministry of Education Pamphlet No. 38, which presents the study of science as a voyage of discovery[3]. Is the offering of facts and the establishment of modern concepts by lecture and film and sophisticated apparatus a substitute for simple first-hand investigation with primitive equipment ? Of course it is not. When teachers are as familiar with the new subject-matter themselves as they are with those topics which experience has shown susceptible to fruitful discovery-voyages (and there is not as wide a repertoire of these as one might think), the right methods will be developed to fit the material. There is no necessary incompatibility; it can be the best chance if we make the best of it.

The first part of the recommendations, covering the basic five-year grammar school course to be taken by all pupils, deals with fundamental ideas of mass and motion and force, the properties of solids and fluids, mechanical energy and heat energy, light and vision, vibrations and waves, electrical energy, atomic structure, and nuclear energy. It omits much that is of purely mathematical interest, such as resolution of forces, kinematic equations, problems on the laws of motion, and work on the inverse-square law. The wisdom of these omissions at this stage is apparent when one considers (*a*) the difficulty they cause, and (*b*) the resulting pictures in the young mind when they are eventually mastered. Later, it will be easy and appropriate to explain that there are convenient alternatives to the entirely general and sufficient principle of moments; that $\ddot{x} = f$ can be integrated; that Newton disclosed a little more than the magic formula $P = mf$; and that the inverse-square law is an essential geometrical property relating fields and particles in space, rather than something that works for sums that prove formulæ about magnets that must be learned as homework.

The second part, for the future scientist (and it must be remembered that very few people taking this sixth-form course will go on to do physics at the university, so that this is the general education of the scientist rather than the early specialization of the specialist), differs essentially from the present Advanced Level course chiefly in the emphasis given to vibrations and waves, to alternating current, and to modern physics, including such topics as the wave-particle duality of matter. But a good deal of purely memory work, and matter more appropriate to technology or applied mathematics, has been removed.

In an appendix, the essential ancillary mathematics has been tabulated, with the comment that this is what all scientists should be expected to master, and that it ought to be possible to cover it in two periods a week. It does not look too terrifying, and with the incentive to apply it to some really interesting physics it could be done.

Finally, it might be mentioned that the Associations have in active preparation (by their "Fourth Panel") sixth-form syllabuses for the general education of the non-scientist. Drafts are available for individuals who wish to see them for comment, but not for publication; and they have the same enthusiasm, the same sense of a pressing need, and the same realization of what can be achieved if it is seriously attempted.

We now come to the opposition. First, there is the admitted incapacity of very many physics teachers to branch out suddenly into new fields. The Panel says: "There are parts of the syllabus which some of us have never taught, and which we do not feel competent to teach. Universities and Technical Colleges are already arranging suitable short courses, and it is hoped that the Ministry of Education and local authorities will realize the importance of assisting in bringing teachers up to date". The widespread and regular running of such courses, starting immediately, would seem to be a first essential. It should be as much of a tradition for the science teacher to go back annually to the university as it is for the linguist to take his annual trip abroad, and all universities and places of higher education should participate in this. Next, there is the question of man-power available. The situation here is beginning to improve, and the new syllabuses should add appreciably to the attractiveness of school-teaching; the young graduate at present feels that he will never need to use his advanced knowledge in school work, and that it destines him for research. The realization that he will be extended, that he can still be a physicist as he understands the term, and that there are unlimited opportunities for research in the field of developing teaching methods and demonstrations, should attract a higher proportion of the best people. The co-operation of university physics departments in presenting this picture, which has always been valid to those who really understand what goes on in schools, could be offered with open eyes.

The matter of equipment would seem to require a combined operation. It would be easy, for a sum in the region of £500, to provide a nucleus of apparatus 'off the peg', which would enable a fair range of basic experiments to be done. It certainly exists, and some firms have specialized in apparatus for modern physics teaching. At the same time, many schoolmasters do not think along these lines, and prefer relatively simple home-made apparatus. Whichever way it is approached, the services of a technician will be not a luxury but a necessity; no such apparatus is 'school-proof', and maintenance and safety precautions in any laboratory equipped for proper teaching cannot be looked after by a full-time teacher. The alternative would be, of course, to use film, and film-strip, and film again . . . but teachers, who have taken the trouble to re-learn their subject in order to make it up to date and stimulating, are not going to be fobbed off with the cheap and easy way. For things such as counters and ionization chambers and gamma-ray sources there must, incidentally, be a good deal of equipment in Civil Defence stores that could easily and safely be passed over directly to schools; it seems to be almost a law of Nature that redundant apparatus must first pass to Government surplus dealers, where it stays because nobody can afford to buy it; but there must be some way of circumventing this in a good cause. The combined operation envisaged, however, is something at the highest level. The Ministry of Education, when it is decided whether these recommendations are desirable, should ask the Minister for Science to take over, and to arrange for the design and supply of suitable equipment. If it can be done for the training of Civil Defence wardens, it can be done for the education of the ordinary citizen.

It is difficult to forecast how the recommendations are going to work out in the matter of teaching time; though it is significant that several topics which lend themselves to a range of orthodox practical exercises, or to tedious mathematical proofs, have been omitted. It would appear that the syllabus has been lightened, particularly for the average sixth-former and for those who would at present be taking Ordinary Level physics as a separate subject. It remains, if the scheme is accepted, for the examining bodies and the teachers to interpret it in the spirit in which it has been framed.

[1] *Science and Education: Physics for Grammar Schools.* A Section of Part I of the Science and Education Report issued by the Science Masters' Association and the Association of Women Science Teachers. (Published for the Associations by John Murray, 1961.)

[2] "The Place of Atomic and Nuclear Physics in Modern Teaching", by J. L. Lewis. Paper delivered at a short course for science teachers at University College, London, April 1960.

[3] *Science in Secondary Schools.* Ministry of Education Pamphlet No. 38 (H.M.S.O., 1960).

PRELUDE TO SOCIOLOGY

Charles Booth

Social Scientist. By Prof. T. S. Simey and M. B. Simey. Pp. x+282. (London: Oxford University Press, 1960.) 30*s*. net.

CHARLES BOOTH (1840–1916), a Liverpool merchant and shipowner, who in his spare time and without any institutional or financial assistance surveyed the life and labour of the entire population of working-class London at the end of the nineteenth century, was indeed a heroic figure. Prof. and Mrs. Simey, in their very readable account of his life and work, have made out a good case for thinking that his contribution to the establishment of sociology on an empirical basis has been much undervalued. The mere magnitude of his achievement still leaves one dazed. To survey every single street, every court and block of buildings in the metropolitan area; to check on the resources of every family; to catalogue and describe every industrial enterprise; and in the final survey of religious influences to visit every chapel, church and social agency; to do all this with a handful of collaborators and with no modern methods of data processing was an incredible achievement.

Booth's survey, *Life and Labour of the People of London* (3rd ed. 17 vols. 1902–3) was the pioneer social survey. Nothing quite like it had been carried out before. Within the limits which Booth set himself it gave a factual picture of London working-class life which was vivid, unsentimental, accurate and detailed. The volumes on poverty, which indicated with irrefutable evidence that more than one-third of London's population were at all times more or less in want, changed our social policies, and led ultimately to the introduction of the Welfare State.

This, perhaps, has been recognized by social historians. The Simeys, however, claim more. Booth was also, they hold, a great social scientist, one of the founders of empirical sociology, and it is this aspect of his work that has been unduly neglected. Sociology before Booth was largely deductive. Booth insisted on the need for facts. "The *a priori* reasoning of political economy", he said in a paper to the Royal Statistical Society, "fails from want of reality. At its base are a series of assumptions very imperfectly connected with the observed facts of life. We need to begin with a true picture of the modern industrial organism". In his collection of facts Booth relied on a combination of two things: statistics and first-hand observations. Accurate statistics were essential to get the picture in objective proportion; but alone they could mislead, and first-hand observation was needed to add colour and life. To this end Booth went endless walks to see for himself; lived in cheap lodgings; personally attended hundreds of religious services; and filled endless notebooks with his impressions and observations. There is no doubt an important lesson here, which in a day of more plentiful statistics may be overlooked. Booth, perhaps, had too many facts. He worked before the introduction of sampling methods, and his theoretical apparatus was not strong enough to enable him to make a meaningful selection. Sometimes from the mass of data important working hypotheses and conclusions emerged, which led to practical action, as in the case of old age pensions, for which Booth was largely responsible; at other times Booth ended up with detailed descriptions from which nothing very conclusive could be derived. Some of the ingredients of a true science of society were still lacking in Booth's work. But no science is possible without facts, and Booth demonstrated in a new way and on a new scale what facts meant in the study of society. The Simeys have made out their case. Booth must properly be regarded as one of the notable pioneers of empirical sociology.

L. S. HEARNSHAW

ELASTIC SHELLS

The Theory of Thin Shells

By V. V. Novozhilov. Translated by P. G. Lowe. Edited by Prof. J. R. M. Radok. Pp. xvi+376. (Groningen: P. Noordhoff, Ltd., 1959.) 36 D. fl.: 9.50 dollars.

Proceedings of the Symposium on the Theory of Thin Elastic Shells

Delft, 24–28 August, 1959. Edited by W. T. Koiter. (International Union of Theoretical and Applied Mechanics.) Pp. ix+496. (Amsterdam: North-Holland Publishing Company, 1960.) 55*s*.

THE theory of elastic thin shells has undergone considerable developments in the past few years, and it is good to see two excellent books on the subject, one concerned to present the elements of the theory to a beginner and the other to mark some of the bounds of our present knowledge.

In *The Theory of Thin Shells*, Prof. V. V. Novozhilov develops the linear theory of the stress and displacement of thin shells in terms of the familiar assumptions of the classical Love–Kirchhoff theory, that normals to the middle surface of the shell remain normal after the displacement, and that stresses on surfaces parallel to the middle surface are small. The relevant parts of the geometry of surfaces are developed *ab initio* in a simple and lucid manner, which should be well within the compass of any engineering undergraduate. With this foundation, Prof. Novozhilov is able to present the elastic equations for a general shell in the simplest practicable form, and so to save the reader the trouble of following a number of separate derivations of equations for special shells. In the succeeding development of the theory, special place is given to the method of complex components, which has not been discussed satisfactorily in previous books in English. The later chapters, on membrane theory, cylindrical shells and shells of revolution, contain most of the important standard solutions in these fields, including those for reinforced cylinders. Special mention should be made of the section on the toroidal shell, which brings out the inadequacy of the membrane theory as a first approximation in this problem, and gives results in a reasonably compact form.

In all, this book can be recommended with confidence as a first course on the subject, and should also prove valuable to experienced workers in the field. The translation is very easy to read, and the type-setting is excellent.

For workers carrying out research on the subject, the proceedings of the symposium on the theory of thin elastic shells will be both stimulating and informative. The subjects of the papers were deliberately limited to problems involving either non-linear behaviour or two independent variables, and the volume has a unity of purpose not always found in a symposium. The papers range from a discussion of the basic approximations involved in a first-order theory of shells (W. T. Koiter) to solutions of particular problems of more immediate practical interest, like the "Bending at the Oblique End Section of a Cylindrical Shell" (A. van der Neut). A detailed discussion of the various papers is beyond the scope of this review, and would, in any event, reflect the tastes of the reviewer more than the importance of the contributions. Some of the work, for example, "On the Unsymmetrical Snapping of Shells of Revolution" (E. I. Grigolyuk) or the beautiful experimental results of W. Schell on the buckling of cylindrical shells, will probably become classical in the literature. Other papers, especially those on the general equations of equilibrium or motion, deal with issues which are still controversial, but pave the way for a more mature understanding of the whole subject at some later date.

The publishers are to be congratulated on the rapid and pleasing production of this volume, and the organizers of the symposium on bringing together so much useful work. J. W. CRAGGS

CELLULOSE PULP FROM WOOD

Pulp and Paper

Chemistry and Chemical Technology. By James P. Casey. Vol. 1: Pulping and Bleaching. Second edition, revised and enlarged. Pp. xix+675. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 147*s*.

THE first edition of this work, published in 1952, has proved so valuable to the industry that there is really little need for the author to justify the present edition by stating in his preface that ideas are changing faster than practice. A measure of the rate at which this is occurring may, however, be obtained from the fact that Volume 1 of the first edition, which dealt with pulping and bleaching and paper-making, now becomes two volumes, of which the first is now under review. Thus, there are now approximately 450 pages on pulp and 120 pages on bleaching, as compared with 250 and 70 before, respectively.

The plan of the book follows the same general lines as previously, but naturally developments which have come to the fore in the past ten years receive due attention in their appropriate places. These include continuous pulping methods, chlorine dioxide bleaching, magnesium-base liquor recovery, the removal of bark from trees by chemical methods, hardwood pulping, and whole wood-fibre (particle board) manufacture. The portions of the book which deal with these are valuable summaries of our knowledge of these important subjects existing to date. Numerous literature references are provided throughout (nearly 1,280 in all).

A criticism levelled in review against the first edition was the almost exclusive American outlook, especially in the literature references and in the treatment of those fibres other than wood which are not commonly used in the United States. This still applies. Thus one might well infer that there have been no advances in the manufacture of esparto, rag and straw pulps, since the sections involved are almost unchanged. Indeed the word 'recent' is used as in the first edition to describe work carried out in 1948. The sections on esparto, bamboo and rag are so sketchy as to be of little or no use to those interested in them; this will apply to many readers outside the United States. Furthermore, the erroneous attribution of low recovery in the straw process to the presence of silica (also pointed out in review) recurs in this edition.

However, it is evident that the author is writing primarily for the American reader, and presumably, therefore, sins of omission cannot be held too strongly against him. As a presentation from the fundamental point of view of up-to-date knowledge on the pulping of wood the second edition of this work will undoubtedly command the same high status as its predecessor. JULIUS GRANT

HETEROMETRY

Heterometry

By Prof. Mordechai Bobtelsky. Pp. viii+229. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 42*s*.

THE heterometric method was introduced by the author, Prof. Mordechai Bobtelsky, of the University of Jerusalem, and the present monograph gives details of the varied and unique applications of this new technique in the fields of inorganic, physical and analytical chemistry. The heterometer was developed as an instrument for observing the instantaneous variations in optical density when a beam of light is passed vertically through a cylindrical absorption cell in which a heterogeneous reaction, for example, precipitation, is taking place. Colour interference is suppressed by placing suitable filters in the path of the light. Variations in the optical density are observed using a photometric cell, the solutions being stirred at a constant convenient rate. Since 1953, Prof. Bobtelsky and his co-workers have studied many thousands of reactions by means of this method. Heterometric titrations can give valuable information relating to the formation of primary or intermediate products during the formation of insoluble complexes, and can thus provide data which are unobtainable by ordinary turbidimetric measurements.

The combination of the heterometric method with the more classical applications of conductimetry and potentiometry are discussed for specific reaction systems. Considerable information is also provided indicating the quantitative analytical semi-micro applications of this new method, the determination of traces of a metal (0·1 per cent) being heterometrically possible even in the presence of more than 99·9 per cent of polyvalent foreign metals. Examples are extensively given of normal salt analyses and of the behaviour of many chelated metal ion–organic compound derivatives as recorded by heterometric analysis.

The final chapters of this monograph deal with the results of numerous researches on the formation of insoluble compounds in various solvents, for example, the metallic derivatives of aliphatic and aromatic dicarboxylic acids, and of allied derivatives of the oxycarboxylic acids, polyphosphate complexes, thio derivatives, interaction of metal salt solutions with dyestuffs, the study of heterogeneous chain reactions and the question of inorganic-organic polymer formation are all topics which are discussed by reference to much graphically illustrated experimental data.

This is the most authoritative compendium of heterometric research investigations which has so far been published, and will, undoubtedly, prove of the greatest utility to the research worker studying heterogeneous phase systems, as well as to the analytical chemist and the university teacher. This is a modern technique which may have much future application, and it is pleasing to note that heterometry forms one of the subjects to be presented at the eighteenth International Congress of Pure and Applied Chemistry in Montreal next August.

D. T. Lewis

ARCHÆOLOGY IN ISRAEL

Hazor II

An Account of the Second Season of Excavations, 1956. By Yigael Yadin, Yohanan Aharoni, Ruth Amiran, Trude Dothan, Immanuel Dunayevsky and Jean Perrot. With a contribution by S. Angress. (The James A. de Rothschild Expedition at Hazor.) Pp. xxiv+174+210 plates. (Jerusalem: Magnes Press of the Hebrew University; London: Oxford University Press, 1960.) 178*s.* 6*d.*

THANKS to the efforts of Dr. Yadin and his army of assistants, Hazor must be one of the most widely known of ancient Palestinian sites. Its importance lies not only in the outstanding interest of its excavated remains, but also in the fact that sufficient was known about the history of the city before the excavations ever began to enable these remains to be related to historical events, and to be dated accordingly with a fair degree of accuracy. In the second season of excavations work was concentrated in two areas on the *tell* proper, and two in the lower 'enclosure', which has now been proved conclusively to have been the site of the Canaanite town of the Middle and Late Bronze Ages, finally destroyed some time in the middle of the thirteenth century B.C. by (so Yadin thinks) the invading Israelites. In this lower city, the most important discovery has been that of a Canaanite shrine, with cultic objects (including a mask and a bronze standard) of outstanding importance for the history of Semitic art and religion, and also of other buildings which Dr. Yadin interprets (not very convincingly) as cultic. On the *tell* the chief buildings excavated were a fine pillared storeroom, attributed to Ahab; the Israelite citadel destroyed by Tiglath-pileser III of Assyria in 732 B.C.; and a casemated fortification wall probably built by Solomon. Individual objects from the *tell* included a beautiful marble incense ladle in the form of a hand, and several highly important Hebrew inscriptions. Space does not permit us to record here any more fully the discoveries of the archæologists. They are all now published lavishly, in this magnificent volume full of drawings, photographs and data, so that students in future will have no excuse for not knowing what has been found at Hazor.

They will, unfortunately, have far more excuse for not knowing exactly where it has all been found. In a review of the first volume of this report (*Nature*, 183, 951; 1959) I questioned the validity of the methods used by Dr. Yadin to unravel and interpret the complicated series of superimposed building remains at Hazor. The appearance of the present volume has, if anything, strengthened my belief that the analysis of the remains is not nearly as accurate and as detailed as it could be. This is the result both of a faulty method of excavation and of a faulty system of interpretation. The method is faulty because too much attention is paid to the levels of individual structures and surfaces, expressed in actual heights, and not enough to true stratigraphy. Where the floors are relatively and consistently level over a wide area, and the succession is regular, this may not matter too much; but such conditions are the exception rather than the rule. Where the ground slopes, and the ancient inhabitants have built on terraces, as they have in area *F*, for example, Dr. Yadin's method of excavation is fraught with danger. The confusion in area *F* is indeed great, and the reader (and, one suspects, the excavator) is quite at a loss to know which structures belong to stratum 1*A* and which to 1*B*. These uncertainties are, admittedly, openly acknowledged by the excavators, and Dr. Yadin is, no doubt, to be highly commended for allowing a member of his staff to publish a 'minority report' expressing serious differences of opinion, as M. Perrot does in a long footnote on p. 128ff.; but such differences of opinion do not inspire the reader with much confidence in the interpretation elsewhere.

This brings me to another point. For all the lavishness of illustration and documentation in this report, the record is still not full enough to allow the reader to form his own judgment about the meaning of the evidence presented. Prof. De Laet has well said (*Archæology and its Problems*, p. 52) that the test of a good excavation report is that the reader "ought to be in a position to reconstruct the whole excavation, almost every turn of the spade, in such a way that he can scrutinize most critically the conclusions reached by the author". The Hazor report fails this test. It is just as important that the reader should see for himself that one floor is higher or lower, and therefore later or earlier, than another, as it is that he should be given illustrations of the forms of Hebrew letters, to judge them for himself. The only way to illustrate stratigraphy is to publish accurately drawn and objective sections; and there is not one in this book. Without them, many of the fine plans are almost useless.

Finally, with regard to the faulty system of interpretation: does Dr. Yadin really want us to believe that all over his site the succession of superimposed floors and 'strata' is regular and orderly; that there was no local destruction and rebuilding; and that the structures under, for example, 'Stratum VI' always belong to 'Stratum VII'? Such is the general impression obtained from this report; though on p. 165 it is admitted that "certain layers in sub-areas which looked at first like real strata are in fact only local phases", while, in his footnote already mentioned, M. Perrot recognizes the presence, in area *F*, of "intermediate" strata, which, however, find no place in the neat synchronization table on p. 165. Such over-simplification is unrealistic, and detracts from the undoubted merits of the Israeli excavations at Hazor.

Peter J. Parr

THE ANATOMY OF MANAGEMENT

Exploration in Management

By Wilfred Brown. Pp. xxii+326. (London: William Heinemann, Ltd., 1960.) 30*s.* net.

The Attitudes of Steelworkers to Technical Change

By Olive Banks. (Social Research Series.) Pp. viii+152. (Liverpool: The University Press, 1960.) 25*s.* net.

THERE are few books that have been written by men who combine a wide experience of the practice of management with a developed system of ideas for the analysis of that experience. Mr. Chester Barnard's *The Functions of the Executive* was alone in eminence in this field until Mr. Brown wrote his latest book; but *Exploration in Management* will be as important in the study of organizations.

Mr. Brown's book has the sub-title *A description of the Glacier Metal Company's concept and method of organization and management.* This is most accurate, for the book is a report on the organizational relationships within that Company today and on the experience that has led to its structure. The book is in several parts, considering structure, policy formulation, communication, the organization of specialists and the sources of managerial authority. It gives us, in great detail, information about the problems that arise within an industrial hierarchy that adds much to our store of empirical knowledge.

Mr. Brown is primarily concerned with the problems of authority in organization; the clarification of superior-subordinate and collateral relations, and the attempt to reconcile the authoritarian concepts of mechanistic organization, writing with the wealth of understanding of human motivation in industry that stems from Elton Mayo, to which the Glacier Metal Company has contributed so much. This book is a part of the new trend in organizational theory in that it seeks a synthesis between these two ways of describing the social system of an organization, and Mr. Brown has developed specific mechanisms in the Company to limit and legitimate the use of authority. It is probably at this point that the book is weakest, for the concept of authority used is over-simplified and almost entirely defined in structural terms. Consequently, the compensatory mechanisms are also too simple and deceptively clear-cut. The Glacier Metal organization may, therefore, not be a generalized model applicable to all firms, or all similar firms, but it will be the stimulus to much new thinking in many.

Dr. Banks's book reports an extension of the previous work at the University of Liverpool on the social factors affecting technical change in a large steel works. She is concerned with an intensive analysis of the attitudes of steel workers to change, and her work is part of an international project. The European Productivity Agency has published a general report on the project "Steel Workers and Technical Progress" and the book under review represents the English contribution to this joint endeavour.

A questionnaire was administered to a sample of 278 men, all of whom had been affected by a major technical change some four years before the administration. The questionnaire was designed to elicit the attitudes of the workers to the specific changes they had experienced, in terms of wages, conditions, and social factors, and also to attitudes to change in general. The responses are analysed in terms of a naïve socio-economic theory, but, although there is some valuable information in this study of perceptions of social change recollected in tranquillity, there is little that could be used as the basis for prediction. The data need to be re-worked in terms of more sophisticated concepts of social structure and industrial motivation before their full value can be realized. There are, scattered in the text, important references to the role of management in this successful change; but the questionnaire is not designed to put dimensions on this apparently crucial factor. This is regrettable, for we might have had more field evidence to consider against Mr. Brown's hypotheses.

F. J. WILLETT

TERRESTRIAL CRUSTACEANS

Isopodes Terrestres (Première Partie)

Par Prof. Albert Vandel. (Faune de France, Nr. 64.) Pp. ii+416. (Paris: Éditions Paul Lechevalier, 1960.) 105 NF.

THE production of this valuable book could have been left in no better hands than those of Prof. Vandel, whose vast knowledge of the terrestrial Isopods represented by the sub-order Oniscoidea, has been collected into this comprehensive work.

The volume is divided into two sections: first an introduction, which contains facts of interest to the group as a whole, and secondly a systematic part dealing with some of the families of the Oniscoidea. In the first part the author outlines the history of the group, and then passes on to points of importance in the morphology of its members. These are dealt with under a number of headings: size, coloration, visual organs, integument (including ornamentation), sensory structures and cutaneous glands.

This is followed by an account of the segmentation of the body, the structure of appendages and sexual characters. A further section is concerned with geographical distribution and ecological factors and the origin and history of the Oniscid fauna of France. The first part ends with a survey of the principles of systematics and the classification of the Oniscoidea. The author points out that, of the twenty-one families, only members of thirteen, which are represented in the fauna of France, are considered in the systematic part. The latter begins with a key to determine these thirteen families, and it is unfortunate that in Section '*K*' of the key 'Armadillidae' occurs in error for 'Armadillidiidae', so that the former appears in both '*K*' and '*L*' sections.

The main body of the work represents a detailed account of the members of four of the families (Tylidae, Ligiidae, Trichoniscidae and Buddelundiellidae); the remaining nine families will be covered in the second volume. Each French species is dealt with under eight heads: synonyms, morphology, affinities, reproduction, parasites, ecology, geographical distribution, and palæontology. Keys for the identification of genera and species are included wherever applicable.

The book is profusely illustrated with clear, well-labelled drawings, as well as a number of distributional maps; it will be indispensable to the specialist and invaluable to ecologists and students not only in France but also elsewhere.

E. M. SHEPPARD

Chemical Analysis of Cast Iron and Foundry Materials

By W. Westwood and A. Mayer. Second edition, revised by W. E. Clarke and H. Green. Pp. 586. (London: George Allen and Unwin, Ltd., 1960.) 63*s*. net.

THE analytical methods presented in this volume have been thoroughly tested in the laboratories of the British Cast-Iron Research Association. Fully detailed instructions are given in each case, and the practical details are accompanied by discussion of the relevant theory. In all cases, a general survey of existing methods is given, and the relative merits of alternative procedures are considered.

In this second edition the revising authors have modified or replaced a number of methods, according to experience and the results of analytical research. Such research has yielded an interesting new scheme for the analysis of cupola slag. A complete analysis of this material can now be carried out in less than one working day, with an accuracy comparable with that obtained by classical methods.

The short section dealing with laboratory practice and apparatus now includes a description of separations by solvent extraction procedures. Instrumental methods receiving brief attention are flame photometry, polarography, spectrography and X-ray fluorescence spectrography. A description of a particular absorptiometer has been replaced by a general account of the basis of absorption spectrophotometry and of spectrophotometric instruments—a particularly sensible modification to the text.

The methods given in this book are capable of wider application than the title suggests. The clarity with which the subject-matter is presented makes it possible for the analyst to assess the applicability of the individual methods to general inorganic analysis, as well as to the analysis of all classes of ferrous materials. F. L. Selfe

Theoretical Elasticity

By Carl E. Pearson. (Harvard Monographs on Applied Science, No. 6.) Pp. xi+218. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1959.) 48*s*. net.

THE writer of this book states that its purpose is in part to discuss modern methods in elastic theory, and in part to provide a compact summary of such methods.

The first four chapters contain a detailed account of vectors and Cartesian tensors, and of their use in describing infinitesimal strain and stress. Chapter 5 gives a summary of the basic equations of classical elasticity, and Chapter 6 a useful and interesting account of the general methods of solution associated with the names Somigliana, Betti, Kelvin, Boussinesq, Papkovitch-Neuber, Galerkin and Maxwell-Morera. Chapter 7 describes variational methods, though without illustration or application. Chapters 8 and 9 deal with topics in thermoelasticity and time-dependent problems, and Chapter 10 gives a brief introduction to non-linear elasticity.

The treatment is somewhat uneven; elementary topics are treated at considerable length and advanced topics sketched briefly. Some references are given, but not always enough to enable the reader to find proofs which have been omitted by the author.

J. F. Baker

Physical Metallurgy of Stress Corrosion Fracture

Edited by Thor N. Rhodin. (Metallurgical Society Conferences, Vol. 4.) Pp. xiii+394. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1959.) 98*s*.

AN engineer of great experience has recently stated that in no less than 27 per cent of all cases submitted to him for investigation the primary cause of the failures was ascribable to corrosion in one form or another, and in the majority of instances while the metal was under stress. The papers presented to the symposium recorded in this book give a very good account of the present state of knowledge regarding stress-corrosion and add to what is already known. In view of the important role of the crystal boundaries in leading to such trouble, it is, for example, valuable to have clear proof that the effect can occur even in single crystals. Since, if a solution of this extremely elusive problem is to be found, it can only arise from fundamental work, it is appropriate that more than half the book is devoted to the more theoretical aspects of the subject. Of the more immediately practical papers, although the stainless steels receive most attention, both mild steels and non-ferrous alloys are considered. This is an excellent account of the complex phenomena concerned with stress-corrosion failures and may be recommended unhesitatingly. F. C. Thompson

The Cathode Ray Tube Memory of the High Speed Electronic Computer of the U.S.S.R. Academy of Sciences

By V. N. Laut and L. A. Lyubovich. Translated from the Russian by Ruth Feinstein. Edited by F. A. Sowan. Pp. xi+90. (London and New York: Pergamon Press, 1960.) 35*s*. net.

THIS book describes the 1,024 word store used in the *B.E.S.M.* digital computer, which operates in parallel with a 39-bit word-length at a pulse-rate of 80 kc./s.

The first two chapters include a block diagram of the store organization, and a description of the storage tube, a barrier grid type using an aluminium back plate which stores 1,024 bits of data as a pattern of electric charges. Chapters 3 and 4 cover the writing and reading circuits used with the storage tube, and the balanced bridge circuit which isolates the reading and writing amplifiers. Chapter 5 describes the decoding of the address digits, the generation of the appropriate storage-tube deflexion voltages, and gives a discussion of the accuracy and stability of the spot position. Chapters 6–8 deal with the control unit, including test facilities, the coupling to the main computer, and the initial setting-up procedure. The final two chapters deal with the mechanical aspect of the store, and its performance in service.

Since the latest reference is dated 1955, the original text was presumably written in 1955 or 1956, and the type of storage described is generally considered obsolete. It has indeed been reported that the *B.E.S.M.* computer was equipped with magnetic-core storage in 1959 to replace the cathode-ray tube store. Thus, although the book gives a comprehensive account of the design and operation of the store, it should be regarded as a contribution to the literature of electrostatic storage tubes, rather than an account of present-day practice in digital computer engineering. J. C. Cluley

FOUNDATIONS OF QUANTUM THEORY AND COMPLEMENTARITY

By Prof. L. ROSENFELD
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THE foundations of quantum mechanics were laid some thirty years ago. Thus, a whole generation has passed since these foundations were established, but for the new generation which is now coming up the situation is perhaps stranger still than it was for the physicists who had to struggle with those problems for the first time. The people who face a problem for the first time and have to think very hard to try to solve it have a more direct and lively impression of what is involved in the new ideas which bring about its solution. The next generation is presented with the finished work and often does not appreciate properly the difficulties that were involved in the solution of the problem. So it happened with relativity theory one generation after Einstein ; some people started then to raise doubts about the very principles underlying the general theory of relativity. This episode is now forgotten; but we see the same phenomenon happening with quantum mechanics. Young physicists are raising doubts about the correctness of the basic ideas of quantum mechanics, and try to do it better. These efforts are, I am afraid, rather futile, because they rest on a complete misunderstanding of the problem, on a lack of appreciation of the really very difficult conceptual situation which quantum mechanics presents to us. This situation is not something that depends on any free choice, about which one might have this or that opinion. It is a problem which is imposed on us by Nature. After all, the existence of the quantum of action is a law of Nature: it is not something we have invented, but an aspect of Nature we have discovered. This discovery created, when it was made, a very embarrassing situation, and quantum mechanics is, in fact, the answer which physicists after many years of effort have been able to give to this challenge of Nature.

The discovery of the quantum of action was made in connexion with properties of electromagnetic radiation. In order to have something definite before us, we can therefore speak about the situation in the theory of light or electromagnetic radiation, although exactly the same can be said of electrons or any other constituent of matter. According to the classical theory of electromagnetism, light or any electromagnetic radiation can be described as a field, in the simplest case a field endowed with periodicity in space and time; the element of radiation is thus represented by a plane monochromatic wave, that is, a field distribution which has strict periodicity in space, with a certain wave-length, and in time a certain period connected with the wave-length and velocity of propagation by the wave equation. In order to have this strict periodicity, the field must be spread out over the whole of space, and it must persist during unlimited time: a pulse which only exists during a finite time does not have a sharply defined frequency, but a whole spectrum of frequencies; likewise, a limitation in space of the 'wave packet' implies a distribution of wave numbers (or inverse wave-lengths).

Now, the discovery of the quantum implied that when radiation interacts with matter, as in the photo-effect or in the Compton effect, the amounts of energy and momentum exchanged in this interaction are not at all related to the distribution of energy and momentum in the radiation field, but are completely determined by the mechanical laws of conservation governing collisions between particles of definite energy and momentum ; in this picture, a monochromatic radiation is represented by a light quantum or photon, behaving like a particle the energy and momentum of which are proportional to the frequency and wave number of the radiation. Thus, one was here led to describe radiation by means of another classical concept, which had hitherto been applied only to matter: the concept of the particle, which represents a concentration of energy and momentum at a single point of space and at any single instant of time. This is also, like the monochromatic wave, an ideal conception, and it is the extreme opposite of the latter; in fact, the two are mutually exclusive. Yet, they are intimately related by the equations:

$$E = h\nu, \qquad p = h\sigma \tag{1}$$

which express the proportionality between the energy and momentum E, p, concentrated in the particle, and the frequency and wave number ν, σ, defined by the plane wave.

These are at first sight very paradoxical equations : they relate together characteristics of the radiation which are contradictory to each other, and yet both are necessary to account for all phenomena (for the interference of waves is just as fundamental a phenomenon of radiation as the scattering of photons). The coefficient of proportionality, Planck's universal constant h, appears in the equations (1) as the product of two physical quantities—the energy of the photon and the period of the wave—which relate to two descriptions of radiation that are mutually exclusive. Thus such a situation demands more than just a mathematical analysis. The mathematics are extremely simple, but the question is to understand what these simple equations mean, as they seem to combine things that are mutually exclusive. What we meet with here is actually a problem of logic: What are we to do when we are confronted with such a situation, in which we have to use two concepts that are mutually exclusive, and yet both of them necessary for a complete description of the phenomena ?

It is this logical problem which confers a very peculiar character on quantum mechanics. The situation was not quite the same with the relativity theory. Of course, in creating this theory, Einstein had also to make a very deep analysis of the fundamental concepts of space and time and of the relation between them, but although the result was quite revolutionary, he could perform this analysis within the framework of classical physics. Here we see that it is this framework itself that is too narrow logically: the task is to find a consistent way of using classical concepts so as to give well-defined meaning to mathematical relations like the equations (1) in spite of the fact that the parameters involved refer

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* Substance of a lecture given at the University of Tokyo on October 6, 1960.

to two contradictory images. The solution of this problem, to which a fundamental contribution was given especially by Heisenberg, received its final formulation from Niels Bohr; the new logical instrument which was created by Bohr is called complementarity. Complementarity denotes the logical relation, of quite a new type, between concepts which are mutually exclusive, and which therefore cannot be considered at the same time because that would ead to logical mistakes, but which nevertheless must both be used in order to give a complete description of the situation.

The idea underlying complementarity and making t possible to surmount the logical contradiction is, n fact, very simple. Contradiction arises when one tries to give a universal validity to the concepts that are mutually exclusive. If one tries to apply both of them to any situation irrespective of the circumstances of the situation, then, of course, one gets into contradictions. However, if one reflects on the use of all physical concepts, one soon realizes that any such concept can only be used within a limited domain of validity. These limitations may be disregarded so long as one is dealing with simple situations, as in classical physics: the constant h, which marks the limit of applicability of classical concepts, is so small compared with the actions involved in macroscopic experiments that we can ignore the limitation. Therefore the physicists of the nineteenth century had no need to think very deeply about this question. But after the discovery of the quantum of action has confronted us with such a situation, we are forced to analyse the logical relations between concepts, and to recognize their limitations. But once one has realized the simple fact that every physical concept is only defined with respect to a certain class of phenomena, to certain well-defined physical situations, it becomes easier to understand that different sets of concepts, although incompatible, may nevertheless be applicable to different physical situations.

Domains of validity of complementary concepts are not, in practice, rigidly exclusive of each other; they may overlap to some extent. Where they overlap, the concepts in question can both be applied, but of course not rigorously. A well-known example is that of a 'wave packet' of radiation. There can be no rigorously monochromatic wave: a real wave is always limited to a finite time-interval, Δt, and a finite region of space, the dimension in one direction of which we shall denote by Δx; such a wave can be analysed into monochromatic components with frequencies and wave numbers (in the direction x) distributed over intervals $\Delta\nu$, $\Delta\sigma_x$, the order of magnitude of which, according to the mathematics of Fourier expansions, is just $(\Delta t)^{-1}$, $(\Delta x)^{-1}$. We may regard such a wave packet as approximating the localization of the phenomenon in time and space with latitudes Δt, Δx, and at the same time, because of the relations (1), fixing the energy and momentum of the radiation within an accuracy $\Delta E = h\Delta\nu$, $\Delta p_x = h\Delta\sigma_x$. Thus, between the latitudes of the complementary pictures there is a reciprocity :

$$\Delta E \,.\, \Delta t \approx h, \qquad \Delta p_x \,.\, \Delta x \approx h \qquad (2)$$

which is an example of the famous 'uncertainty relations' holding for any pair of physical quantities complementary to each other.

This reciprocity illustrates a fundamental feature of the complementarity relationship. On one hand, there is no absolute limitation to the possibility of defining either of the complementary quantities: by making the wave packet infinite we obtain the idealization of a photon of well-defined energy and momentum; by making it infinitely small we have the ideal localization of the radiation at a single point of space–time. On the other hand, when we approach either of these extreme idealizations, we see that the complementary one becomes less and less defined, and is finally excluded : the fixation of the energy and momentum of the photon precludes its localization, and conversely, if we localize the radiation field, we lose the definition of the energy and momentum contained in it. Such reciprocal limitations correspond, of course, to real physical effects, and the complementarity relationship just expresses the fact that the experimental set-ups required for the determination and recording of each of the quantities in question are such as to impose on the complementary quantity the lack of definition expressed by a relation of the type (2). To realize quite clearly what is involved here, we must sharpen our language and ask ourselves, more searchingly than was necessary in classical physics, what we understand by a physical phenomenon. The precise definition of a phenomenon must include the description of the apparatus which defines the conditions of the experiment, and also some recording process (an index reading, a spot on a photographic plate, etc.) by which alone the phenomenon can be regarded as terminated : the physical quantities we are dealing with in our mathematical theories are in fact just such records of phenomena. Phenomena so defined can be in a complementarity relationship, and the accuracies of their recordings then stand in a reciprocal uncertainty relation.

Reciprocal uncertainty relations are in fact precisely what we must expect, from a purely logical point of view, if we are, as in quantum theory, in a situation in which contradictory pictures of the phenomena have to be resorted to for a complete account. In order to avoid trivial mistakes which would arise from an indiscriminate use of such contradictory concepts, we must have some way of delimiting their domains of validity, some prescription enabling us to decide in each case how far we can go with the help of each of them. The uncertainty relations tell us, so to speak, the price we have to pay for applying to the description of a phenomenon two sets of concepts which are strictly contradictory. It will be realized, without going into further detail, that the conception of complementarity means a very considerable extension of our way of speaking about Nature and about natural phenomena. It involves a new logical framework within which to arrange our concepts, compared with which the classical way of speaking appears very narrow because it is confined to the simple classical logic, governed by the principle of contradiction. Of course the classical logic remains the foundation of any argument we use, and certainly of our mathematics. But we find that when we deal with quantum phenomena it is too narrow : we must have a wider way of connecting together the various concepts, and this is provided by complementarity.

The most considerable change in our view of natural phenomena which is implied by this widening of our outlook is related to our conception of causality. In classical physics the expression of causality is the result of the mathematical properties of the equations expressing the laws of motion of particles and of propagation of the electromagnetic field. When we are given the initial conditions, involving the initial values of the quantities and perhaps some of their derivatives, the solution of the equations is completely determined; and accordingly, the type of causality of classical physics is determinism. This is no longer the case when we have a complementary relationship, as is already suggested by the fact that we cannot then speak of all physical quantities with unlimited accuracy. One of the most obvious examples illustrating the new situation is the emission of the spectral lines: according to the quantum postulate, an atom in a high state of excitation can effect transitions to any one of the lower excited states; but there is nothing in the conditions of the phenomenon to tell us which of these possibilities will be realized in a definite case. Therefore, all we can do is to try to find the relative probabilities of their occurrence; it is just to cope with such situations that the concept of probability has been invented. The argument applies quite generally to all complementary situations; if we consider one set-up, in which, let us say, the field is localized within a certain space–time region, we have a probability distribution for all possible values of the energy and momentum, and conversely. These distributions are such that the corresponding average fluctuations are connected with the complementary time and space latitudes by reciprocity relations: if the field distribution is very narrow in time, it is very wide in energy, etc. In other words, the kind of causality that is implied by the existence of complementary relations between concepts is statistical causality.

As is known, statistical causality was introduced, historically, in dealing with the affairs of human societies or states, which are notoriously undetermined : that is the origin of the word 'statistics'. It was for the first time applied in physics in connexion with problems of atomic theory, though not those concerned with the individual properties of atoms ; it was introduced in thermodynamics, that is, in the study of the properties of very large systems of atoms, and later also of systems composed of a very large number of monochromatic waves of radiation. This occurred in the second half of the nineteenth century, when, of course, nobody dreamt of the possibility of such queer things as complementarity. At that time the justification for introducing statistical causality was simply this. We aim at a complete description of a gas as a mechanical system of molecules; but this could only be obtained if we could know at a certain time the exact positions and velocities of all the molecules of the gas; then we could solve, at least in principle, the mechanical equations for the system, and deduce in a unique, deterministic way the state of the gas at any future, or past, time. However, this ideal aim is obviously unattainable, and therefore the best we can do is to speak of the states of the system in terms of probability, just as, in view of the impossibility of predicting the exact time of a man's death, we speak of the probability of his living for so many years.

The concept of probability was thus used with a sort of apology, in default of something better. This attitude was of course quite natural for people believing in determinism, believing that the Creator had imposed on Nature, once for all, rigid laws that had to be obeyed and were so contrived that they could only yield a single, predetermined, result. Unprejudiced observation of the world around us, however, will soon reveal that this idea of rigid determinism is a fiction, an unwarranted extrapolation from a very limited experience, and that there is no real justification for it. On the contrary, if we want to describe immediate experience, surely we must say that all our judgments about Nature or about ourselves are judgments of probability: we are never in a situation in which we know all the conditions for applying laws which would give us a unique answer. Thus, statistical causality appears in fact as more natural, more directly adapted to our actual needs than determinism; and it is much better to look at determinism as a limiting case of statistical causality, corresponding to statistical distributions very sharply peaked around the average values. From this point of view we are better prepared to accept the fact that the type of causality of quantum mechanics is fundamentally statistical; and we are even in a position to give exactly the limit of validity of classical determinism: the statistical deviations from classical behaviour become negligible in phenomena involving a large number of quanta of action.

Now, returning to the case of statistical thermodynamics, it is instructive to consider an objection to the point of view just outlined which is still often heard. It is all right to say that statistical mechanics is a self-contained theory, as I have tried to suggest; nevertheless it is conceivable that, if not human beings, at least imaginary beings, which Maxwell and his friends called demons, could be so small (and yet endowed with sense organs similar to ours) that they could observe the motions of the individual molecules of a gas; such beings could then give a deterministic description of it. I think the answer to this was already given very pointedly by Clausius, when Tait tried to use this fiction of the Maxwell demon to question the universal validity of the second law of thermodynamics by pointing out that those demons could bring about situations contradictory to the second law. Clausius's answer was: "We are not concerned with what demons can do, but with what heat can do by itself", which clearly means: with the phenomenon of heat which we can observe. I think there is a very deep truth in that answer, deeper perhaps than Clausius himself could realize at the time.

In fact, what the situation in quantum mechanics has helped us to realize is that science is primarily concerned with the communication of experience about phenomena, in other words, with statements made by human beings, and which other human beings are able to understand. For this very reason all such statements must make use of the concepts of classical physics, because classical physics is just the language describing the results of direct observation, the phenomena which we can observe with our senses, either directly or with instruments to increase the sensitivity, in any event, with recording systems composed of many atoms. If, then, we talk in this language about atoms which do not follow the laws of classical physics, we meet with the limitations of its use expressed by relations of complementarity. We thus see that this complementarity has its source just in the peculiar situation in which we find ourselves, as macroscopic systems, trying to describe the possible macroscopic interactions of our apparatus with the smallest elements of which Nature consists.

Now, if we look back at thermodynamics from this point of view, we see that it presents a logical problem which is just the same as that of quantum mechanics. Here also we are trying to describe atomic structures in terms of macroscopic concepts, and we may do this in two different ways; we may be able to give a more or less detailed dynamical description of the atomic system, in terms of the motions of its parts; or we may describe its state in terms of the macroscopic quantities, such as volume, pressure, temperature, entropy, which are related by the laws of thermodynamics. Between these two modes of description there is a relation of complementarity; this is very clear if we consider the temperature and the energy, representing the thermodynamical and the dynamical points of view respectively. These two quantities are complementary to each other in the sense that if a system has to have a well-defined energy, it must be thermally isolated, and we cannot then ascribe to it any temperature, because in order to define the temperature we must establish thermal contact between the system and another, very large system, serving as a thermostat; conversely, if the temperature of the system is defined in this way, it exchanges energy with the thermostat, which means that its energy is not determined; in fact, we know that in this case this energy has a definite statistical distribution, the canonical one, the modulus of which is proportional to the temperature of the system.

This complementarity we can formulate in a more general and precise way, in wide-going analogy with the case of quantum mechanics, by setting up reciprocal relations between the statistical fluctuations affecting macroscopic quantities. Of course, the reciprocal limitation of the dynamical and thermodynamical modes of description has nothing to do with the quantum of action: the similarity of the two cases is on the logical level only; physically, they are completely independent of each other.

The fluctuations we are here considering are those which affect the macroscopic parameters defining the state of a system which is a small part of a larger, but finite, system; then, even intensive quantities, like the temperature, will exhibit fluctuations, for they are only exactly defined for infinite systems. It is easy to see that one must expect reciprocal relations between fluctuations of extensive and intensive quantities, that is, those quantities which (for a homogeneous system) are proportional to the number N of constituent elements of the system, and those which are independent of N. In fact, the fluctuation of an extensive quantity, the energy, for example, is proportional to $N^{1/2}$, while that of an intensive quantity, the temperature, say, is inversely proportional to $N^{1/2}$; the product of the two is therefore independent of N and cannot be made arbitrarily small by a change of the number of degrees of freedom of the system. A more detailed calculation shows that the products in question are of the form kC, where C is a quantity independent of the size, but depending on the physical properties of the system considered, and k is Boltzmann's universal constant. Thus, it is this constant which here plays a part analogous to that of Planck's constant in quantum theory: it is inversely proportional to Avogadro's number, which is thus seen to define a critical size of the system, for which the complementarity between dynamical and thermodynamical description becomes important.

This consideration is not as artificial as it may seem: it is easy to give examples of systems of this critical size, which can be considered from both the dynamical and the thermodynamical points of view. If we take as the systems under investigation droplets of emulsion distributed under gravity in a solution, we have a case in which the systems are large enough for us to see them and to follow their motion, which is a Brownian motion, and yet small enough for us to speak of their statistical distribution in the solution. As a representative thermodynamical quantity we may take the entropy S, which will fluctuate on account of this statistical distribution of the droplets. Now we need an intensive quantity characteristic of the individual motions of the droplets; for this let us choose the velocity v, which is subject to the well-known Brownian fluctuations due to the collisions with the molecules of the solution. A simple calculation gives in this case for the product of the two fluctuations:

$$\Delta S\,.\,\Delta v = kv_0$$

where v_0 is a quantity of the order of the mean atomic velocities inside the droplet. We see that the pattern of complementarity applies to the present case in all details just as in quantum mechanics.

So far, therefore, we have found that the atomistic view of the world involves in its description two stages of complementarity. The first and most fundamental one occurs in the account of the properties of individual atomic systems, the second when we want to describe the behaviour of systems of large numbers of atoms. In the first case, a relation of complementarity exists between two sets of concepts which were used indiscriminately in classical theory: the space–time localization and the energy-momentum balance; it becomes significant when the mechanical action in the process considered is of the order of Planck's constant. At the second stage, we have complementarity between the dynamical and the thermodynamical behaviour of large systems, the critical size being given by Avogadro's number. In both cases, as we have seen, complementarity essentially implies a specific kind of statistical causality.

Let us now venture for a moment into the field of biology. We here meet with a very peculiar situation: what characterizes the biological behaviour, in contrast with the behaviour of inanimate matter, is the existence of a certain structural order, either definite spatial structures such as those of the protein molecules, or more formal, topological structures such as the interconnexions of nerve cells in the brain. In any event, we notice the appearance of some kind of order which has a remarkable stability, and which can be reproduced. Now, the great event of the past few years has been the discovery, at the very lowest stage at which the phenomena of life appear, of possible mechanisms by which the maintenance and perpetuation of such a type of order can be understood in simple terms of physics. There is certainly no reason to expect that, with the most elementary, also the most elaborate forms of biological behaviour will not eventually be reduced, in a certain sense, to the laws of physics and chemistry, that is, in the last instance, to quantum mechanics, since according to the famous saying of Dirac quantum mechanics explains a large part of physics and the whole of chemistry. Now we may perhaps add: the whole of biology.

Yet this is not the whole story. It is quite obvious that, when we are presented with such complicated structural problems, it is practically impossible, even with the most perfect knowledge of all the laws of physics and chemistry, to predict what kind of organism will result from a given structure of such complexity. We are here in the same situation as somebody who would know all about mechanics and is presented with a blueprint of some machine which he has never seen and which he does not know. It would be very hard for this person to guess the function of the machine, to understand why this particular combination of motions has been set up. We must realize that in order to get complete understanding of organisms we need the concept of function. We must somehow know beforehand what the function of a certain organ is in order to be able to understand its structure. We here meet with another type of causality, functional causality, which is obviously complementary to the ordinary causality of physics and chemistry, from which this concept of function is absent. Thus, we see that at the biological level we have another stage of complementarity, which again presents the same characteristics of mutual exclusiveness as the previous ones: on one hand, we have the complete description of organs in terms of physics and chemistry, on the other the functional mode of description, which will always be necessary for a complete account of such complicated arrangements of molecules as living beings. This complementarity is concerned with another aspect of atomic systems than just the size, although the size is rather critical, inasmuch as biological structures can only occur at a definite stage of molecular complexity, allowing on one hand for the necessary stability and on the other for the possibility of reproduction; but the main point here is the type of spatial ordering of the constituent atoms, which distinguishes biological systems from inanimate ones.

At this point I may perhaps be allowed to venture a little further still on the path of speculation, and raise the following question. The whole point of the logic of complementarity, in its application to the atomistic description of the world, is that the mutual limitations in the use of the classical concepts it implies, at each of the three stages we have recognized, are related in the last resort to the size of the sense organs of human beings. To be sure, there is no direct reference in physics or even in molecular biology to the human observer as such, but only to registering appliances which are entirely controllable physical systems. Still, the size of these appliances is dictated by the requirement that the complementary limitations be negligible in the account of their behaviour, and this effectively brings them to macroscopic size, that is, to a size adapted to our sense organs. There was a Greek philosopher, the sophist Protagoras, who used to say that "man is the measure of all things". There is a deeper truth in this than he could possibly fathom. This brings us to the question: Is the recourse to complementarity logic so to speak an accident, another of the many imperfections of human nature, and is it not conceivable that other rational beings, some kind of Maxwellian demons, could develop a different mode of description of Nature not affected by any complementary limitations ?

The tentative answer I should like to submit is that there is nothing more accidental in the emergence of complementarity logic than in the emergence of man himself as a product of organic evolution. My point is that the concept of a thinking Maxwellian demon is self-contradictory. I suspect that the development of a computing and communication system like our brain demands about that complexity of organization which has been reached by our own species in the course of evolution. After all, it is an empirical fact that rational thinking has only arisen at the stage of mankind, presumably because it was only at this stage that a brain developed with a sufficient number of interconnexions between nerve cells to make possible this very peculiar behaviour which we call rational thinking. Hence the suggestion that no animal other than man or some being of equivalent complexity can possibly have any physics; and this degree of complexity, as we have seen, necessarily implies the introduction of complementarity in physical theory.

The view of physics, and in fact of science, which results from the development of quantum theory is very different from the scientific ideal of the nineteenth century; but it is by no means, or so it seems to me, inferior to it. The ideal for which such people as Huxley and Tyndall fought, the supremacy of human reason over all forms of irrationality, was based on the deterministic view of a Nature governed by inflexible laws, which the task of science was to discover and to formulate. These laws, by their very nature, would have absolute validity, and they would give a picture of the universe, in the literal sense of the word, a picture which could be contemplated, but from which the spectator, the human spectator, would be excluded, or at least reduced himself to an object of contemplation. The present conception, on the contrary, stresses the fact that at every stage the human observer intervenes in a very fundamental way by determining the very language in which the laws of Nature are formulated. The significance of the laws of Nature, from this point of view, is not that of drawing a picture of the world independent of who is looking at it, but rather of giving an analysis of the interaction between ourselves and the external world. At first sight it would seem that one thus introduces in science a subjective element. But the fear that the objectivity and rationality of the old view would be abandoned is entirely unfounded. True objectivity is simply the possibility of guaranteeing that the account of the phenomena will convey equivalent information to all observers, that it will consist of statements intelligible to all human beings. This objectivity is indeed ensured in quantum theory by the existence of transformations which allow you to pass at will from the point of view of one observer to that of another, who makes some other type of experiment. It is just the invariance of the laws of physics for such transformations that guarantees their objectivity, and nothing is changed in this respect by the advent of quantum theory.

Far from marking a retreat of human reason from the ambitious aim of the great pioneers of the preceding centuries, the new ideal of science appears as a tremendous advance towards this very aim. Compared with the old one, it is much broader of scope, much richer of content. It is more truly adapted to the actual situation that scientists have to face in their quest; the logic of complementarity enables us to cope with the dilemmas which this situation presents to us, and which were insoluble from the old point of view. I may therefore conclude on an optimistic note by assuring the younger generation that the instrument of rational analysis we are handing over to them contributes to a further increase of our power of understanding our position in Nature and of coping with the problems which it raises.

COMETS AND THE FORMATION OF BIOCHEMICAL COMPOUNDS ON THE PRIMITIVE EARTH

By Prof. J. ORÓ

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INTERACTIONS of comets with the Earth or the Moon have been suggested by Urey[1], Fensenkov[2] and more recently by Whipple[3] in order to explain several terrestrial phenomena. It is estimated that about 40 million comets are now present in the solar system[4], and it has been calculated that about 100 comets have collided with the Earth since the formation of our planet some 5×10^9 years ago. (The value of 100 comets is obtained from Urey's comet-Earth collisional probability assuming a constant rate of comet formation. This value would be larger if the number of comets when the solar system was formed were several thousand times greater than at present[4].)

I suggest that one of the important consequences of the interactions of comets with the Earth would be the accumulation on our planet of relatively large amounts of carbon compounds which are known to be transformed spontaneously into amino-acids, purines and other biochemical compounds.

First, the amount of cometary matter (made mostly of carbon compounds) trapped by the Earth during its first 2×10^9 years can be calculated to range from 2×10^8 to 1×10^{12} tons on the basis of Urey's collisional probability[1] and the estimates of 1–10 km. for the diameter, and 0·01–0·05 gm. cm.$^{-3}$ for the density of the nuclei of comets[1,5]. The assumptions made here are that most of the cometary matter, neutral or ionized, was retained by the gravitational and magnetic fields of the Earth, and that the Earth's environment during the first 2×10^9 years was essentially reducing[6] and perhaps abiotic[7,8]. It is further considered that the high temperatures developed as a result of the collision impact would not essentially have altered the potential reactivity of the chemical species present in comets, since spectroscopical observations of pre- and post-perihelion emission[5], and chemical experimentation[9–11], attest to the thermal stability of the cometary carbon compounds. The high pressures developed on impact would, if anything, tend to condense the monocarbon species into linear chains of 2–6 carbon atoms[12].

Secondly, if terrestrial accretion of cometary matter from comets passing sufficiently close to our planet is considered, the amount of carbon compounds trapped by the Earth would be even larger. It is possible that this second mechanism of mass transfer which would result in a more continuous accretion of cometary matter was as important as the one by direct collision. Analogous mechanisms to those above have been offered for the terrestrial capture of non-volatile meteoric matter[3,8,13–15].

Thirdly, it is known from work on cometary spectra carried out for more than half a century and compiled by Swings and Haser[5] that comets contain large amounts of hydrogen, carbon, nitrogen and oxygen compounds, as indicated by the fluorescence emission bands of diatomic and triatomic radicals or molecules such as CN, CH, CH_2, C_2, C_3, NH, NH_2 and OH, and ions such as CH^+, OH^+, CO^+, N_2^+ and CO_2^+, observed in the heads or in the tails of comets when these bodies are at distances of less than 3 A.U. from the Sun. The CN band is generally the first molecular emission to appear on the tails of comets during the travel of these bodies toward the Sun, and it is also the band with the largest degree of extension into the comets' heads, followed in intensity by the C_2 and the C_3 bands. It is possible that the nuclei of the heads of comets contain frozen free radicals which are volatilized by radiant heat from the Sun[16,17], and it is also possible that they contain frozen ordinary molecules which are vapourized and photodissociated into radicals by the solar radiation[5,18]. These two points of view do not necessarily exclude each other, since it has been found recently that the NH radical can coexist with reactive carbon compounds[19]. In general, it is considered that the parent molecules of CN, NH_2 and OH are hydrogen cyanide or cyanogen, ammonia and water, respectively[5,18].

On the basis of the above considerations, it would be of interest to find out by experiment the products formed from chemical mixtures approaching cometary composition. This has partially been done by studying the products formed in reaction mixtures containing hydrogen cyanide, ammonia and water. Recent studies in my laboratory have provided evidence for the synthesis of adenine[20,21], 4-amino-imidazole-5-carboxamide, 4-aminoimidazole-5-carboxamidine and formamidine[22], and glycinamide, glycine, alanine and other amino-acids[23] from the reaction of hydrogen cyanide in aqueous ammonia. All these compounds and other unidentified products were formed more or less simultaneously in the same reaction mixture in a spontaneous manner. More than fifteen experiments have been carried out to the present time using concentrations of hydrogen cyanide of 1 to about 11 *M* and temperatures of 30–100° C. The synthesis of adenine, imidazoles, amino-acids and the other compounds has been demonstrated every time that the reaction product has been analysed for them.

In addition to hydrogen cyanide, ammonia and water, other reactive molecules such as formaldehyde, acetaldehyde, hydroxylamine and hydrazine could be expected to be formed by intercombination of cometary radicals. Experiments carried out with aqueous solutions of these compounds have also resulted in the formation of biochemical products. Thus, formaldehyde and hydroxylamine or hydrazine have yielded amino-acids, amino-acid amides and aliphatic acids[24–26]. Formaldehyde in the presence of calcium oxide is known to yield C_3 to C_6 monosaccharides[27]. Recently the synthesis of deoxyribose from formaldehyde and acetaldehyde has been observed for the first time in my laboratory[28].

Since glycine and glycinamide were two of the most frequent products obtained in the above amino-acid synthesis, the polymerization of these compounds in aqueous ammonia systems was also studied. They were both found to give polypeptides which were characterized as polyglycines I and II by the infra-red spectra and chemical properties[29,30]. It may be added here that the synthesis of pyrimidines, such as uracil and cytosine, should also be possible by reaction of urea with certain C_3 compounds resulting from the symmetrical C_3 species of comets.

The mechanisms of synthesis of most of the above compounds have been elucidated, and in general follow lines in common with known chemical and biochemical syntheses. They have been briefly reported[31–33], and they will be published in detail elsewhere.

From the foregoing it appears reasonable that a large number of compounds of biochemical significance such as amino-acids, polypeptides, purines, pyrimidines, imidazoles and the pentoses, ribose and deoxyribose could have been formed from cometary matter trapped by the primitive Earth.

The present hypothesis of cometary origin of biochemical compounds on the primitive Earth is not to be considered as an alternative hypothesis to that formulated by Oparin[34], Urey[35] and Miller and Urey[36] and others, but rather as a complementary one since in both cases essentially reducing conditions for the primitive Earth's environment are assumed. This assumption of reducing conditions, or at least non-oxidizing, is borne out by geochemical evidence[6].

It can thus be visualized that after the Earth was condensed to approximately its present volume, having lost practically all the gases of the protoplanet's atmosphere[37–40], collisional and non-collisional mass transfer from the comets resulted in the accumulation on the Earth of significant amounts of reactive carbon compounds, which were mixed with similar, but perhaps less-reactive, compounds of magmatic origin[41,42], forming the early atmosphere and hydrosphere where organo-geochemical syntheses took place.

An important feature of this hypothesis is that it gives a plausible mechanism for the partial replenishment of the Earth's atmosphere by the continued accretion of gaseous compounds on the planet after all the Earth's original gases had escaped.

A second feature is that cometary collisions would lead to the concentration of relatively large amounts of carbon compounds in isolated regions of the primitive planet, making it irrelevant to the central biopoietic problem the extent of replenishment of the Earth's atmosphere. It need only be realized that a single comet would be equivalent to more than one million tons of organic matter, and that the effective concentration in the vicinity of the impact area would presumably be quite large due to the limited dilution caused by the small amount of water thought to be present on the primitive Earth[41,42].

A third, and perhaps most important feature, is that the reactivity of the chemical species present in comets and trapped by the Earth would be such that, under the increased gravitational field, and the other physical conditions developed on capture, they would be rapidly transformed into organic and biochemical compounds without the need of additional sources of energy ; making the synthesis possible even if the energy input from electrical discharges, ultra-violet light, ionizing radiation and magmatic processes were limited.

As a final thought it is interesting to note that the predominant elementary composition of comets, and for that matter that of the Universe as a whole if we exclude the inert gases[43], is precisely identical to the elementary composition of proteins, that is, hydrogen, carbon, nitrogen and oxygen. The corollary is that given appropriate planetary conditions[44], the phenomenon of biopoiesis should be a probable event in our universe rather than a very improbable one.

In order to formulate more precisely the quantitative aspects of this hypothesis it will be necessary to carry out more detailed work on the statistics of the cometary population of the solar system, on the chemical composition of comets, and on the nature of the dust cloud about the Earth.

I wish to thank Drs. L. F. Biermann, G. H. Herbig, H. C. Urey and F. L. Whipple for valuable information.

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OBITUARIES

Lord Stopford, K.B.E., F.R.S.

John Sebastian Bach Stopford was born near Wigan on June 25, 1888, and died on March 6, 1961, at Arnside, Westmorland.

He was educated at Liverpool College, Manchester Grammar School and the University of Manchester, graduating M.B., Ch.B. with honours in 1911, after gaining several distinctions and prizes during his medical course. After serving as a house surgeon at Rochdale Infirmary and assistant medical officer at Manchester Royal Infirmary, he became, in 1912, a demonstrator in anatomy in the University of Manchester. He was promoted lecturer in 1915 and in the same year he received the degree of M.D. with a Gold Medal for a thesis of outstanding merit on "The Arteries of the Pons and Medulla". He succeeded to the Manchester chair of anatomy in 1919 on the resignation of Prof. (later Sir) Grafton Elliot Smith, and in December 1934 he was appointed vice-chancellor in succession to Sir Walter Moberley. He combined his duties as vice-chancellor and professor of anatomy until 1938, when he resigned his chair and was appointed to a personal chair of experimental neurology.

His ability as a scientist was recognized by his election to the fellowship of the Royal Society in 1927, and he was the first Manchester medical graduate to achieve this high honour. Under the inspiration of Elliot Smith, his research work was predominantly neurological, and he collaborated successfully with his surgical colleagues (Sir Harry Platt, Sir Geoffrey Jefferson and Profs. E. D. Telford and John Morley) in the elucidation of various problems. His work can be divided roughly into three phases—his investigations on the blood supply of the brain, his anatomical and clinical studies on sensation, based mainly on the observation of many patients with peripheral nerve injuries, and his researches on the structure and function of various parts of the autonomic nervous system. He published more than sixty papers and an important monograph on "Sensation and the Sensory Pathways" (1930), besides contributing sections to several anatomical text-books. As vice-chancellor he was unable to indulge his flair for correlating basic and clinical research, but had he continued as a scientist he would undoubtedly have enhanced his already high reputation.

In a short notice one cannot even mention all that Stopford did for education and his University; but he was the best vice-chancellor Manchester has ever had and according to Sir Hector Hetherington (1956) "the best of us all" (the "all" refers to the members of the Committee of Vice-Chancellors and Principals). His worth was recognized nationally by his appointment as chairman, vice-chairman or member of such influential bodies as the General Medical Council and the University Grants Committee, the Commonwealth Fund, the Nuffield Foundation, the Interdepartmental Committee on Medical Schools, the Universities Bureau of the British Empire and the Council of the Royal College of Physicians, while locally he served as chairman of the Manchester Regional Hospital Board (1948–53), of the John Rylands Library, of the Royal College of Music, and on many other bodies. He was awarded honorary degrees by the Universities of Dublin, Leeds, Cambridge, Liverpool, Durham and Manchester, and honorary fellowships by the Royal College of Physicians and Royal College of Surgeons. He was knighted in 1941, created a K.B.E. in 1955, and he was included in the first list of life peers (1958).

He was a fine and imaginative teacher who helped to rescue anatomy from the topographical morass into which it had fallen, and who did much to close the gap which had developed between the basic medical sciences and clinical practice. He was a pioneer in the use of radiological methods in teaching living anatomy, and he was the first to take over from physiology the full responsibility for teaching histology. Both as a professor and vice-chancellor his sympathy with students and their problems was evident; and he was appreciated as highly by his colleagues in the Arts and other Faculties as by those in his own profession. His familiarity with education and research in all their manifold aspects was utilized, as the abbreviated list of his appointments given above indicates, by many Government and other bodies; and he was the chief inspiration for many of the far-sighted reforms in medical education recommended in the Goodenough Report.

He married Lily Allan, another Manchester medical honours graduate, in 1916, and they had one son. Theirs is the greatest loss; but those who served under him honour the memory of a distinguished scientist and supreme administrator and marvel that one who had gained so many honours could yet remain so human, humble and kind.

G. A. G. Mitchell

Miss Ethel F. Bellamy

The death of Ethel F. Bellamy on December 7 at the age of seventy-eight removes one of the last of those self-trained and dedicated workers who have done so much for astronomy and seismology in Great Britain. Miss Bellamy went to the Oxford University Observatory in 1899 as a personal assistant to her uncle, the late F. A. Bellamy, and was able to relieve him of some part of the work in the preparation of the Oxford zones of the *Astrographic Catalogue*. On the completion of these zones and under the direction of Prof. H. H. Turner, Miss Bellamy then undertook the reduction of the measures for the Vatican zones, work for which she was awarded a commemorative silver medal by the Pope in 1920.

At the end of the First World War, Prof. Turner had agreed to continue at Oxford the seismological work of John Milne. Miss Bellamy assisted Turner, and in this connexion spent some months in 1919 at Shide in the Isle of Wight in charge of Milne's instruments. This responsibility she continued when the improved Milne–Shaw seismographs were transferred first to the Boys's basement in the old Clarendon Laboratory and ultimately in 1928 to the University Observatory itself. Her great contribution to seismology lay, however, in the preparation of the *International Seismological Summary* (prior to 1923 the

Bulletin of the British Association Committee on Seismology). Her part was to collate the readings, in some cases measuring the original records, of earthquakes as recorded initially by 200, afterwards nearly 600, seismological stations throughout the world, and to maintain year after year a correspondence with these stations which did much to improve the accuracy of time-keeping. Originally, Turner computed the epicentres from these collated readings, but from 1923 a greater and greater part of this work was taken over by J. S. Hughes, now the director of the *International Seismological Summary* at Kew. From these epicentres and the computed residuals for each station Miss Bellamy then prepared the copy for the press and read the proofs. This work she continued until the transfer of the *Summary* to Kew in 1946, adding to it during Hughes's war service the computation of the epicentres for six quarterly issues of the *Summary*. Time also was found to prepare a catalogue of epicentres from the *International Seismological Summary* for the years 1925–30, and in 1936 a complete *Index Catalogue of Epicentres* for the years 1913–30 (Isle of Wight County Press) together with a frequently reproduced equal-area map showing the location of these epicentres on the Earth's surface. A brief account of this work with some of the original maps appeared in *Nature* (143, 504; 1939).

Dogged throughout her life by ill-health and working in discomfort for many years in an old wooden hut in the Observatory grounds, nothing was ever allowed to stand in the way of the quality and quantity of her work. It was only fitting that she should have been awarded an honorary M.A. by the University of Oxford, whose faithful servant she had been for so many years. Retiring in 1947, she spent her last years in Upwey, near Weymouth.

H. H. Plaskett

NEWS and VIEWS

Element 103, Lawrencium

The creation of lawrencium, the eleventh new man-made element beyond uranium (element 92), was announced on April 13 by the University of California and the U.S. Atomic Energy Commission. An isotope of this element was formed on February 14 by bombarding 3 µgm. of californium (element 98) with boron-10 or boron-11 nuclei in a heavy-ion linear accelerator at the Lawrence Radiation Laboratory of the University of California; the four nuclear scientists who carried out this experiment, Drs. A. Ghiorso, T. Sikkeland, A. Larsh and R. Latimer, propose to name the new element lawrencium in honour of the late Ernest Orlando Lawrence, inventor of the cyclotron and founder of the laboratory which bears his name. Californium, used as the target material, is made by the prolonged irradiation of trans-uranium elements in a nuclear reactor by the successive addition of neutrons. Since each new heavy element is more unstable than the one before, this procedure is inapplicable to the heaviest elements and heavy-ion bombardment is the only route whereby they may be created. In addition, the identification of the heaviest elements prepared in this way becomes progressively more difficult with increasing atomic number owing to their instability and to the very few atoms of the product which are formed. Although it may be possible to create one or two more new elements by this technique, it is improbable that elements beyond number 105 will be synthesized and satisfactorily identified.

Ministry of Aviation : Mr. J. R. Mills

Mr. J. R. Mills has been appointed head of the Radio Department, Royal Aircraft Establishment, Farnborough. Educated at the Grammar School, Kingston-on-Thames and King's College, London, he completed in 1939 the special honours course in physics. Shortly after war broke out Mr. Mills joined the Air Ministry Research Establishment, Dundee (formerly Bawdsey Research Station). It was here that he began his connexion with radar. Between 1940 and 1944 he divided his time between the Ministry of Aviation Radio Department, the Royal Aircraft Establishment and the Telecommunications Research Establishment, working throughout most of this period on the problems of secondary radar systems, mainly in the metric wave-bands. In 1946 Mr. Mills was a member of the team at the Telecommunications Research Establishment which had been set up to develop electronic systems for use in civil aviation, his particular interest lying in the development of distance measuring equipment. During this period his group was one of the first in the United Kingdom to investigate the use of overtone crystals as frequency sub-standards. In 1950 he returned to the military field and concentrated on techniques for measuring range to a very high order of accuracy. From 1952 he led a team set up in the Telecommunications Research Establishment to carry out research into new forms of airborne surveillance radar and, in particular, to exploit the new wave-band (Q) centred on a wave-length of 0·86 cm. During 1954–60 Mr. Mills was the superintendent in the Airborne Radar Department, Royal Radar Establishment (formerly Telecommunications Research Establishment and the Radar Research Development Establishment), in charge of research and development concerned with the offensive airborne operational needs of the Royal Air Force and Royal Navy. For a brief period commencing in October 1960 he was the assistant director at Ministry of Aviation Headquarters concerned specifically with the electronic problems of civil aviation.

Mr. J. H. Briggs

Mr. J. H. Briggs has been appointed director of electronics, research and development (civil aviation) in the Ministry of Aviation. Mr. Briggs was educated at King Edward's School, Birmingham, and obtained a major open scholarship in natural sciences at St. John's College, Cambridge, where in 1935 he graduated with honours in the Mathematical Tripos Part I and Natural Sciences Tripos Part II (physics). After a year and a half in the carrier communications development laboratory of the General Electric Co. he joined the Government service at the Royal Aircraft Establishment, where for three years in the Radio Department he was engaged on direction-finding research and development. On transfer to the Air Ministry Research Establishment at Swanage in 1940 initial work on radar aerial systems led to anti-surface vessel development and then to a period of liaison work with Coastal Command to investigate the utilization of anti-surface vessel Mk. 2 and prepare for the introduction of centimetre anti-surface vessel for the Battle of the Atlantic. On return to the Telecommunications Research Establishment at Malvern a period in charge of test-gear development was followed at the end of the War by the organization and running of a research programme on microwave measurements in conjunction with the Radio Research Station of the Department of Scientific and Industrial Research. During this period he was for a time chairman of the Inter-services Radio Measurement Committee (later the Radio Electronic Measurements Committee).

In 1950 Mr. Briggs undertook a programme of work involving simulation and computation to establish a mathematical model of a homing test vehicle for comparison with the results of flight trials. In 1955 he became a superintendent in the Ground Radar Department, Radar Research Establishment, in charge of the development of data handling systems, and as such was intimately concerned with the overall design of air defence systems. These interests resulted in participation in extensive negotiations within the Nato framework in connexion with the co-ordination of European defence requirements—particularly in the field of military data-processing and exchange. The recently renewed interest in co-ordination of defence and air traffic control requirements brought him into close touch with civil aviation problems so that he is particularly well qualified for his new appointment.

United Kingdom Atomic Energy Authority Appointments

The Atomic Energy Authority and the National Institute for Research in Nuclear Science have agreed that the development of particle accelerators hitherto carried out by the Accelerator Division, Atomic Energy Research Establishment, Harwell, should be the responsibility of the Rutherford Laboratory of the National Institute for Research in Nuclear Science. Mr. L. B. Mullett, the head of the Accelerator Division, has joined the Rutherford Laboratory as assistant director for accelerator and applied physics. Other appropriate staff transfers have already been made.

Consequent on the appointment of Dr. R. Spence as deputy director of the Atomic Energy Research Establishment, Harwell, Dr. W. Wild has been appointed head of the Chemistry Division. He has responsibility for the scientific work of three branches: Chemical Processing and Reactor Chemistry; Radiation Chemistry and Solid State Chemistry; Radiochemistry. Mr. A. A. Smales, head of the Analytical Chemistry Branch, has been given the status of a division head and will be directly responsible to the director of the Establishment for the scientific work under his control. His branch will continue to use the general administrative services of the Chemistry Division.

Day-release Students in England and Wales

In a written answer in the House of Commons on April 14, the Minister of Education, Sir David Eccles, gave the number of day-release students under 18 years of age enrolled in major establishments of further education in England and Wales in 1959–60 as 191,526 boys and 50,976 girls. Of these, 91,009 boys and 23,419 girls were in the English counties (including London), and 91,070 boys and 26,247 girls in the English boroughs; only 6,501 boys and 857 girls were in the Welsh counties, and 2,946 boys and 453 girls in the Welsh boroughs.

Educational Statistics for England and Wales

As a complement to the national statistics which appeared in his annual report, "Education in 1959", the Minister of Education has now issued a wide range of statistical material concerning local education authorities in England and Wales (Selected Statistics relating to Local Education Authorities in England and Wales, Educational Year 1958–1959. Pp. ii+25. (London: H.M. Stationery Office, 1960.) 3*s*. net). The booklet contains estimates of the total population of the two countries and statistics of the total number of scholars in primary and secondary schools, and in training colleges in counties and county boroughs in England and Wales. There is also interesting information about the number of medical officers, dental officers and nurses serving each local authority; the number of pupils receiving milk and school meals; the number of awards made by local authorities to students wishing to study at a university or elsewhere—the latter show that sufficient discrepancies occur to make implementation of the full recommendations of the Anderson Committee a matter of urgent necessity.

U.S. Project Mohole

The National Academy of Sciences and the National Science Foundation announced recently that the experimental drilling ship, *Cuss I*, operating in 12,000 ft. of water off the west coast of Mexico in the Project Mohole, obtained, for the first time, a sample of the so-called second layer under the softer sediments of the ocean floor. This layer, the presence of which has been previously known only by reflexions of seismic soundings, has been revealed as basalt. This hole, which was begun at Guadalupe Island on March 31, reached a depth of 490 ft. after 11 hr. of drilling. Coring then began, and cores of soft, grey-green clay of Miocene age were obtained to a depth of 560 ft. At that depth the drilling-rate abruptly decreased and when the core barrel was retrieved, it was found to contain a hard, fine-grained basalt; the drilling had achieved threshold to the sima.

Animals for Cancer Research

The provision of animals for cancer research was discussed at a symposium sponsored by the Laboratory Animals Centre of the Medical Research Council Laboratories on May 23, 1960. Discussion was mainly concerned with the desirability of providing suitable animals from a central organization or whether users should provide their own locally. The dilemma was posed by Prof. J. S. Mitchell, of the University of Cambridge, who acted as chairman of the symposium, and indicated that the provision of animals for research needs expert knowledge, including that of mammalian geneticists and veterinary experts. The problem is not only one of size but also involves many technical problems, including. for example, continued testing to make certain the strains are authentic, and maintenance of the production of disease-free animals. The great contribution to cancer research has been the development of inbred strains of animals, especially mice, and the use of $F1$ hybrids between two inbred strains. At the present time, about eighty inbred strains of mice are known. Much less seems to be known about the genetics of rats. There are a few, perhaps not more than five, pure strains of rats. It is questionable whether the maintenance of all these strains, that is, about eighty inbred strains of mice and perhaps five inbred strains of rats, is necessary for cancer research. Prof. Mitchell believes that there is need to establish a large organization with expert staff and proper financial support to provide animals and tumours needed for cancer research. A full report of the symposium has now been published by the Centre (Collected Papers, Vol. 9, 1960. Pp. 116. Carshalton, Surrey: Laboratory Animals Centre, Medical Research Council Laboratories, 12*s.* 6*d.*).

Paranormal Phenomena

ALTHOUGH almost all physiologists regard the brain as in some sense the 'organ of mind', it has to be admitted that our knowledge of its action is still distressingly incomplete. In his fourteenth Frederic W. H. Myers Memorial Lecture, Dr. W. Grey Walter provides a most useful survey of recent work on the neurophysiological aspects of hallucinations and illusory experience (*The Neurophysiological Aspects of Hallucinations and Illusory Experience.* Pp. 24. (London: Society for Psychical Research, 1, Adam and Eve Mews, W.8, 1960.) 2*s.* net). As he points out, it is now possible to evoke by experimental techniques a variety of bizarre subjective states long thought to be peculiar to epilepsy or kindred nervous disorder. These techniques include rhythmic photic stimulation, sensory deprivation and the use of psychotomimetic drugs; the results obtained go far to justify Dr. Grey Walter's hope that many phenomena formerly consigned to the occult may come to achieve reputable neurophysiological explanation. At the same time, he finds little to say about those aspects of hallucinations which particularly interested Myers, such as their occasional (though apparently well-authenticated) telepathic character. It would seem, then, that neurophysiology has still far to go before it can provide an acceptable explanation of paranormal phenomena.

The Raffles Museum, Singapore

The Bulletin of the Raffles Museum, Singapore (No. 26; February 1961), maintains the high standard of research results that is now associated with this well-known publication. All the papers deal either with zoological or palæontological subjects based on Malaya and are well and clearly illustrated. An interesting experiment relates to an attempt to hatch the eggs of the leathery turtle, and a brief account of the geology of the limestone hills of Malaya is an important contribution to the stratigraphy of the region.

Museums Association: Monthly Bulletin

AFTER publishing the *Museums Journal* each month for 60 years without a break it has now been decided to issue a monthly *Bulletin* and a quarterly *Journal.* The first issue of the former (No. 1, April 1961) has just appeared. It is a 4-page production giving essential and current museum news and notices, obituary notices, recent appointments, letters to the editor, a list of temporary exhibitions in certain museums and details of posts now vacant in museums and art galleries.

'Araldite' in Museum Work

Technical Notes, No. 218, issued by C.I.B.A. (A.R.L.), Ltd., gives some interesting examples of the use of 'Araldite' in the numerous problems confronting museum staffs ('Araldite' in Museum Work. Pp. 10. Duxford: C.I.B.A. (A.R.L.), Ltd.; February 1961). The resin can be used either to assemble broken and fragile articles or to reinforce and consolidate museum objects and thus preserve them for posterity. The examples quoted include the Kentmere boat unearthed in Westmorland during 1955 strengthened by the use of a formulation consisting of 'Araldite' *CY* 219 (50 parts), hardener *HY* 219 (25 parts), accelerator *DY* 219 (1 part) and dibutyl phthalate (10 parts). Other objects dealt with in a somewhat similar manner were a Nigerian ceremonial mask, the St. Ninian's hanging bowl dating from about A.D. 800, and a Belgic wrought-iron frame. Other museum objects quoted are from the Royal Scottish Museum, Edinburgh, where repair work with 'Araldite' has been carried out with success including colour matching if the glue line is rather wide.

Poultry Keeping

KEEPERS of fowls, ducks and geese will welcome the publication by the Ministry of Agriculture, Food and Fisheries of its two Bulletins on these birds. One of these, entitled *Poultry Breeding* (Bulletin No. 146. Third Edition. Pp. vi+146. By G. E. Mann. London: H.M.S.O., 1960. 7*s.* net), contains 146 pages of valuable information written by G. E. Mann, poultry advisory officer of the National Agricultural Advisory Service, on the genetics of poultry, the inheritance of breed and economic characteristics and on mating systems and systems of breeding, with a glossary explaining technical terms. One of its aims is to give the smaller breeder practical information about the methods used by the larger breeders. The other Bulletin, entitled *Ducks and Geese* (Bulletin No. 70. Pp. v+93+8 plates. Fourth Edition. London: H.M.S.O., 1960. 6*s.* net), will interest those who find that these birds are not only interesting in themselves, but also profitable either as a large-scale enterprise or as a side-line. Their rate of egg production and of food conversion is high, and this Bulletin takes the reader step by step through the intricacies of choosing a good breed, methods of breeding, management, rearing and fattening, and of the marketing of eggs and birds. There is also some information about by-products, and the diseases of ducks and geese and their prevention and control. Written by experienced contributors, this Bulletin provides sound, practical advice. It has five appendixes on nutrition, the preparation of the birds for the oven, feeding trials, losses of weight due to plucking and trussing ducklings and a summary of the main breeds and their characteristics. Its eight pages of excellent photographs include portraits of ten breeds and pictures of how to handle birds when they are being sexed. Its twelve line-illustrations are all also of practical value.

Test on Recording Spectrophotometers

THE Photoelectric Spectrometry Group is organizing a collaborative test on commercially available recording ultra-violet spectrophotometers which will be based, so far as possible, on the successful test on manually operated instruments that was reported in the *Photoelectric Spectrometry Group Bulletin*

No. 4 (1951). A pilot run to establish the conditions of the test has been successfully conducted on approximately 10 instruments of 5 different makes. It is hoped that as many separate examples of all commercially available instruments can be included in the full-scale test, which it is expected will be initiated in October of this year. Sample solutions will be supplied to those who agree to enter the test and they will be asked to run spectra (*a*) under predetermined instrumental conditions, (*b*) under their own preferred conditions. The analysis of the results of the test will be published in the *Bulletin*. Anyone who would like to enter a recording ultra-violet instrument should write to the honorary secretary, K. A. Macdonald, Unicam Instruments, Ltd., Arbury Works, Cambridge.

The Devon Naturalists Trust

A BODY, to be known as the Devon Naturalists Trust, has been formed with the support of many national and local bodies including the Nature Conservancy, the Council for Nature and the Devonshire Association. Lord Roborough is to be president of the Trust, and Mr. H. G. Hurrell chairman of its executive committee. The aims of the Trust are to protect places of interest to naturalists, to preserve the county's wild-life against encroachment, and to encourage the study of natural history. These aims should appeal to many who are concerned at the tide of change that has already much reduced the natural amenities of other counties, and is beginning to do so in Devon, too. It will be one of the main concerns of the Trust to see that developments of this sort are kept under constant review. There will be a meeting in Exeter to invite support and to publicize the Trust on May 16. Further information can be obtained from the honorary secretary, C. P. E. Grant, Chain House, Modbury, Ivybridge, Devon.

Grants for Field Work in Natural History

AT the suggestion of the Council for Nature, the Carnegie United Kingdom Trust has set aside a fund from whch it will make grants to approved societies for assistance with field work. These grants will be made on recommendations submitted by the Council for Nature, and will be contributions towards expenditure incurred in carrying out specific projects that give amateurs experience of field work in natural history under skilled direction. Approved expenditure is likely to include fees paid to experts for instruction and leadership, the incidental expenses of such people and the cost of essential equipment subject to certain conditions. Grants will not be available for the expenses incurred by members of the societies taking part in any scheme or for the general support of a society. Full particulars of the conditions for the award of grants can be obtained from the Council for Nature, 41 Queen's Gate, London, S.W.7.

Library of Recorded Animal Sounds

A LIBRARY of recorded animal sounds is being set up by the Committee for Biological Acoustics in collaboration with the British Institute of Recorded Sound, 38 Russell Square, London, W.C.1, in the archives of which the records will be stored. The records will be made on magnetic tape and recorded at a standard speed of 7½ in. per sec.; each record will contain a commentary giving physical and biological data in relation to the recording. The recordings will be available free on loan to bona fide research workers anywhere in the world and the only expenses will be postage of the tapes. The library is being supported by a grant for equipment from the Royal Society. As soon as a reasonable number of recordings have been amassed a catalogue will be prepared and sent free of charge to all interested workers, departments and institutions. It is hoped that all workers in the field will give their support to the project, and those willing to allow their recordings to be copied for the library are asked to write to Dr. P. T. Haskell, Committee for Biological Acoustics, c/o Anti-Locust Research Centre, 1, Princes Gate, Kensington, London, S.W.7.

Japan Association for Study of Congenital Anomalies

THE Japan Association for Study of Congenital Anomalies held its initial meeting at the Kyoto University, Faculty of Medicine, on February 25, 1961. For the time being, the Association has been established on an informal basis, by an organizing group consisting of the following members: Prof. Chisato Araki (Kyoto University); Prof. Tameyoshi Baba (Osaka Municipal University); Prof. Ichiro Hayashi (Nagasaki University); Prof. Yoshiki Mikami (Mie Prefectural University); Shigeru Mitani (Tokyo Women's Medical College); Prof. Ujihiro Murakami (Nagoya University); Prof. Hideo Nishimura (Kyoto University). Prof. Nishimura and a member of the organizing group will be in charge of international affairs.

Association of Consulting Scientists

AT the annual general meeting of the Association of Consulting Scientists, held in London recently, the following honorary officers and members of council were elected: *chairman*, Dr. M. Barent (Barent and Johnson); *vice-chairman*, Mr. F. G. Sarel Whitfield (Avebury Research Laboratories, Ltd.); *honorary treasurer*, Dr. G. W. Ferguson (Parry and Ferguson); *honorary secretary*, Mr. W. H. Stevens (W. H. Stevens); *members of council*, Dr. H. H. Chambers (Sondes Place Research Laboratories, Ltd.), Dr. J. G. Davis (Dr. J. G. Davis and Partners), Dr. Julius Grant (Hehner and Cox, Ltd.), Dr. R. F. Milton (R. F. Milton), Mr. O. W. Roskill (O. W. Roskill).

1961 Waverley Gold Medal Essay Competition

THE Waverley Gold Medal essay competition is sponsored by *Research* to encourage scientists to write clearly and comprehensibly about their work. The Medal, together with £100, will be awarded for the best essay of about 3,000 words describing a new project or practical development in pure or applied science, giving an outline of the scientific background, the experimental basis and the potential or actual application of the idea to industry or its importance to society. A second prize of £50 will be awarded and also a special prize of £50 for the best entry from a competitor under the age of thirty on July 31. Entry forms and further information can be obtained from the Editor of *Research*, 88 Kingsway, London, W.C.2. The last date of entry is July 31.

Film-Strips on Physical Science

A CLASSIFIED list of their film-strips and wall-charts in physics, chemistry and general science has been issued by Educational Productions, Ltd., of East Ardsley, Wakefield, Yorks. The strips deal with such topics as the structure of matter, electricity and light, while there is also a whole series of wall-charts linked to the products of prominent industrial organizations and produced in collaboration with them. Wall charts are also available to describe lessons in astronomy and space exploration.

Guide to Careers

As a link with the Commonwealth Technical Training Week which is to be held during May 29–June 4, the 1961 edition of the *Guide to Careers* published by the National Union of Teachers is mainly concerned with the training of young people in industry and commerce (Pp. viii+80. London: National Union of Teachers. 1961). In a foreword to the Guide, H.R.H. the Duke of Edinburgh directs attention to the need for careful choosing of careers. Many experts describe the different careers available to young people, and these are supported by even more advertisements in which the advantages of a particular company or organization are displayed. School-leavers themselves are likely to find the mass of generalizations somewhat overwhelming, and will seek refuge in the related experiences of some young people who have already tried out certain jobs for themselves. Even these tend to be over-glamorized and should be balanced by descriptions from young people with experience of jobs somewhat more mundane than that of staff manageress or management trainee.

Postgraduate Courses in London

PARTICULARS of full-time postgraduate courses of one or two years duration which lead to a degree or diploma are contained in a booklet *Postgraduate Courses*, 1961–62, issued by the Imperial College of Science and Technology (Pp. xii+114. London: Imperial College of Science and Technology, 1961). These courses, which have been accepted by the Department of Scientific and Industrial Research as suitable for the tenure of advanced course studentships, are indicated, and besides outlining the content of the individual courses, their length and the respective lecturers, the booklet includes the regulations for the diploma of the Imperial College of Science and Technology.

Gordon Research Conferences 1961

THE Gordon Research Conferences have justly achieved international fame as effective instruments for the exchange of ideas and information between research scientists drawn from industrial, Government and academic laboratories, and the organizers have been paid the great compliment of having their methods copied in other countries. Papers are presented at morning and evening sessions over a period of four and a half days and the formal programme is often very slight, for example, in the 1961 Petroleum Conference only six papers are scheduled for formal presentation. There is ample time for discussion, the environmental and residence conditions are all favourable and the selected membership ensures that the discussion is free and of a high level. As in previous years a conference will be held each week during June 12–September 1 at each of three residential schools in New Hampshire, and the subjects cover a wide range in the physical and biological sciences. Residence and registration charges for a week amount to 100 dollars. Further information can be obtained from Dr. W. George Parks, Department of Chemistry, University of Rhode Island, Kingston, R.I. Applications for attendance should be submitted before June 12.

University News:

Bristol

THE following appointments to senior lectureships have recently been announced: Mr. R. Bassindale (zoology); Dr. A. Couper (inorganic and physical chemistry); Dr. F. G. Hannell (geography); Dr. J. R. Holmes (veterinary medicine); Dr. W. K. Metcalf (anatomy); Mr. W. H. Dearden (metallurgy); Dr. M. S. G. Cullimore (civil engineering).

Cambridge

THE Arnold Gerstenberg studentship for 1962 is open to students in the University of Cambridge who, going into residence not earlier than 1956, have obtained honours in the Natural Sciences Tripos and who wish to pursue a course of philosophical study. The studentship will be awarded for an essay, which must be submitted before May 1, 1962. Further information can be obtained from Mr. Gerd Buchdahl, Whipple Science Museum, Free School Lane, Cambridge.

London

THE following announcement has been issued by the Imperial College of Science and Technology: Prof. D. M. Newitt, pro-rector and head of the Department of Chemical Engineering and Chemical Technology, who retires on September 30, has been appointed senior Research Fellow of the College. Prof. P. M. S. Blackett has been appointed pro-rector as from October 1, and Prof. A. R. Ubbelohde as head of the Department of Chemical Engineering and Chemical Technology. The following have been elected Fellows of the College: Prof. O. M. B. Bulman, Woodwardian professor of geology, University of Cambridge; Prof. A. C. Chibnall, professor emeritus, former professor of biochemistry; Mr. T. G. N. Haldane, member of the Governing Body of the College; Dr. D. T. A. Townend, director-general of the British Coal Utilization Research Association; Dr. J. H. Watson, chief assayer, Royal Mint.

Dr. A. H. Chilver, a director of studies in engineering, University of Cambridge, has been appointed to the Chadwick chair of civil engineering tenable at University College. Dr. F. G. Bailey, lecturer in Asian anthropology at the School of Oriental and African Studies, has been appointed to the University readership in Asian anthropology tenable at that School. Dr. E. G. Gray, lecturer at University College, has been appointed to the University readership in cytology tenable at that College.

Titles have been conferred as follows: Sir William Collins professorships, on Mr. G. W. Causey (anatomy) and Dr. G. J. Cunningham (pathology) in respect of the posts held by them at the Institute of Basic Medical Sciences; reader in veterinary physiology at the Royal Veterinary College, on Dr. F. R. Bell; reader in anatomy at St. Mary's Hospital Medical School, on Dr. A. S. Breathnach; reader in physics at University College, on Dr. F. F. Heymann; reader in chemistry at University College, on Dr. J. Lewis; reader in chemical engineering at University College, on Dr. J. W. Mullin.

Sheffield

THE title of Dr. G. Weber's readership has been announced as reader in biophysics. J. E. Enderby has been appointed as a lecturer in physics; and Dr. J. V. S. Mani has been appointed an Imperial Chemical Industries research fellow in the Department of Fuel Technology and Chemical Engineering.

Southampton

THE origins of the University of Southampton lie within the Hartley Institute, established from a bequest by H. R. Hartley in 1862. Towards the University's centenary appeal, Hawker-Siddeley Aviation, Ltd., is contributing a sum of £28,700, over a period of seven years, for a readership and a lectureship in the Department of Aeronautics and Astronautics; Alcan Industries, Ltd., is contributing a sum of £10,000.

The title of senior lecturer has been conferred on Mr. K. N. C. Bray (aeronautics and astronautics), Dr. W. A. Gambling (electronics), Dr. N. S. Grassam (mechanical engineering) and Dr. A. C. Riddiford (chemistry). The following new appointments have been made: Mr. V. J. D. Baston, Dr. H. Holstein and Mr. C. R. F. Maunder (lecturer in mathematics); Mr. W. S. Hall and Mr. T. Sprinks (Research Fellows in mathematics); and Mr. G. C. Spencer (lecturer in mechanical engineering).

Announcements

SIR HAROLD ROXBEE COX, formerly chief scientist of the Ministry of Fuel and Power and at present chairman of the Metal Box Co., Ltd., has been appointed chairman of the Council for Scientific and Industrial Research for five years from October 1, 1961, in succession to Sir Harry Jephcott, who completes his term of office in September.

MISS M. J. BISHOP, headmistress of the Godolphin and Latymer School, Hammersmith, has been appointed a member of the University Grants Committee in succession to Dame Kitty Anderson, who resigned from the Committee on her appointment as a member of the Committee on Higher Education. Miss Bishop's appointment runs to December 31, 1963.

THE United Nations Food and Agriculture Organization is organizing a technical meeting on "Plant Exploration and Introduction", to be held in Rome during July 10–20. Further information can be obtained from the Food and Agriculture Organization, Viale delle Terme di Caracalla, Rome.

A TECHNICAL meeting of the United Nations Food and Agriculture Organization to discuss "Plant Exploration and Introduction" is to be held during July 10–20, at the headquarters of the Organization in Rome, from which further information can be obtained.

THE summer conference of the Society for Applied Bacteriology will be held at Wye College, Kent, during July 11–13. Included in the programme is a symposium on "Bacteria of the Intestine". The conference is open to all, and further information can be obtained from Dr. Ella M. Barnes, Low Temperature Research Station, Downing Street, Cambridge.

THE second congress of the International Federation of Societies of Cosmetic Chemists, organized by the Society of Cosmetic Chemists of Great Britain, will take place in London during July 2–5. Further information can be obtained from A. Herzka, c/o Pressurized Packaging Consultants, Ltd., Ashbourne House, Alberon Gardens, London, N.W.11.

THE sixty-sixth annual congress of the South-Eastern Union of Scientific Societies will be held at Haslemere, Surrey, during May 26–28. The theme of the congress will be "The Changing Country-side" and the programme will include a number of lectures, a brains trust and various excursions. Further information can be obtained from the general secretary and treasurer, Frank Edwards, 53 The Drive, Shoreham-by-Sea, Sussex.

THE eighth International Cancer Congress will be held in Moscow during July 22–28, 1962, under the auspices of the International Union against Cancer. The Congress will meet at the Moscow State University and will consider both experimental and clinical aspects of the problem. Further information can be obtained from the general secretary of the Soviet National Organizing Committee, Prof. L. Shabad, or the assistant general secretary, Dr. N. Perevodchickova, Academy of Medical Sciences of the U.S.S.R., 14 Solyanka, Moscow.

AN international exhibition, devoted to measurement control, regulation and automation, is being held in Paris during May 9–17. The exhibition is being organized by the MESUCORA Association in conjunction with the Fédération des Industries Mécaniques et Transformatrices des Métaux, the Syndicat Général de la Construction Electrique, and the Fédération des Industries Electroniques. This event will also include the annual exhibition of the Société Française de Physique. Further information can be obtained from Congress International, MESUCORA, 40 Rue du Colisée, Paris, France.

HATFIELD TECHNICAL COLLEGE is organizing a series of lectures on the general theme "Technology, Science and Art—Common Ground". The details are as follows: "Science in History" by Prof. J. D. Bernal (May 5); "Poetry and Science" by T. R. Henn (May 12); "Science and Ethics" by Dame Kathleen Lonsdale (May 26); "Science and the Classics" by Prof. B. Farrington (June 2); "Art and Engineering" by Prof. M. Black (June 23). All lectures will be held in the Old Library of the College on the dates indicated. Further information can be obtained from Mr. I. O. McLuckie, chairman, Teaching Staff Association, Hatfield College of Technology, Hatfield, Herts.

THE 1961 Conference on Medical Electronics, which is to be held at the Waldorf Astoria Hotel, Park Avenue and 49th Street, New York City, N.Y., during July 16–21, will be a combined meeting of the fourth International Conference on Medical Electronics and the fourteenth Annual Conference on Electrical Techniques in Medicine and Biology. It is to be sponsored by the Joint Executive Committee on Medicine and Biology under the auspices of the International Federation for Medical Electronics, and is organized by the Institute of Radio Engineers. Further information can be obtained from the Secretary-General, Fourth International Conference on Medical Electronics, Institute of Radio Engineers, 1 East 79th Street, New York 21, N.Y.

A COURSE in tropical hygiene for laymen will be held by the Ross Institute during July 3–7. The course is designed specially for planters, mining engineers and other non-medical people who may be responsible for the health of industrial labour forces in the tropics. Admission is not restricted to such people, however, but is open to any non-medical men and women proceeding to the tropics. Further information can be obtained from L. G. Ponsford, organizing secretary, Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London, W.C.1.

MALNUTRITION

ON March 18, the Nutrition Society held, at the London School of Hygiene and Tropical Medicine, a symposium on "The Place of Food Science and Technology in the Campaign against Malnutrition". The symposium was primarily concerned with malnutrition in the under-developed countries, and in particular with malnutrition in children in the 'toddler' stage.

There is much evidence that malnutrition associated with lack of protein and calories is common in countries in which supplies of foods of animal origin, especially animal milk, are low; it is, in fact, the most common of all food deficiency states in the world as a whole. Its manifestations range from retardation in growth to serious disease and death. The West African word 'kwashiorkor' is now widely used to describe a form of the disease, with characteristic signs, occurring in children whose diet is grossly deficient in protein and less deficient in calories. The wider term 'protein–calorie malnutrition', covering the effects of both lack of calories and lack of protein, usually found together in the victims, is preferred by many workers, but it should be added that at present there is some confusion with respect to nomenclature.

As the result of intensive research during the past ten years in many parts of the world, the factors underlying protein–calorie malnutrition—dietary, agronomic, economic and social—are now well understood, but relatively little progress has as yet been made in prevention. Through food science—meaning in this context the study of foods and food values in relation to requirements—appropriate measures for correcting deficiencies can be demonstrated, and food technology, wisely applied, can increase supplies of nutritious foods pending, and in association with, the expansion of food production.

The symposium began with five papers, under the heading of "Problems and Some Solutions", given by workers from India (Dr. K. K. Halder), Turkey (Dr. O. Köksal), Iraq (W. Frankul), Pakistan (S. M. Ali) and Ghana (R. Orra ca-Tetteh) respectively, who are engaged in research at the Human Nutrition Research Unit at Mill Hill, under the direction of Prof. B. S. Platt. Each speaker outlined the background of the problem in his country and described some locally feasible method or methods, based on recent research, of making diets more satisfactory. These papers brought out on one hand the basic similarity of the situation in various parts of the world, and on the other, the differences from country to country which call for a local approach in applying food science and technology effectively. In introducing these contributions, Prof. Platt commented on the value of providing postgraduate experience in nutrition research and its application to workers from countries in which there are serious problems of nutrition and a shortage of trained personnel to deal with them.

A paper on "Some African Problems" by Dr. F. Aylward (Department of Chemistry and Food Technology, Borough Polytechnic, London) reflected the author's experience as adviser to the Ministry of Agriculture in Ghana in the capacity of a technical assistance expert for the Food and Agriculture Organization. He analysed the contributions which food technology can make in increasing supplies of nutritious foods available for consumption, suggesting various categories into which these contributions can be grouped. His paper was concerned with the application of food technology in ways which do not demand big capital outlay, rather than with the development of large-scale food industry. He considered that under African conditions there is an important place for small food-processing plants in rural areas, with low maintenance costs and minimum requirements for technical supervision. Dr. J. W. Corran (J. and J. Colman, Ltd., Carrow Works, Norwich), who dealt with "Factors Affecting Development of Food Science and Technology and Food Resources in Pakistan", also drew on experience gained during a Food and Agriculture Organization assignment as a technical assistance expert. He directed attention to the lack of accurate data about food consumption and state of nutrition and of reliable agricultural statistics, which handicapped attempts to formulate and execute programmes to develop food production and food technology. For example, there were widely conflicting estimates of supplies of fish, an important food in East Pakistan. While Pakistan greatly needed trained food scientists and technologists, the number of active workers in these fields could at present be counted on the fingers of one hand. Facilities for training such workers in Pakistan did not exist, and even those available in the United Kingdom were far from satisfactory. Pakistan urgently needed the help of food technologists from abroad to foster the growth of food industry.

Dr. E. Wokes (Vegetarian Nutritional Research Centre, Research Laboratory, Stanborough School, Watford, Herts) discussed "India's Food Problems" on the premise that substantial increase in supplies of animal protein could not be expected in the foreseeable future. Attention must therefore be focused on plant proteins, which are cheaper than animal proteins, and may be obtained from pulses, groundnut preparations, leaf protein concentrates, and other sources. The lack of vitamin B_{12} in plant proteins might be corrected by fortification with the synthetic vitamin itself. He referred to deficiencies of lysine and methionine in average Indian diets and to possibilities of making these good by suitable combinations of vegetable foods. In conclusion, he stressed the need for more clinical and nutritional research, and for developments in food technology, food control, agriculture, transport and education.

An account was given by Dr. W. F. J. Cuthbertson (Glaxo Laboratories, Ltd., Greenford, Middlesex) of "The Commercial Development of 'Amama', a protein-rich Supplement in Nigeria". This preparation, based on groundnut flour to which casein, yeast, minerals and vitamins are added, is manufactured in a factory near Lagos. Tests made in Nigeria, where protein malnutrition is common, showed that 'Amama' was as effective in treating malnourished children as skim milk powder. Cuthbertson emphasized that the manufacture and distribution of a new food to wholesalers and retailers were not enough to ensure its use; an advertising and educational programme was essential so that the public can become aware of its availability and how to use it. He outlined the methods followed in promoting the sale of 'Amama', and the difficulties encountered.

Throughout the symposium there were many references to the need for popular education in nutrition, which must accompany all other measures to eliminate malnutrition. The last paper, by Dr. A. T. M. Wilson (Unilever House, Blackfriars, London), on "Nutritional Change; Some Comments from Social Research", dealt mainly with this subject. He quoted effectively a passage in a World Health Organization report about the "baffling maze of tradition, taboo and magic, and the whole system of belief of the people about health and the causes of disease" of which educators must be aware. "In the past", the World Health Organization report continued, "most workers have tried, by repeated blows of didactic teaching, to hammer their way through the maze and break down the resistance to unaccustomed foods and eating practices—with very little success . . . because beliefs about food are part of the whole fabric of the life of a people". It follows that the social and psychological aspects of the campaign against malnutrition are of essential importance, an example being the complex social factors which influence lactation and weaning. Wilson also commented that the campaign called for co-operation between different disciplines and services, always difficult to secure. The epigram "God has not seen fit to divide our problems into the same categories as university chairs" was highly applicable to the problem of nutrition.

The papers and discussions at the symposium gave at least some answers to the questions implied in its title. While the general attitude was optimistic, the speakers recognized that immediate and facile solutions cannot be expected. There was emphasis on the need for action based on research and on an understanding of the complex factors which cause malnutrition.

W. R. AYKROYD

GROWTH AND GENETICS OF HIGHER FUNGI

THE higher fungi commonly known as mushrooms, toadstools and bracket fungi and classified in the Basidiomycetes, have certain distinctive biological features which make them a favourable group in which to study their physiology of growth and their genetics. The large fruiting body with its size, shape and form, and in many species with its extraordinary rate of growth, offers opportunities for studying the factors affecting growth, translocation and morphogenesis. The mycelium with its regular growth-pattern, its production of asexual spores and its ability to grow on simple minimal medium has all the properties of mycelia in other groups of lower fungi. The regular formation of a stable dikaryon from the compatible mating of monokaryons provides a system for studying gene action which is intermediate between the regular haploid–diploid cycle of yeast and the variable heterokaryon and homokaryon in *Neurospora* and *Aspergillus*. The complex mating system in the higher fungi, although it can be compared in its genetics with certain incompatibility systems in higher plants, has features that are not found in any other group of plants.

In a symposium organized by the British Mycological Society, under the chairmanship of its president, Miss G. M. Waterhouse, five aspects of the higher fungi were examined: (1) the effects of light and gravity on the growth and form of a fruiting body; (2) the factors, both internal and external to the hyphæ, which govern the regular pattern of growth and branching of the mycelium; (3) the extent of gene action and the transport of exogenous substances along the hyphæ; (4) the genetic and possible biochemical control of the complex mating system; (5) the mosaic structure of populations and sterility barriers in higher fungi.

Dr. B. E. Plunkett (Birkbeck College, London), using the fruiting-bodies of *Polyporus brumalis*, demonstrated components of the complex system of stimuli that operate to keep the cap horizontal and to ensure the production of spores on the lower side. These are factors of importance if the fungus is to distribute its spores efficiently. The stalk of the young fruiting body, before the cap has expanded, grows towards a laterally placed light, but as the cap expands it is no longer attracted to the light but grows upwards under the influence of gravity on the horizontal stem. Light of low intensity obtained by shading the young stem with an artificial cap resulted in upward growth due to gravity. This showed that the geotropic response was present in the early as well as in the later stages of development. The weaker geotropic response is at first overruled by the strong photo-response, but later, as the perceptive region is shaded by the expanding cap, the geotropic response expresses itself. Rotation-with-light experiments demonstrated that the spore-bearing region develops away from the light and the cap develops at right angles to the incident light. These interesting studies in growth and morphogenesis were paraphrased by the statement that according to Corner the cap was an umbrella to keep rain from the spores, but now it could also be a parasol.

Turning from the fruit body to the mycelium, Dr. G. M. Butler (Birmingham) discussed the problems of the hyphal branching system in *Coprinus disseminatus*. Three types of hyphæ could be distinguished, main, primary branch and secondary branch. In Petri dish cultures the main hyphæ had the highest extension-rate and the greatest diameter, the secondary branch had the lowest rate and diameter. The monopoidal branching system and the radial growth of a colony depend on these differential extension-rates of leading and branch hyphæ. A steady rate of growth of a whole branch system is maintained, from which it is concluded that the hyphal tip, the region where extension occurs, is not autonomous but is subject to competition and interaction occurring within the hyphal system. Experiments with two cultures separated by 'Cellophane' and growing out of step showed that under certain conditions there existed hyphal competition or inhibition transmitted in the culture media, but this external effect, even at its greatest intensity, was too small to account for the difference between the main and branch hyphæ in extension-rate and diameter. Little is known about the main control of hyphal growth, which is internal, either in its extent along the hyphæ or in its mode of action, but it appears to operate on cell extension rather than on nuclear division.

Prof. D. Lewis (University College, London) discussed the problem of hyphal tip autonomy from a genetical approach and came to the same conclusion that the tip is not autonomous. A methionine-requiring mutant *me*-1 of *Coprinus lagopus* produced prototrophs not requiring methionine at a frequency of 2 in 10^5 asexual spores. These asexual spores are uninucleate and are cut off from the tips of hyphæ. The prototrophs are the result of recessive mutants of suppressor genes. Monokaryotic mycelia of the *me*-1 stock, when transplanted to minimal media, do not produce prototrophic sectors of growth despite the fact that hyphæ grow for 1 mm. into the minimal medium. The recessive mutant therefore is not expressed in the articulated hyphæ but is expressed in the isolated spore.

A dikaryon which is homogenic for *me*-1 and heterogenic for a suppressor gene will not grow on minimal medium, but each hypha resolves into a monokaryon with the nucleus carrying the suppressor gene. This difference between the monokaryon and dikaryon indicates that the sphere of gene action extends well beyond its own cell in a monokaryon, but in a dikaryon the same gene has a limited field of action and may be limited to its own cell. Experiments on the transport of exogenously supplied methionine in monocaryons and dicaryons did not reveal any difference between the two types of mycelia. Transport was limited to 1–3 mm. in both; this is a fact which may have a direct explanation for the lack of fruiting ability of many biochemical mutants when homogenic.

The unique mating system in the higher Basidiomycetes is controlled by two genetic loci *A* and *B*; one gene controls the migration of the donor nucleus in the receptor hyphæ and the other gene controls the regular synchronous division of the two nuclei and the formation of the clamp connexions which are peculiar to the Basidiomycetes. The *A* and *B* loci in *Schizophyllum commune* have been analysed into two genes in each.

Dr. P. R. Day (John Innes Institute) described a genetical analysis of the *A* complex in *Coprinus lagopus*. Using two auxotrophic mutants linked on both sides of the *A* locus, recombinants of the outside auxotrophic mutants were selected on minimal medium, and among these were a few *A* alleles which were different from the two parental alleles. The frequency of recombination to give these new alleles was 0·07 per cent. The recombining parts have been called sub-units; but whether these sub-units can be considered as two different functional genes or as different sites of the same functional gene cannot be decided. The frequency of 0·07 per cent is a value which is consistent with either alternative, and the important functional complementation test is not possible with this *A* locus in the present lack of knowledge of its action. But if the *A* locus is similar in *Coprinus* and in *Schizophyllum* which has two genes 4–10 per cent units apart, then two genes are indicated. It may be significant for the working of the incompatibility system that in all four species of higher fungi where recombination within the *A* and *B* loci has been tested on a sufficient scale, two sub-units or genes have been found.

Mutations affecting the *A* function were obtained in heterokaryons which had a common *A* allele. These were either changes at the *A* locus itself or at a gene suppressing the *A* function. All the mutants appeared to result in loss of function because monokaryons with these mutations produced spontaneously the clamps which are normally confined to dikaryons. A further analysis of the *A* locus using recombination and mutation should reveal further important details about its structure and function.

Prof. R. A. Raper (Harvard), who has been mainly responsible for the detailed analysis of the *A* and *B* loci in *Schizophyllum commune*, in his paper "An Enquiring Look at Tetrapolar Incompatibility", gave a penetrating and exhaustive review of the possible control systems which could be accommodated into the known facts about the *A*–*B* mating system in higher Basidiomycetes. Such a control system has to account for the high degree of specificity, and at the same time the great variation in the specific control. It also must be consistent with the genetic system, but as was pointed out, such a genetic system would have been subjected to exceptional selective pressures because of the unusual nature of the system, and it is possible that exceptional or even unknown gene action and interaction may be revealed by the system.

Three basic modes of biochemical control were considered: (1) an inhibitor system which is produced by like alleles to cause incompatibility; (2) an inhibitor which is present in the monokaryon, but which is removed by unlike *A* and *B* alleles to give a compatible dikaryon; (3) a complementary action between unlike alleles to elaborate an essential substance for the compatible dikaryon to become established. With our present knowledge of gene structure, function, interaction and complementation there are some difficulties with all three schemes, but Prof. Raper hoped that a solution might be forthcoming from a serological analysis which had already given promising results.

Prof. J. H. Burnett (Newcastle) described his results of surveying natural populations of several species of higher Basidiomycetes for their mating type *A* and *B* alleles. The survey included bipolar and tetrapolar species. The number of different alleles was large, but varied significantly between different groups. The genus *Nidularia* stands out from the rest by having a small number of alleles.

Fruit bodies sampled from the same tree and from the same clump revealed that different dikaryons were mixed together in a mosaic. Several examples of contiguous fruiting bodies were found to be genetically isolated from one another as shown by the failure of their spore colonies to mate. Mosaic cultures were reconstructed in artificial conditions, and it was concluded that the mycelium is not often a single ecological unit but a genetical mosaic.

The mosaic nature of the mycelium, together with the genetic sterility barriers between the components of a mosaic, may have its causative connexion. Genetically isolated components could only form a mosaic, but components not so isolated could fuse to give a dominant single dikaryon. But, like many aspects of higher fungi, whether this frequent isolation barrier has its cause in the unique mating system is not known.

D. Lewis

BARLEY AND BREWING

THE European Brewery Convention belongs wholly to the post-war period and, in retrospect, it is surprising that, prior to its inception, no organization existed for the regular international exchange of scientific information and experience in relation to malting and brewing. There were of course various journals of high repute, but the differences in standards, industrial methods and laboratory procedures from country to country were not widely appreciated. Consequently, valuable though many published investigations were, they tended more to describe particular experience than verifiable fact. With the present volume in mind*, it can be still more clearly seen that the selection of barleys and of malting methods even in a single country could scarcely have been done scientifically so long as the lack of properly documented experience made it impossible to co-ordinate the economic and material requirements of the farmer, maltster and brewer.

The tenth annual report reveals that these earlier deficiencies have been largely remedied by a degree of integration in international investigation never before achieved in this connexion. For example, it gives, mostly in tabular form, the results of trial comparative cultivations, often with relevant malting and brewing trials, of some 12 new varieties of barley the majority of which were grown under highly controlled conditions in 14 western European countries. The data extend far beyond the expected facts on such matters as yield, nitrogen-content, dormancy, grind characteristics of the malts and head retention of the final beers making up the series of part-reports, each under separate authorship. Thus they include numerous details of the lay-out of each trial, the nature of its location, agricultural conditions, field observations and meteorological data. The report is therefore a reference work rather than a book to be read cursorily. There can be no doubt that it reflects what is, in its way, an exceptionally important step to find improved varieties of what is the basic raw material of an unusually widespread industry.

It may seem strange to some that this effort is so disseminated when much of the work could apparently be carried out centrally. Actually, however, this type of inquiry is unexpectedly complicated because it involves comparison not only of barleys as such but also of the conditions under which they are grown and processed. Broadly, therefore, the question arises as to whether the differences between varieties can outweigh the differences arising from varying methods of treatment, for example, of malting method. The European Brewery Convention Barley Committee is in an unrivalled position to sponsor investigations of this kind, and its report has therefore a scientific value beyond that of a factual record of a unique but continuing international exercise.

A. H. COOK

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* European Brewery Convention. *Rep. Barley Committee*, 1959. Pp. 170. (London: Institute of Brewing, 1960.)

ADMISSION TO UNIVERSITIES IN BRITAIN

THE third report of an *ad hoc* Committee on Procedure for Admission of Students to Universities, appointed by the Committee of Vice-Chancellors and Principals of Universities of the United Kingdom in January 1958, has now become available*. The Committee was set up to consider what steps need to be taken to reduce difficulties experienced by candidates for admission and by the schools. The report contains the findings of a working party established under the chairmanship of Dr. A. W. Chapman to report on the methods and techniques by which the multiplication of applications for admission and the consideration of candidates could be reduced, the kind of central organization involved and any changes of principle as regards relations between universities and candidate. The Committee has been forced to conclude that the time has come when universities must consider the establishment of a central organization and a central office in connexion with applications for admission, and it was this conclusion which led to the establishment of the working party. Further, having been satisfied as to the technical feasibility of such central arrangements, the committee also concluded that it must now make recommendations which would cause the universities as a matter of urgency to consider the rapid establishment of co-operative arrangements, and this is the whole burden of the report.

The working party quickly recognized that it would be essential to have some kind of central office with information about every candidate and every institution, but equally that a candidate must have liberty to apply to a reasonable number of institutions of his own choice, and that the choosing of their students must remain entirely a matter for the universities.

Three schemes were submitted by the working party for consideration by the *ad hoc* Committee. One scheme looks to the maintenance of the present system, and the central office would only act as an information centre, as a means of checking candidates stated priorities and acceptances of places, and, in so far as time permitted, as a clearing house. Acceptance of this scheme is not recommended.

The other two schemes provide for a central office, equipped with data-processing machines, to which each candidate would make his or her application, naming a limited, but reasonably generous, number of institutions, to which he or she wishes to apply. It would be the duty of the central office to distribute the applications to the institutions concerned, to act as the channel through which all offers, whether firm or provisional, or rejections are conveyed to the candidate, to ensure that no other application remains in force once a candidate has accepted a firm offer, to provide a general clearing-up service operating from the earliest possible date in the admissions season, and to make full information to those who need it. Under the scheme preferred by the *ad hoc* Committee, though both are recommended to the consideration of the Committee of Vice-Chancellors and Principals, a candidate's applications would be released two or three at a time in an order chosen and specified by the candidate and a second pair of applications would ordinarily be released only after the results of the first pair had proved unacceptable. It provides that the selector must make a definite decision on an application within a given time-limit and can no longer wait until after his or her General Certificate of Education results before reaching a decision. Either scheme could be made effective for the intake of October 1964 if immediate steps were taken, and the Committee recommends the convening of a conference of representatives of the universities to consider the two recommended schemes and their application to the needs of the individual institutions.

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* Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom. Third Report of an *ad hoc* Committee on Procedure for Admission of Students. Pp. v+41. (London: Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom, 1961.) 2*s*. 6*d*.

Neither scheme need adversely affect the direct relation between candidate and institution. In the light of the discussion at the conference, the Committee of Vice-Chancellors and Principals would invite universities to agree to establish a standing advisory council, and to authorize the necessary steps to establish a central office, including a small executive committee which would act as its governing body and control its policy and operation. These proposals and recommendations are limited to universities in England and Wales other than Oxford and Cambridge, but it is hoped that the colleges of Oxford and Cambridge will also consider entering the scheme and that, even if they do not enter fully, they will at least arrange to complete their selections for admission by the end of December or not later than mid-January. The proposals also seem to be applicable without difficulty to the Scottish universities.

Figures appended to the report show that for the institutions covered 151,000 applications were lodged for 1960–61, against 134,000 for 1959–60, 121,000 for 1958–59 and 70,000 for 1955–56, and in all 22,650 students were admitted to first degree courses in October 1960, compared with 20,300 in October 1959. Of the applications, 14,000 were from overseas candidates. Except for technological courses, the number of applications increased substantially compared with the previous year, though the number admitted to such courses increased by about 12 per cent. There is a notable decrease in the number of vacant places, except in technology, and there seems to be no doubt that, at present, too few adequately qualified candidates are applying for courses in the technologies and especially in the classical engineering subjects. It would appear that the clearing-up houses, established in the summer of 1960, as recommended in the *ad hoc* Committee's second report, have already contributed to the decrease in the number of vacant places.

CÆSIUM-137 IN AIR, PRECIPITATION, DRINKING-WATER, MILK AND BEEF IN NORWAY DURING 1959 AND 1960

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CÆSIUM-137 in air, precipitation and milk has been regularly measured at Bergen, Røros and Lillestrøm. The results for January 1959–December 1960 are shown in Fig. 1 as mean values over periods of two months.

Cæsium-137 in beef-samples from the same districts has also been measured. Cæsium-137 in drinking-water has been regularly measured at several places.

The Bergen district is a mountainous coastal area, Røros district is a mountainous inland area and Lillestrøm district is a lowland, inland area. The locations are shown on the map in Fig. 1.

As shown in Fig. 1*B* the concentration of cæsium-137 in ground-level air is at nearly the same level at the three measuring sites, and the variations occur simultaneously. There is one maximum during May–June 1959 and another, but much lower, in May–June 1960. The yearly average values are:

District	1959	1960	
Bergen	0·020	0·0042	$\mu\mu$c. cæsium-137/m.^{3}
Røros	0·019	0·0045	,,
Lillestrøm	0·019	0·0044	,,

The concentration of cæsium-137 per litre precipitation, shown in Fig. 1*C*, also varies in much the same way, but with a somewhat larger difference from site to site. The maximum concentrations in 1959 are found during the periods March–June, and so are the maximum concentrations in 1960. It appears, nevertheless, from Fig. 1*B* and 1*C* that the concentration of cæsium-137 per litre precipitation to a first approximation is proportional to the concentration in ground-level air.

The average cæsium-137 concentration in the total yearly precipitation is:

District	1959	1960		
Bergen	17	3·5	$\mu\mu$c. cæsium-137/l.	precipitation
Røros	13	3·4	,,	,,
Lillestrøm	13	3·3	,,	,,

The rate of deposition of cæsium-137 fall-out, shown in Fig. 1*D*, depends on the amount of precipitation, which is very different from site to site. The maximum fall-out rates in 1959 are found during the periods March to August, and so are the maximum rates in 1960.

The yearly average rate of fall-out is:

District	1959	1960		
Bergen	67	13	$\mu\mu$c. cæsium-137/m.^{2}	per 24 hr.
Røros	10	4·1	,,	,,
Lillestrøm	29	8·9	,,	,,

Fig. 1*E*, showing cumulative cæsium-137 fall-out during January 1959–December 1960, reflects the difference in precipitation from site to site. The total yearly fall-out is:

District	1959	1960	
Bergen	24,300	4,800	$\mu\mu$c. cæsium-137/m.^{2}
Røros	3,800	1,500	,,
Lillestrøm	10,600	3,300	,,

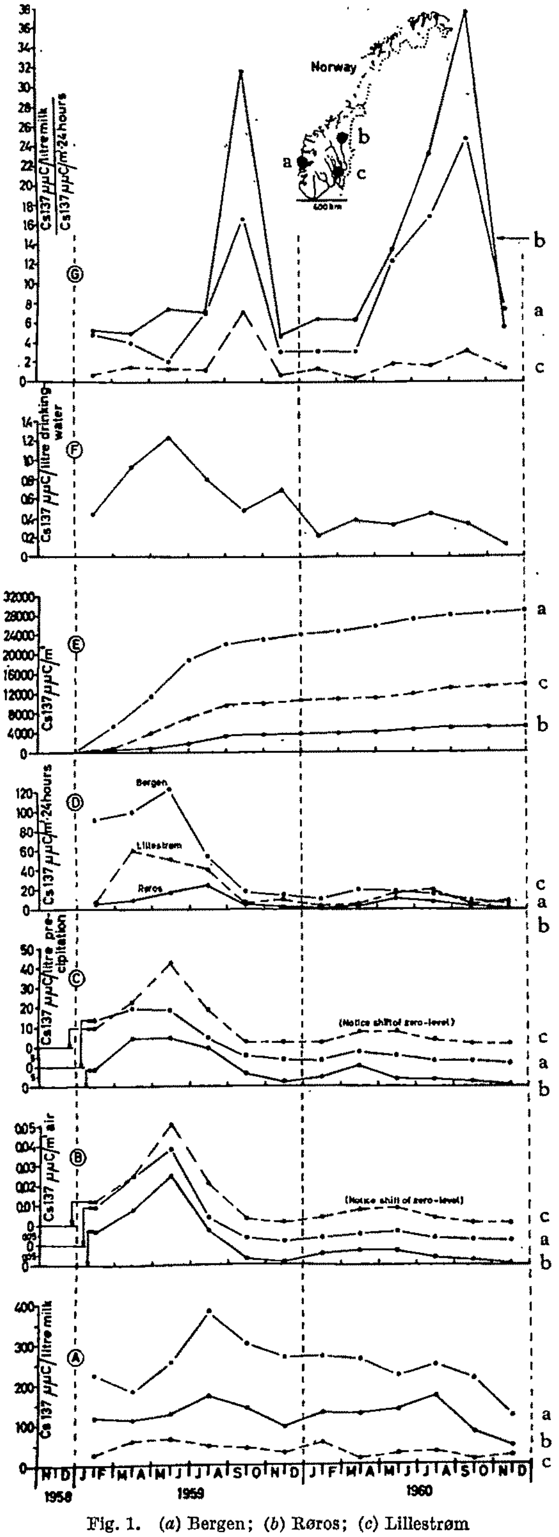

Fig. 1. (*a*) Bergen; (*b*) Røros; (*c*) Lillestrøm

It appears thus that the fall-out at Bergen is about 5 times higher than at Røros and about twice as high as at Lillestrøm.

The average concentration of cæsium-137 in drinking-water samples from 13 places in southern Norway, including Røros, is shown in Fig. 1*F*. The yearly average value is:

1959	1960	
0·8	0·3	μμc. cæsium-137/l. drinking-water

The average cæsium-137 concentration in drinking-water thus is only a few per cent of the average cæsium-137 concentration in precipitation.

The concentration of cæsium-137 in milk, shown in Fig. 1*A*, is also very different from site to site. The yearly average values are:

District	1959	1960	
Bergen	270	230	μμc. cæsium-137/l. milk
Røros	130	120	,, ,,
Lillestrøm	47	29	,, ,,

The concentration of cæsium-137 in milk at Bergen is about twice as high as at Røros and about 7 times higher than at Lillestrøm. The difference thus does not follow the difference in cæsium-137 fall-out.

At all three sites the concentration of cæsium-137 in milk shows a tendency to decrease towards the end of 1960.

The cæsium-137 concentration found in samples of beef during winter and early spring 1960 is:

Bergen	1,760 μμc. cæsium-137/kgm. meat	(average of 2 samples)
Røros	590 ,, ,,	(average of 9 samples)
Lillestrøm	180 ,, ,,	(1 sample)

The variations in cæsium-137 concentrations in meat from district to district appear to follow nearly the same pattern as for milk.

The concentration of cæsium-137 in milk and meat corresponds to an intake by the cow of up to several thousand μμc. cæsium-137 per day. The intake may be through air inhaled, through drinking-water and through fodder. As the air inhaled by a cow is of the order of 200 m.^{3} per day, it is evident that the concentration of less than 0·1 μμc. cæsium/m.^{3} air does not contribute significantly to this intake. The drinking-water intake by a cow is of the order of 50 litres a day. The concentration in drinking-water of less than about 1 μμc. cæsium-137/l. therefore apparently does not represent any important contribution to the daily cæsium-137 intake by the cow. Even during summer time when the cow may be drinking water directly from pools, rivers, lakes, etc., with a somewhat higher concentration than ordinary drinking-water (but still lower than in precipitation), the intake of cæsium-137 by drinking-water does not amount to a large part of the total daily intake by the cow.

Apparently the main contribution to the intake of cæsium-137 by the cow is from cæsium-137 in grass, hay and possibly other fodder. The uptake of cæsium-137 by grass may so far have been mainly direct from rain and air, and less through soil and roots. It might be expected then that the concentration of cæsium-137 in grass and hay and therefore also in milk and meat to a first approximation would be proportional to the rate of cæsium-137 fall-out.

The ratio of cæsium-137/l. milk to cæsium-137/m.^{2} per 24 hr. is shown in Fig. 1*G*. During the winter months November–April, the hay eaten by the cow is from the previous summer. The ratio shown in Fig. 1*G* for the winter months therefore is based on average cæsium-137 fall-out rate during the period May–August of the preceding summer. The average cæsium-137 fall-out rate during these months of 1958 was 47 μμc./m.^{2} per 24 hr. at Bergen and 30 at Lillestrøm. The value at Røros has been calculated to be 23, based on rainfall data from Røros and average concentration per litre precipitation at Bergen and Lillestrøm during May–August 1958. The calculation adds an extra uncertainty to the two first points of the Røros curve in Fig. 1*G*.

It appears from Fig. 1*G* that the ratio of cæsium-137 in milk to cæsium-137 fall-out rate is different from site to site. The yearly average of the 2-monthly values is:

District	1959	1960	
Bergen	6·3	11	$\frac{\mu\mu\text{c. cæsium-137/l. milk}}{\mu\mu\text{c. cæsium-137/m.}^2\text{/24 hr.}}$
Røros	10	15	
Lillestrøm	2·1	1·6	

The difference in ratio may be taken to represent to a first approximation the difference in grazing-field qualities of the three districts, a low ratio corresponding to high grazing-field quality.

For all three districts the ratio-curve shows a maximum during the period September–October, both in 1959 and in 1960. It seems reasonable to relate this increase to the decreasing quality of the grazing fields in the late months of autumn, the slower growing rate of grass giving an increased cumulative effect in the cæsium-137 uptake by the plants.

At Bergen and Røros the ratio is somewhat higher in 1960 than in 1959. As Bergen had an abnormally dry summer in 1960, while Røros had a rather wet and cold summer, this may again be related to lower grazing-field quality during 1960.

On the other hand, the variations in the ratio of cæsium-137 in milk to cæsium-137 fall-out rate may depend on a number of other factors in addition to the grazing-field quality, such as variations in precipitation and fall-out amounts within each district, variations in feeding conditions including amount and type of additional fodder, delay between variation in cæsium-137 intake and resulting variation in cæsium-137 in milk, redistribution of fall-out, and also uncertainties in sampling and measurements.

NUCLEAR MATERIALS AND NUCLEAR CHANGE IN DIFFERENTIATION

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IN a noteworthy series of experiments, Brink[1,2] has demonstrated that certain alleles at the *R* locus, affecting pigmentation in maize, are capable of showing two different kinds of change. One of these is the familiar phenomenon of mutation. It is sporadic, occurring in this case with frequencies of the order of 1 in 10^5; it is stepwise, in that the products of the mutation do not fall into a graded series; it is undirected; and it results in new alleles which are as stable as that from which they arose. The second type of change differs in all these respects. It is determinate, in that it occurs in 100 per cent of cases under certain conditions; it appears to be capable of giving rise to a graded series of products; it is directed in that a given allele always changes in the same way under the same conditions; and the changed alleles to which it gives rise are metastable, rather than stable, as attenuation of the effect has been observed. Brink terms this second type of change 'paramutation', and shows that the chromosome material on which it depends cannot be the same as that the changes of which appear as normal mutations. In other words, the *R* locus must be dual, containing orthochromatin, which provides the basic gene structure and which by its changes gives rise to true mutations, and parachromatin, which though not of the essence of gene structure nevertheless affects the expression of the gene's action and which by its changes gives rise to paramutations. Paramutation has been established as occurring at one further locus, at least, in maize, and there are indications if not full proof of it in other species[2].

Although at least partial reversion of the *R* paramutants has been observed in maize, the circumstances in which this occurs are not yet clear. In the fly *Sciara coprophila*, however, a similar type of change is to be inferred and the cycle is unambiguous. In the male flies meiosis is replaced by a division which results in one haploid set of chromosomes being eliminated, and this is always the set the male received from his father. The set originating from his mother is handed on to his progeny in which, if they are male, these chromosomes will now be eliminated. The chromosomes have thus changed from 'maternal' to 'paternal' behaviour following passage through the male. Should, however, they be passed by the male to a daughter (from which they are, of course, not eliminated) they will regain 'maternal' behaviour as will be made evident if this daughter in turn passes them on to a son[3].

Further evidence of the complete dependence of change in behaviour of the chromosomes on the sex of the fly through which they passed in the previous generation is supplied by the so-called *X* chromosomes. Each zygote, whatever the sex of the individual destined to develop from it, receives three *X*s, one from the mother and two from the father. The differences between the sexes become apparent at the fifth or sixth cleavage division in the somatic line, for in females one *X* and in males two *X*s are then eliminated from the nucleus. The *X*s to be eliminated are always of paternal origin. The sole remaining *X* of a male is thus maternal, but if it is passed on to a son in the next generation it will show paternal behaviour. One of the remaining *X*s of a female is, of course, maternal but the other is paternal. When passed on by the female to her offspring the two will behave alike as maternals, but if the offspring are male the chromosomes, while continuing to behave alike, will be changed to paternal behaviour as will be exemplified in offspring to which those males give rise in their turn. It has recently been shown that the *X*s owe these curious properties to heterochromatin at the centric end of the chromosome[4].

These changes in the chromosomes of *Sciara* are clearly comparable to paramutation (as indeed Brink points out) in three respects. They are directed; they are determinate, and indeed inevitable according to the sex of the fly which passes the chromosomes to its offspring; and they are metastable, being changed by passage through a fly of the opposite sex. The changes in chromosome behaviour are thus regular consequences of the processes of development and may in fact be described as parts of these processes. We cannot, however, be sure that they in their turn affect subsequent development, for we do not know that the fly would develop differently if a maternal *X* chromosome were to be eliminated instead of a paternal. Indeed, the significance of this differential elimination of paternal chromosomes in *Sciara* is quite obscure.

Various other types of nuclear change are known to occur during development. Some of these, as, for example, the widespread phenomenon of endopolyploidy, are most readily understood not as adaptive changes playing a determinate part in development itself, but simply as the consequences of breakdown in the adjustment of the mechanism of nuclear division in cell-lineages which have come to the end of their history of cell multiplication[5,6]. No question of paramutation arises in this interpretation of endopolyploidy. In other types of change a kind of paramutation may be important. There is cytological evidence, some of it obtained by the use of tritium-labelled thymine[6,7], that the activity of the genes in *Drosophila* changes, and changes differentially, during development. The observations further suggest that these changes must be associated characteristically with the different tissues so that they are determined by the state of the cells in the tissues and indeed are inevitable under the condition of the tissue, just as the chromosome changes of *Sciara* are determined by and inevitable in flies of a given sex. Similar changes in gene action are indicated by King and Briggs's observations[8] on the nuclei of developing frog embryos. I have earlier suggested[9] that these changes are to be interpreted not as changes in the basic content of the genes of the nucleus, but as brought about by the depletion from or accretion to the chromosomes of what I called 'ancillary materials', which, while not of the genes, affect the action of the genes—an interpretation bringing in the same type of change as that which Brink now terms paramutation, with his parachromatin corresponding to my ancillary materials.

In all these cases change is consequent on development, but in none of them is there clear evidence (as opposed to arguable presumption) that the change plays a positive and constructive part in determining the course of future development. The evidence from King and Briggs's frogs is of nuclei which have lost the capacity to sustain the full range of development when put back into the cytoplasm of a fertilized egg. This could indicate (though it does not necessarily do so) no more than a characteristic loss of gene function, just as the cytoplasmic changes associated with certain aspects of differentiation in the fungus *Aspergillus nidulans* are losses of certain capacities rather than constructive gains[6]. There would seem, however, to be no reason in principle why changes of a paramutational type arising during development should not actively and constructively determine the further developmental history of the cells and tissues in which they occur. It is especially tempting to attribute to paramutation the stability which is reached in cells at the end-point of their differentiation. The mechanical control which the chromosome system exercises over nuclear genes gives to these a regularity of behaviour and precision of balance which the cytoplasm could not be expected to emulate, and the evidence from maize and *Sciara* shows the parachromatin or ancillary materials to behave as regularly and be transmitted as precisely as the genes themselves, except, of course, when they are undergoing their determinate changes from one metastable state to another.

Clearly, therefore, paramutational types of change in the chromosomes could play an important part in development. They could not, however, of themselves offer any interpretation of differentiation, for even if we attribute alteration in the behaviour of a cell to such directed and determinate change in its chromosomes, we are still not enabled thereby to see why two sister cells, derived by division of a single mother cell, follow different courses of development. If the parachromatin or ancillary materials share with the other and fully permanent materials of the chromosome the property of equal division in cell reproduction they can no more account of themselves for sister cells following different paths than can those other chromosome materials. So we must still return to the cytoplasm if we are to understand differentiation. The divergences of differentiation must spring basically from differences in the cytoplasmic materials[6,11] even though secondarily it may involve nuclear differences, including paramutational changes, arising as responses to the initial cytoplasmic differences.

We are thus led to inquire whether changes in the cytoplasm can bring about changes in the nucleus, and more particularly paramutational changes. Changes of gross nuclear behaviour during differentiation are, of course, well known and some of them are demonstrably responses to cytoplasmic differences, as, for example, the polymitotic behaviour of nuclei in a mutant strain of maize and the differences in rates of condensation between generative and vegetative nuclei in pollen grains[5,11]. It is known, too, that in bacteria material can exist at times as particles in the cytoplasm and at others as an integral part of the chromosomes, the transition from one state to the other being promoted by specifiable conditions[12]. Furthermore, it is difficult to avoid the conclusion that both Brink's paramutations in maize and the similar changes of chromosomes in *Sciara* involve something outside the chromosomes as an intermediary. In maize, paramutation of the allele on one chromosome is dependent on the presence of an allele not in the same chromosome but in its homologue, so that the paramutagenic action must be exerted extra-chromosomally. In this case the effect may be exerted through the nucleoplasm, as opposed to the extra-nuclear cytoplasm. With the changes of chromosome behaviour in *Sciara* the cytoplasm is more directly implicated since the sex of an individual, and therefore the type of behaviour which its chromosomes will come to assume and express in its offspring, is dependent not on its own but on its mother's genotype and thus on the presence of genes which it itself does not always possess. The cytoplasm would therefore appear to be the intermediary, being conditioned by the genotype of the mother but exerting its effect, of determining the state of the chromosomes, in the offspring. Thus paramutation, like any other nuclear change in development, must be due directly to an action of the cytoplasm (or at least extra-chromosomal plasm) on the nuclear materials and only indirectly on the genes which acted to change and mould that cytoplasm in the first place. The significance of paramutation in development would thus be that it fixed, so to speak, the initially cytoplasmic changes, and gave to them a precision in cell heredity that purely cytoplasmic transmission could not guarantee. In this way paramutational changes, depending on the presence and changes of parachromatin or ancillary materials in the chromosomes, could be of great significance in development. But in differentiation they could have only a derived significance springing from and dependent on the reflexion—one might almost say crystallization—by paramutation of primary changes in the cytoplasm to which indeed differentiation must still ultimately be referred.

It is worth noting, too, that if the normal cytoplasmic progression of differentiation is reflected at least partly in a progression of materials in the chromosomes, these chromosome materials, like the cytoplasm, must either remain unchanged in the cell lineages from which gametes are derived or their progression must come full circle. Otherwise the individuals of the next generation would not start off their development from the same point as did the parents. If developmental change occurs at all in the germ-line, there must be a parachromatinic re-standardization—a clean-up of the ancillary materials in the chromosomes—just as there is of the cytoplasm in the sexual cycle of *Aspergillus glaucus*[10]. This re-standardization is not indeed observed in paramutation at the *R* locus in maize; but here the changes are not characteristic components of development, and indeed if there had been a re-standardization Brink could never have recognized or investigated the phenomena.

Clearly there is need for further studies of paramutation in its relation to the cytoplasm in development and differentiation. This relation may also be of importance in another connexion, for if cytoplasmic change can bring about paramutational alterations of the chromosomes it is possible that external intervention may, through its effects on the cytoplasm, also bring about directed changes of the hereditary materials. The remarkable changes observed by Durrant[13] may be of this kind. He 'conditioned' flax plants by growing them in artificially fertilized soils and found that the plants transmitted the change type not only to the offspring they gave by self-fertilization but also to their crossed progeny, the transmission being equal through pollen and egg. The changes were determinate and specific yet apparently nuclear in transmission: they are capable of obvious interpretation in terms of ancillary materials or paramutation as I have been discussing it. Other alterations of a similar kind can be interpreted in the same way, as may be wider observations of cytoplasmic effects which somehow seem to become attached in transmission to the nucleus (see Roper[14]). In these cases, however, the interpretation is less clear and must remain so until the results of further experiments become available.

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THE IONIC CHARACTER OF CHEMICAL BONDS

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IN recent years various attempts have been made to determine the effective charges on the atoms of semiconducting compounds from a discussion of the chemical bonding in these materials. While these attempts have not succeeded in reaching their goal, they have led to considerable confusion about the partial ionic character of covalent bonds[1]. In an effort to clear up this confusion we have had many vigorous discussions with our colleagues, and since we believe that the conclusions drawn from these discussions might be of interest to other scientists, we would like to summarize them here.

One of the causes of confusion is the synonymous use of the words 'covalent' and 'homopolar', a mistake which is readily understandable since the simplest examples of covalent bonding—the bonds in the hydrogen molecule and in diamond—are also examples of homopolar bonding, that is, bonding between 'uncharged' atoms. However, the definition of a covalent bond as first given by Langmuir[2] and as adopted by Pauling[3] states that such a bond arises from the 'equal sharing of two electrons' between the two bonded atoms, and thus makes no reference at all to the effective charges on the atoms.

The two distinctly different meanings at present attributed to the term 'ionicity' or 'ionic character of a bond' present a second more serious source of confusion. Lest we contribute further to the confusion we will therefore, in the following, distinguish between an 'effective' and a 'formal' bond ionicity.

Discussing the 'effective' bond ionicity first, we note that an ionic bond results "from the Coulomb attraction of the excess electric charges of oppositely charged ions"[4]. It readily follows from this definition that if two neighbouring atoms (ions) in a molecule or solid carry opposite charges, then the bond between them will have some effective ionic character. Unfortunately, it is difficult to define an unequivocal scale of effective bond ionicity, because whereas zero ionic character obviously corresponds to zero charges on the ligands, it is sometimes not immediately clear to which degree of ionization of the ligands 100 per cent effective bond ionicity has to be ascribed. However, by convention a bond between two atoms is normally called 100 per cent ionic if the atoms are ionized to the extent that at least one of them acquires a noble gas shell. This convention allows us to call a compound purely ionic if all its bonds have 100 per cent effective ionic character. Moreover, because an ion with a noble gas shell cannot form any covalent bonds, the convention assures that a bond with 100 per cent ionic character cannot have a covalent component.

It follows from the above discussion that the percentage i_e of effective ionic character of the bond between two atoms A and B is closely related to the 'effective charges' q_A and q_B on these atoms. Indeed, if q_{0A} and q_{0B} are the charges which the two atoms would carry if the bond between them were completely ionic, then:

$$i_e = \frac{q_A}{q_{0A}} \times 100 = \frac{q_B}{q_{0B}} \times 100 \qquad (1)$$

We note that the definition of the effective ionic character of a bond as given by (1) is identical with that used by Pauling[5] in discussing the relationship between bond ionicity and dipole moment μ of the hydrogen halogenide molecules (see also Hannay and Smyth[6]). Indeed, if r_0 is the equilibrium distance between the two atoms of such a molecule, we find for the effective charges $q_A = -q_B = q$ on the atoms:

$$q = \mu / r_0 \qquad (2)$$

and hence for the effective bond ionicity:

$$i_e = \frac{\mu}{r_0 q_{0A}} \times 100 = -\frac{\mu}{r_0 q_{0B}} \times 100 = \frac{\mu}{r_0 e} \times 100 \qquad (3)$$

where e is the electronic charge.

Contrary to the effective bond ionicity, the 'formal bond ionicity' is not related to any quantity such as the effective charge, which is at least in principle measurable. (For diatomic molecules the effective charge is defined by (2) and hence can readily be determined experimentally. Unfortunately, this definition cannot be extended to include complex molecules and solids. Nevertheless, any discussion of effective bond ionicities implies that effective charges can, at least in principle, be measured, because the ionic bond itself is, after all, defined in terms of such charges.)

The formal bond ionicity is a purely theoretical concept, and its numerical value depends on the approximation used in describing a bond and not on the actual properties of this bond.

Because no method is available for solving many-electron problems accurately, it is often assumed[3,7] that the bonds in molecules or solids can, with sufficient accuracy, be described as resulting from the resonance of these molecules or solids among different valence structures. Let us discuss briefly a diatomic molecule AB the components of which have one valence electron each (monovalent atoms). Chemical and physical intuition tells us that it is reasonable in this case to consider the following valence structures:

$$A:B \qquad A^+B^- \qquad A^-B^+$$

Corresponding to equal sharing of the valence electrons by the two partners, the first structure is called 'covalent'. For obvious reasons, the other two structures are both called ionic.

Let $\Psi_{A:B}$, $\Psi_{A^+B^-}$ and $\Psi_{A^-B^+}$ be three properly chosen two-electron wave-functions corresponding to the three valence structures. With these functions we form the linear combination:

$$\Psi = a\Psi_{A:B} + b\Psi_{A^+B^-} + c\Psi_{A^-B^+} \qquad (4)$$

and determine the coefficients b/a and c/a such as to minimize the integral:

$$E = \frac{\int \Psi^* H \Psi d\tau}{\int \Psi^* \Psi d\tau} \qquad (5)$$

Substituting these coefficients in (4), we obtain the best wave-function which can be constructed by combining linearly $\Psi_{A:B}$, $\Psi_{A^+B^-}$ and $\Psi_{A^-B^+}$. The formal ionic character of the bond $A - B$ is then determined by the extent (measured by the coefficients b/a and c/a) to which the structures A^+B^- and A^-B^+ contribute to Ψ.

It is obvious from this discussion that the formal ionic character of a bond is defined only with respect to the particular approximation used in describing the bond. Thus if, for example, the electronegativity of atom B is much larger than that of atom A, it is sufficient to consider only the first two of the above valence structures, and (4) takes the form:

$$\Psi = a\Psi_{A:B} + b\Psi_{A^+B^-} \qquad (6)$$

The corresponding percentage i_f of formal ionic character of the bond then is given by:

$$i_f = \frac{(b/a)^2}{1 + (b/a)^2} \times 100 \qquad (7)$$

The two valence structures considered here in describing the bond in a molecule of monovalent atoms are obviously the same as those defining the scale of effective bond ionicity in such a molecule. It is permissible therefore to put:

$$i_f = i_e \qquad (8)$$

and one can in this case determine the only free parameter in (6), namely b/a, from the experimental value of i_e, thereby avoiding the cumbersome process of minimizing (5).

Equation (8) is perhaps the cause of all the confusion about bond ionicities. We would like to emphasize, therefore, that in spite of the conditional validity of (8), formal and effective bond ionicities are not identical, and one does well to distinguish carefully between them, particularly when discussing solids. Moreover, (8) should never be interpreted as implying that the effective ionic character of a bond can be derived from a formal bond ionicity. Indeed, if the effective ionicity of a bond is not known, the value of any formal ionicity attributed to this bond is always arbitrary, because it is necessarily based on arbitrary mixing of arbitrary, if plausible, valence structures.

To demonstrate the confusion which can arise from the indiscriminate use of the term 'bond ionicity', let us briefly dwell on a fallacious but often repeated discussion of the bonding in semiconductors of the zincblende type. This discussion, which has perhaps been instigated by Pauling's somewhat misleading account of the bonding in AlP (ref. 8), will here be given for zinc blende itself. The tetrahedral bonds in this compound are said to arise from resonance among the following two valence structures:

$$Zn^{2-}S^{2+} \qquad Zn^{2+}S^{2-}$$

The first of these is referred to as the 'extreme covalent' structure, because it leaves each of the components with four valence electrons which can be shared in the formation of the maximum number of four tetrahedral bonds. The second structure is called 100 per cent ionic since it corresponds to a noble-gas configuration on both the zinc and the sulphur ions. These valence structures are taken to define the scale of bond ionicity (one is left guessing whether it is formal or effective ionicity), so that the bonds in the hypothetical 'neutral-bonded' Zn^0S^0 obtain 50 per cent ionic character[1]. Since sulphur is more electronegative than zinc, it is then concluded that in the crystal each sulphur carries some negative and each zinc the same amount of positive charge. Hence, the argument goes on, the bonds in ZnS are more than 50 per cent ionic. Such a statement is very misleading indeed, and matters are only made worse if, as has been done, one goes a step further and calls ZnS a compound with more than 50 per cent ionic character.

We should, perhaps, recall here that the generally accepted definition of the covalent bond as given earlier leaves the effective charges on the ligands unspecified. To define the zero of effective bond ionicity by an arbitrary valence structure such as $Zn^{2-}S^{2+}$ is therefore meaningless, even though one might choose to call the structure 'extreme covalent'. Finally, we should like to repeat that out of any discussion of formal bond ionicities one can never obtain more information than was originally put in. In particular, any attempts such as have been made to determine effective charges from formal bond ionicities are bound to fail.

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EFFECT OF TEMPERATURE ON ADHESION TO GLASS AND HISTOGENETIC COHESION OF DISSOCIATED CELLS

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ONE of the chief attributes of metazoan cells is their ability to cohere and interact mutually into integrated, multicellular systems. Though the mechanisms of cell cohesion and the role of cell contact in development and in tissue function have had a long history of interest and speculation, recent reviews of these problems[1] clearly underscore the paucity of basic information on various important aspects. Among the theoretically and practically more obvious and pressing questions is that of the effects of temperature on the capacity of cells to adhere to various substrates and to each other. Though such information would seem prerequisite for a systematic inquiry into the mechanisms of adhesion and interaction of cells, the point has been surprisingly little studied. The methodology of dissociation and aggregation of embryonic cells[2] affords a direct approach to these problems; in connexion with present work on tissue-synthesis *in vitro* from dissociated cells[3,4], the question of temperature and cell adhesion was examined and some of the findings are reported here briefly.

Chemically dissociated cells[2,4] from neural-retina, liver and kidney (mesonephros) of 7-day chick embryos were used throughout this work. The cells were suspended in a standard culture medium (Eagle's basal medium with 1 per cent glutamine, plus 10 per cent horse serum, 2 per cent fresh embryo extract in Tyrode's solution, and 50 μ/c.c. penicillin-streptomycin).

Adhesion to glass. 2 c.c. aliquots of cell suspension (10^4 cells/ml.) were distributed into flat, 15-ml. T-flasks, filled with 5 per cent air containing carbon dioxide and sealed. Groups of flasks were kept at 38°, 30°, 25° and 15° C. The cells settled rapidly on the floor of the flasks and were not disturbed for 24 hr. They were then examined and the number of attached and unattached cells determined.

The three kinds of cell populations tested gave essentially similar results. At 38° C., 85–90 per cent of cells adhered firmly to the glass and were not dislodged by gentle flushing. The individual attached cells were flattened or rounded; others were in small spheroid clusters also stably attached to the glass. At 30°, about 60 per cent of the cells were firmly attached; at 25°, less than 40 per cent adhered stably. At 15°, cells did not adhere; even when left for 72 hr. in continuous contact with the floor of the flasks there was no attachment. When cultures from sub-optimal temperatures were transferred to 38°, there was rapid and abundant attachment of cells to the flask. It appears, therefore, that in the present case adhesion of cells to glass depended on temperature-sensitive processes which were completely, but reversibly, inhibited at 15° C. Interpretations of the mechanism of cell adhesion in such and similar systems must, therefore, account for the temperature-dependence of this cellular activity.

Histogenetic cohesion. To find out whether cohesion and histogenetic bonding of dissociated cells also were dependent on temperature, a procedure was sought which would: (1) render it possible to bring discrete cells rapidly into contact, independently of their capacity for locomotion, and without forceful packing (as by centrifugation); (2) reveal differences in stability and effectiveness of intercellular contacts under diverse testing conditions; (3) yield consistent results in a form amenable to quantitation. These requirements are adequately met by the 'rotation-mediated cell aggregation' procedure, described in detail elsewhere[4]. Erlenmeyer flasks with cell suspension are rotated on a gyratory shaker with a ¾-in. diameter motion. Due to the spinning flow of the liquid, all the cells are rapidly concentrated in the middle of the vortex and, if capable of cohesion, become soon bound into compact aggregates in which they construct tissue. The size distribution of aggregates thus formed reflects an equilibrium between cell cohesiveness under the given conditions, and the shearing forces of the system: the lesser the capacity of cells to cohere (or the fewer the number of cohesive cells, or the stronger the shearing forces), the smaller the resulting aggregates. All experimental parameters in this system are rigorously controllable, and under standard conditions diagnostically consistent 'aggregation patterns' are obtained, characteristic for diverse cellular and environmental situations.

In the present case, aggregation patterns produced at 38° C. by suspensions of 7-day embryonic chick cells (as above) served as a basis for testing the effects of lower temperatures on cell cohesion.

3-ml. aliquots of cell suspension (3×10^5 cells/ml.) were dispensed into 25-ml. Erlenmeyer flasks and rotated at 70 r.p.m. at 38°, 30°, 25° and 15° C. After 24 hr., size distribution of aggregates and number of free cells in each culture were determined. At 30° and 25° there was a significant and consistent decrease in size of aggregates of all cell types tested, illustrated for neural-retina cells in Figs. 1 and 2. This was accompanied by an increase in number of free cells. At 15° the cells did not cohere at all, and aggregates were not formed; even when maintained in continuous contact for 72 hr. at this temperature, the cells remained entirely discrete. Transferred to 38° C. such cells cohered into histogenetically effective aggregates. It should be pointed out that 15° C. is not the starting temperature for complete inhibition of cell cohesion; more recent results indicate that neural-retina cells may not cohere at about 18° C. and that sensitivity in this respect varies with cell type, state and culture medium.

The foregoing results reveal a clear correlation between temperature and ability of freshly dissociated avian embryonic cells to adhere to glass or to cohere mutually. The finding that at 15° C. cells are completely unable to become attached, though in prolonged and continuous contact, appears particularly useful towards further study of the mechanism of cell adhesion. It indicates that simple reactions between divalent cations (calcium, magnesium), or other first-order physico-chemical effects cannot be exclusively responsible for cell adhesion, as such reactions are unlikely to be persistently inhibited at 15° C. It also suggests that the exogenous molecules prerequisite for normal adhesion of cells (divalent cations, serum proteins[3,5]) provide only parts of the actual mechanism or contribute otherwise partially to its effective function, and that endogenous thermo-sensitive processes may have a highly important, perhaps primary, role in this cellular activity[6]. It is therefore proposed that cell adhesion may depend on metabolic sequences and closely reflect the metabolic economy of the cells ; therefore, that it is not merely a result of coupling between stable surfaces, but also a function of the synthetic activities of cells. Assuming this, cell adhesiveness would be expected to vary with different functional states of cells ; evidence to this effect is forthcoming, and can also be inferred from various, indirectly related, data in the literature.

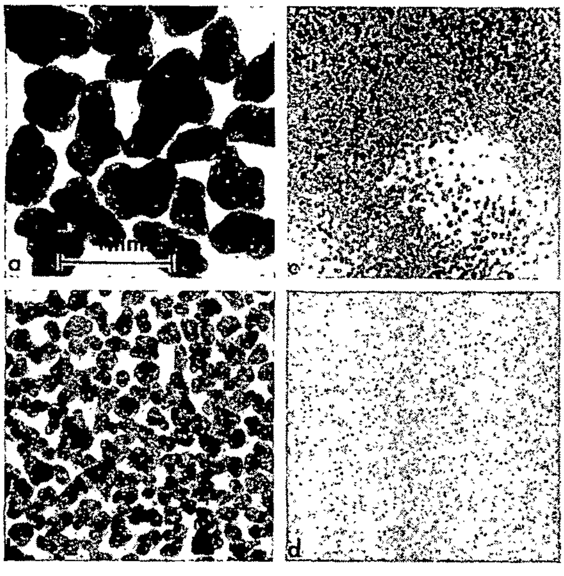

Fig. 1. 24-hr. aggregates of dissociated neural-retina cells (7-day chick embryo) formed in rotating cultures (70 r.p.m.) at 38° (*a*); 30° (*b*) ; 25° (*c*) and 15° C. (*d*); the cells remained separate

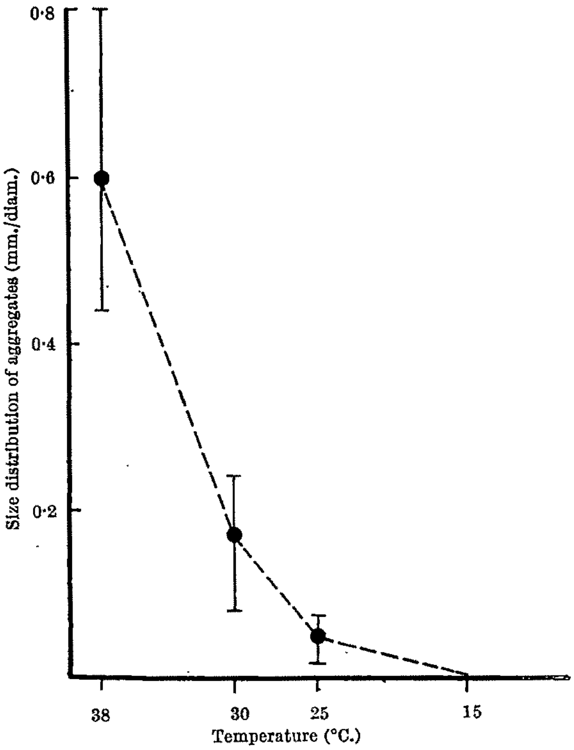

Fig. 2. Effect of temperature on size distribution of aggregates of dissociated neural-retina cells

The present findings appear to fit in with the working assumption[3] that (1) adhesion and histogenetic bonding of cells involve the production and organization between cells of mucoprotein-containing extracellular materials, the formation or effective function of which requires divalent cations and certain large molecules in the cellular environment ; (2) rate of formation and properties of extracellular materials vary with type, age, metabolic pattern and treatment of cells; at low temperatures synthesis of extracellular materials is impaired resulting in failure of dissociated cells to become effectively attached.

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MAGNETIC SUSCEPTIBILITY OF NORMAL LIVER AND TRANSPLANTABLE HEPATOMA TISSUE

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IN 1948, Barnothy[1] found that young female mice, when placed in a (3–6) × 10^3 oersteds magnetic field, underwent a temporary retardation of growth. The magnetic field apparently hindered mitosis. Barnothy *et al.*[2] reported a significant drop in the segmented leukocyte count in mice while confined in a magnetic field, whereas when they were removed from the field the count increased to peak values more than 100 per cent higher than the normal value. From these and other related experiments[2–5] with biological materials in magnetic fields, it can be inferred that a magnetic field may have a significant effect on growth of cancer cells. In support of this thesis, Barnothy reports[6] that at the University of Budapest he obtained good recovery results on albino mice inoculated with *T* 2146 adenocarcinoma. Of the animals placed in an inhomogeneous magnetic field of 3,000–3,500 oersteds, there was loss of tumour in 5 and complete recovery in 4 cases out of 6, whereas there was 100 per cent mortality in the control animals. It has thus become increasingly clear that as a next step a more fundamental study of the magnetic properties of cancer and normal cells should be made. A comparative study was therefore undertaken of the magnetic susceptibilities of both types of cells. The results in this article indicate that the magnetic properties of transplanted hepatoma and normal liver tissue are different, and that the difference is probably associated with the way in which the water is held by the respective cells.

As an exploratory experiment, measurements were made on the normal and host tissue from the same organ (human larynx) which had been preserved in formaldehyde. The specimens were thoroughly dried, and several milligrams of tissue were removed with a glass knife for each measurement. No steel instruments were used in order to avoid unnecessary ferromagnetic contamination. The average magnetic susceptibility at room temperature of the tumour and normal tissue from this organ was $(-0{\cdot}61 \pm 0{\cdot}008) \times 10^{-6}$ e.m.u./gm. and $(-0{\cdot}57 \pm 0{\cdot}007) \times 10^{-6}$ e.m.u./gm. respectively. The difference is considered significant as it is several times the experimental error.

In view of these results, two series of controlled experiments were performed with rats. By means of trocar inoculation, a transplantable hepatoma was allowed to develop in some of the rats for 4–8 weeks. The tumour-bearing and control animals were then killed and the liver and transplanted tumour tissue removed. As soon as the tissue was removed from an animal, it was quick-frozen in liquid nitrogen to prevent any serious deterioration of the cells and remained there until the measurements could be made. The specimens were then removed from the liquid nitrogen and a small sample ($\approx$10 mgm.) was removed by cracking the frozen organ in a porcelain mortar. The entire sample preparation was performed in a refrigerated room held to within a few degrees of 263° K. so as to preclude any thawing of the specimen.

Although a steel scalpel was used to remove the tissue from the animal, non-ferromagnetic instruments were used thereafter in handling the samples to prevent contamination. By selecting a sample of frozen tissue from below the surface of the specimen ferromagnetic contamination could be minimized. However, to ensure further against this effect, each sample was measured at several magnetic field strengths. The apparent magnetic susceptibilities were then plotted against the reciprocal field (see Fig. 1) at each field strength after the manner of Owen[7] and Honda[8]. As ferromagnetism is dependent on field, the ordinal intercept is thus the susceptibility corrected for ferromagnetic impurity.

The magnetic susceptibility measurements were made by a modified Faraday method using a sensitive quartz helical spring balance described elsewhere[9]. The balance was placed in the same refrigerated room described above. Considerable difficulty was encountered in making the measurements due to the change in mass caused by evaporation of moisture at certain temperatures. Because the moisture content of the cold room was reasonably constant, little difficulty was experienced in making the measurements at 263° K. in air. At lower temperatures, however, carbon dioxide and some of the rare gases will condense out of the air, and hence helium had to be used as a heat transfer gas. As the helium was very dry, the sample tended to lose water. For each of the measurements in helium at temperatures between 170° K. and 263° K., the mass of the sample was remeasured using the quartz helix without a magnetic field, that is, as an analytical balance. In this way, a correction for loss of moisture could easily be made. At temperatures below 170° K., the vapour pressure of ice is so low that the change in mass due to loss of moisture is negligible.

In the first experimental series, two rats of the $A \times C$ strain were used for the tests. The tumour was allowed to grow for four weeks after inoculation in one rat, the other was used as a control. At the end of this period, the animals were lightly anæsthetized, and the liver and tumour tissue removed and

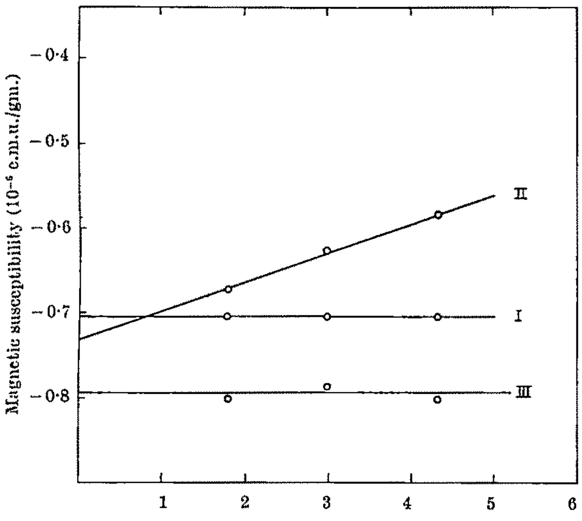

Fig. 1. Magnetic susceptibility of normal liver tissue from control animal (I) and liver (II) and transplanted hepatoma tissue (III) from tumour-bearing rat as a function of the reciprocal magnetic field

frozen. After loading a small sample on the balance, helium was admitted to the system and the temperature immediately dropped to liquid nitrogen temperature. By using this technique on each specimen of this series, a minimum amount of water was lost by evaporation. Re-measurement at room temperature in moist air allowed a correction to be made for this small loss.

All the measurements of this series were made at liquid-nitrogen temperature for another reason. If the cancer cells contained an abnormal amount of some paramagnetic impurity as, for example, some free radical, one would expect a paramagnetic component in addition to the basic diamagnetism of the cells. As the positive magnetic susceptibility of many paramagnetic substances increases as the temperature is lowered, it was thought worth while to take advantage of this enhancement if it existed in these specimens.

The susceptibilities plotted against the reciprocal field are shown in Fig. 1. The susceptibilities corrected for ferromagnetism (ordinal intercept values) of all the specimens are diamagnetic, the tumour tissue being more diamagnetic than the normal tissue as it was in the previous measurements. Although the results of the previous experiment on larynx tissue should not be compared too closely with these because of the differences in kinds of tissue, the pattern of the results is similar. The fact that the susceptibilities at the lower temperature are more diamagnetic would seem to suggest that the major differences between the normal and tumour tissue are not due to the presence of a paramagnetic substance.

The dependence on field shown in Fig. 1 for the liver from the tumour-bearing host rat indicates ferromagnetic impurity. At first this was thought to be a significant property of the tissue, but subsequent repeated measurements on samples of liver from other tumour-bearing host rats lead us to believe that this impurity was probably contamination.

To substantiate the results of this experiment, tissue from three more rats with transplanted hepatoma No. 3683 and three normal control animals was measured. To obtain a better temperature comparison with the first series, the measurements in this second series were first made at 263° K. in air. The results shown in Table 1 again show the same pattern, but in this instance are comparable with those in Fig. 1. The diamagnetic susceptibilities are lower, and the differences between the susceptibilities of the two kinds of tissue are smaller.

Table 1. MAGNETIC SUSCEPTIBILITY OF TUMOUR AND NORMAL TISSUE OF RATS*

Sample No.	Magnetic susceptibility (in 10^{-6} e.m.u./gm.)	
Transplanted hepatoma Morris No. 3683		
II-1	− 0·680	− 0·688 ± 0·0046
II-2*a*	− 0·695	
II-2*b*	− 0·675	
II-3*a*	− 0·691	
II-3*b*	− 0·700	
Liver tissue from tumour-bearing rat		
II-1	− 0·668	− 0·670 ± 0·0012
II-2	− 0·671	
II-3	− 0·672	
Liver from normal control animals		
II-4	− 0·629	− 0·637 ± 0·0059
II-5	− 0·649	
II-6	− 0·634	

* All the results have been corrected for ferromagnetic impurity, and have been taken at 263° K.

To explain these results better, the susceptibilities of duplicate samples of the same specimens listed in Table 1 were determined as a function of temperature from 77° K. to 263° K. No change was observed in the force on the sample as the temperature of the system was dropped from 263° K. to about 160° K. Therefore, independence of temperature was assumed in this region. Below 160° K., the force on the sample began to change and more measurements were made down to 77° K.

The results shown in Fig. 2 throw considerable light on the problem. The results for the tissue from the tumorous animals resemble a change-of-state curve exhibited by many substances[10]. As the change in the susceptibility at about 130–140° K. is so pronounced, it is not likely that it could be caused by a substance present in trace amounts. Due to high concentration in cell tissue, water or ice must be suspected. The magnetic susceptibility of ice is not well known in this temperature-range, so a series of measurements on ice was made following the same general procedure as outlined here. The

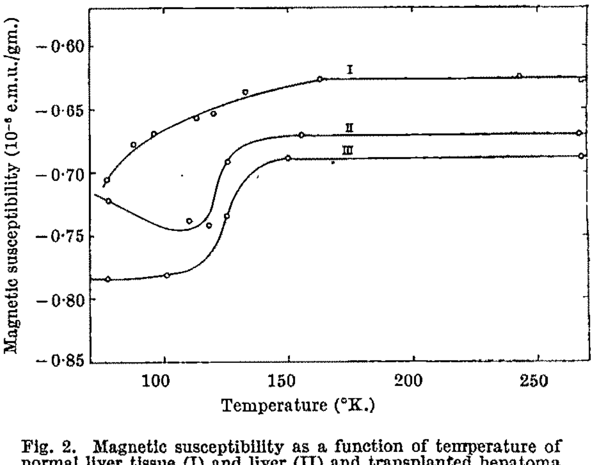

Fig. 2. Magnetic susceptibility as a function of temperature of normal liver tissue (I) and liver (II) and transplanted hepatoma No. 3683 tissue (III) from tumour-bearing rat

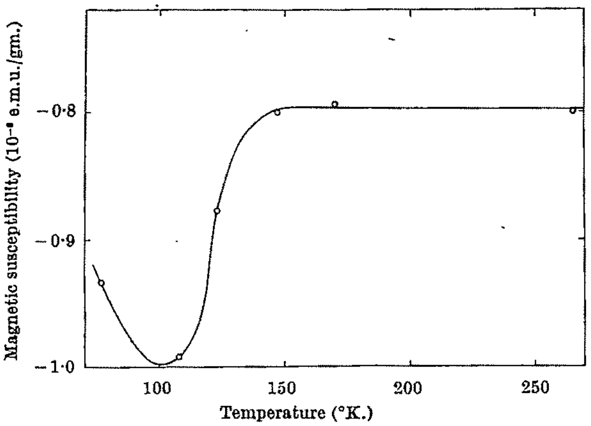

Fig. 3. Magnetic susceptibility of ice from distilled water as a function of temperature

results are shown in Fig. 3. The shape of the curve is so similar to those of the hepatoma and liver of the tumour-bearing host that one is led to conclude that the general feature of these curves is primarily due to the magnetic properties of ice rather than of the tissue itself. The break in the curve at about 130–140° K. is undoubtedly due to the change in state. Ice changes from hexagonal to the low-temperature cubic form at some temperature near 173° K. [11]. However, below 133° K., Lisgarten and Blackman[12] have shown that the X-ray diffraction pattern becomes diffuse. It is probably a change in crystal size or strain which accounts for the diffuse X-ray pattern and also the increase in diamagnetism of ice at about the same temperature.

To substantiate further the fact that changes in the magnetic properties of water are the chief reason for the characteristic form of the magnetic susceptibility curve of tumour tissue, an attempt was made to remove the water in the tumour cells by evacuation at 263° K. The residual tissue, however, was so spongy that it was extremely difficult to get enough material for the measurement in the homogeneous part of the magnetic field of the magnets. As an alternative, the effects of water in egg whites was measured for comparison. Curve I of Fig. 4 shows the change in the magnetic susceptibility of fresh egg white as a function of temperature. The results are similar to that of pure water and also the tumour tissue. The egg white was then slowly evacuated for 48 hr. at room temperature, and a 14·7-mgm. sample was loaded on the magnetic balance. The balance chamber was then further evacuated for an additional 18 hr. until constant weight was achieved. The subsequent susceptibility measurements (curve II, Fig. 4) show the absence of the magnetic properties of water. From these experiments it is reasonable to assume that the general features of the susceptibility curves of the tumour tissue are essentially due to the effects of water.

As water is diamagnetic ($-0{\cdot}7^{+} \times 10^{-6}$ e.m.u./gm. at 273° K.) and as livers of tumour-bearing animals are known to contain more water than normal liver tissue[13–15], the more diamagnetic character of the liver tissue from the tumour-bearing host compared with normal tissue is reasonable. Apparently, the same is true of hepatoma, although some tumours are known to contain less water[15]. From the magnetic susceptibility of water at 263° K. (Fig. 3) and of normal and tumour tissue (Fig. 2), an increase in the water content of the host tissue over the normal tissue of 5·8 and 8·5 per cent can be calculated for the liver and hepatoma respectively. This agrees with the fact that hepatomas generally have a higher content of water than normal liver[15,16]. It is therefore very probable that the increased diamagnetism of the liver and tumour tissue in the host is due to an increase in water content compared with the normal tissue.

An interesting feature of the results in Fig. 2 is the shape of the susceptibility curves for the tissue from the control and host animals. The change in state pattern of water does not appear in the normal tissue, and yet it is known that normal liver tissue contains about 70 per cent water. At least part of the water in the normal and tumour tissue does not go through the normal phase changes, that is, the molecules do not appear to be free to associate in the usual manner. As discussed by Gortner[17], many experiments have been performed which show that forces at the surface of lyophilic colloids tend to bind water with greater force than the usual forces of association which ultimately arrange water molecules in an ice crystal lattice. Bound water in gelatin has been shown to remain unfrozen down to temperatures as low as 170° K. (ref. 18), and probably will remain unfrozen to much lower temperatures. The perplexing problem in trying to explain the magnetic susceptibility of normal tissue by bound water is the amount present. It is difficult to see why essentially all the normal tissue water is bound, as would have to be the case to explain the results in Fig. 2. Most investigators have reported bound-water values of 25–30 per cent or less for colloidal materials[17], which are considerably

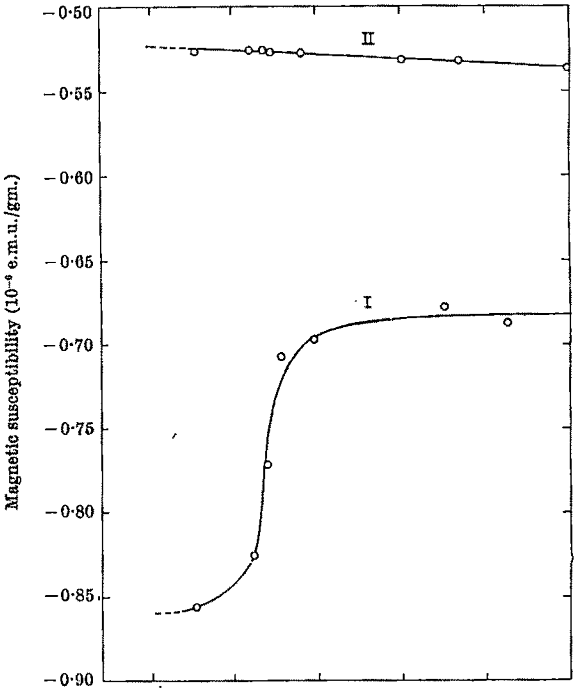

Fig. 4. Magnetic susceptibility of fresh egg white (I) and vacuum dehydrated egg white (II) as a function of temperature

less than is indicated by these measurements. Further work will have to be done to confirm the amount of bound water in normal tissue.

The decrease in the diamagnetism below 110° K. in the liver tissue and not in the tumour of the tumour-bearing host is not surprising. A small amount of paramagnetic or ferromagnetic impurity could easily account for the rise in the curve at the lower temperatures. It is known, for example, that both iron and cobalt are present in increased concentrations in the liver of tumour-bearing hosts.

In summary, it has been shown that the magnetic properties of transplanted hepatoma and normal liver tissue are different, and that the difference is probably due in a large part to the amount and form of the water held by the cells in the two types of tissue. The water in the normal cells appears to be bound, whereas that in the tumour tissue appears to be partially free water; however, this hypothesis will have to be confirmed by further experiment. If the pattern of magnetic susceptibility is found to be generally true for various kinds of tumour tissue, the measurement of magnetic susceptibility may be used as a diagnostic tool. With some refinements the method could be used on specimens as small as 1–2 mgm.

We wish to acknowledge the interest and help of various members of the National Cancer Institute. In particular, we wish to thank Drs. R. Kaiser, S. Inghram, A. Hilberg and Mr. P. Lawrence for their stimulating discussions, Dr. H. P. Morris for supplying the test animals and transplanted hepatomas, and Mr. D. Trainor who helped to prepare the specimens. We are also grateful to Dr. H. Branson, of Harvard University, for his continued interest in this work.

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ACTIVATION OF FERRITIN SYNTHESIS AND INDUCTION OF CHANGES IN FINE STRUCTURE IN HELA CELLS *IN VITRO*: IMPLICATIONS FOR PROTEIN SYNTHESIS

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WORK in my laboratory has provided evidence that the synthesis of the iron-containing protein, ferritin, can be activated in cells grown *in vitro* in tissue cultures. HeLa cells growing in a semi-synthetic medium produced ferritin within 4 hr. following addition of small quantities of ferrous sulphate to the medium. 5γ of iron per ml. of medium sufficed to bring about this result.

A strain of HeLa cells was obtained from Microbiological Associates, Inc., Bethesda, Maryland, and the cells were grown in an Earle–Eagle medium containing amino-acids, vitamins, minerals (excluding iron), streptomycin, penicillin, and horse serum (10 per cent by vol.). No iron was demonstrable in this medium by standard analytical tests sensitive to concentrations as low as 0·03 γ/ml.[1]. Control tests for iron, performed on acid digests of packed HeLa cells, grown during 2 weeks in 5 ml. of this medium, revealed minute amounts of iron, consistent with the presence of iron-containing enzymes.

Ferritin was demonstrated by means of electron microscopy on sectioned cells[2,3], and by chemical isolation using cadmium sulphate[4]. Furthermore, using established procedures[5,6], both ferritin and apoferritin were isolated from cell homogenates with an ultracentrifuge, and were later identified in the electron microscope[7] and by means of precipitin tests with specific antisera, obtained from rabbits after immunization with solutions of crystalline human ferritin. Trivalent iron in the cytoplasm—and occasionally in the nuclei—of cells treated with ferrous sulphate was shown to be present by means of the Prussian blue test.

Sub-cultures carried in individual flasks or bottles for approximately one week were used. Measured quantities of freshly prepared sterile solutions of ferrous sulphate were added to yield final concentrations of iron ranging from 2 to 15 γ/ml. Afterwards, cells were taken for examination after 2, 4, 24 hr., and at intervals up to 2 weeks. In cultures kept longer than 3 days the medium was changed periodically, and to some of these ferrous sulphate was added a second time. Cells from control cultures, to which no iron had been added, were examined concurrently.

Representative results obtained with HeLa cells are shown in Table 1. The findings indicate clearly that ferritin synthesis occurred within 4 hr. after addition of ferrous sulphate. As judged from electron micrographs and from yields of chemical

Table 1. Production of Ferritin (Apoferritin) by HeLa Cells

Ferrous sulphate γ/ml. medium	Interval	Tests for ferritin (apoferritin)			
		Cell extracts		Crystallization (cadmium sulphate)	Electron microscopy of cells (*e*)
		Precipitin tests	Electron microscopy (*d*)		
0	0 (*a*)	–	–	–	–
5	2 hr.	–	*A*	not done	–
5	4 hr.	±	*F* and *A*	,, ,,	+
5	24 hr.	+	*F* and *A*	,, ,,	+ +
5	48 hr.	+	*F* and *A*	,, ,,	+ + +
5 (*b*)	5 days	+	*F* and *A*	+	+ + + +
5 (*c*)	14 days	+	*F* and *A*	+	+ + + +

Notes: (*a*) Control cultures, same age as others. No iron added. (*b*) Medium changed after 3 days without addition of iron. (*c*) Medium changed twice. Ferrous sulphate added each time. (*d*) *F*, ferritin present; *A*, apoferritin present. Apoferritin identified by means of negative staining (ref. 7). (*e*) Only ferritin can be identified with certainty by this method.

extractions, the quantities of ferritin accumulating in HeLa cells increased as time went on. But it was also found that some HeLa cells from cultures grown in the presence of ferrous sulphate contained little or no ferritin. It is not clear at present whether these were cells that had synthesized little or no ferritin; whether they had synthesized it and disgorged it; or whether only apoferritin—not clearly visible in electron micrographs of sectioned cells—was present.

The earliest accumulations of ferritin were located within discrete cytoplasmic bodies, up to 1μ in cross-section, and similar in appearance to the 'siderosomes' described previously by me[2]. However, these bodies varied considerably. Some resembled 'myelin figures', others had a complex system of tubules, and still others also contained a relatively homogeneous material of low density to electrons (apoferritin ?). Frequently, they were situated in a cell segment close to an indenture in the nucleus. Cytoplasmic bodies of a novel kind were noted in HeLa cells taken three or more days after addition of iron (Fig. 1). Characteristically, those bodies were delimited by membranes and contained material of high density to electrons. In profile this material appeared in the form of circinate structures, with hollow centres and with walls measuring up to 200 Å. in cross-section. Arrays of ferritin molecules often adjoined the dense walls. Cytoplasmic deposits of partly hydrated ferric oxide[8] were also noted. In samples taken from cultures 24 hr. after addition of ferrous sulphate a small proportion of cells contained intranuclear collections of ferritin molecules.

No ferritin or apoferritin was found in cells from control cultures to which no iron had been added.

Thus, the addition of ferrous sulphate to the HeLa cell cultures had two consequences: apoferritin synthesis, and the reaction of the apoferritin with iron, yielding ferritin.

Further results have indicated that apoferritin synthesis can be initiated in HeLa cells by very small concentrations of iron (< 1 γ/ml.), and that apoferritin is synthesized within 2 hr. after addition of iron—before ferritin iron micelles are detected in cells with the electron microscope. This is in agreement with evidence showing that in the liver apoferritin is synthesized first, and iron incorporated later into apoferritin[9].

It is not clear yet whether any of the aforementioned cytoplasmic organelles are sites of apoferritin synthesis, whether they are involved in the incorporation of iron into apoferritin, or whether they are concerned with the disposition of ferritin or apoferritin following synthesis elsewhere in the cell. Judging from present hypotheses on the mechanism of protein synthesis, the production of apoferritin might be initiated by intranuclear synthesis of specific RNA. The latter may then be moved into the cytoplasm[10].

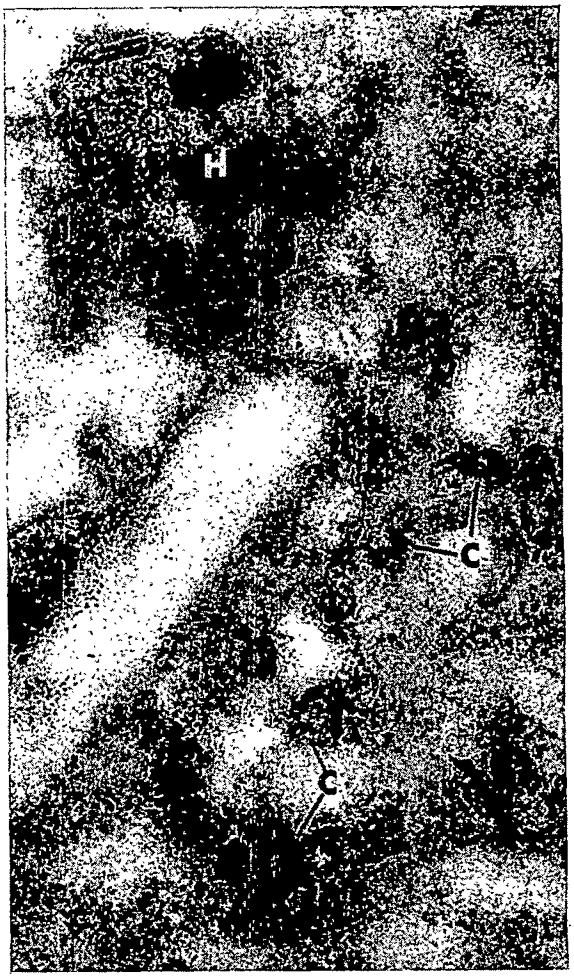

Fig. 1. Part of cytoplasm of HeLa cell from culture kept 3 days in medium containing 5γ of iron per ml. Note organelle (*H*) at upper left, delimited by membrane and containing: (1) ferritin molecules represented by small particles, ~ 60 Å diam. (FeOOH micelles); (2) larger opaque bodies. A larger organelle (lower right) contains bodies (*C*) with opaque walls (~ 200 Å.), and non-opaque centres. Ferritin molecules are situated close to these bodies and within them. (× 100,000)

One can infer that the HeLa cells possess an inherent capacity to synthesize ferritin; that this capacity has been retained after many years of cell division *in vitro*, and that therefore it is connected in some way with the cell genome. Further experiments are in progress to determine whether the primary effect of iron is intranuclear or intracytoplasmic, and to learn more about the mechanisms of the phenomena reported.

It is evident that the methods described here are applicable to controlled studies of protein synthesis in relation to the fine structure of cells, to their chemical constitution, and to their function. The methods are also relevant to investigations of immune mechanisms in which ferritin can be used as antigen.

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RADIATION CARCINOGENESIS AT HIGH DOSE-RESPONSE LEVELS: A HYPOTHESIS

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CARCINOGENESIS and cytotoxicity are two of the most important biological effects of ionizing radiation. The present hypothesis is an attempt to unify the concepts of their action[1]. It states that carcinogenesis and cytotoxicity act in opposition and together produce the overall relationship between radiation dose and tumour incidence that is found experimentally. The hypothesis has application to radiation therapy, and has been used to estimate the relative carcinogenic potencies of radium and strontium-90.

No assumptions are made regarding the detailed mechanism of radiation carcinogenesis, irrespective of whether in a given situation it is a primary process caused by a somatic mutation, or a secondary process caused by, for example, liberation of an oncogenic virus, or by creation of hormonal imbalance. Similarly, no assumptions are made regarding the normal defence mechanisms of the host against incipient cancer cells. Instead, consideration is confined to factual presence or absence of developing cancer cell clones. Complexities arising from the widely varying response to a carcinogenic stimulus depending on hormonal status[2] and functional integrity of the reticulo-endothelial system[3] will be discussed in a future publication.

The hypothesis assumes that the carcinogenic change is a rare but specific type of cell damage that occurs short of death of the cell. Since damage to cellular material increases as the dose of radiation is increased[4], so does carcinogenesis and the resultant incidence of tumours[5], until a maximum is reached. Beyond this maximum, the cell-killing effects of radiation would be expected to outweigh increasing carcinogenesis, resulting in a progressively lower incidence of tumours.

In fact, this expected behaviour occurs both with external radiations and with internal emitters. External cobalt-60 γ-rays given to mice in various constant daily doses resulted in a peak incidence of thymic lymphoma at 32 r./day, while at 56 r./day tumour incidence had dropped again to the level of unirradiated controls[6]. External X-rays given in a single dose to mice resulted in a peak in leukæmia incidence[7]. Injections of internal emitters such as radium, strontium-90 or calcium-45 into mice gave curves for bone tumour incidence showing sharp maxima[8]; injections of iodine-131 into rats gave similar incidence curves for thyroid tumours[5,9].

The lower portion of Fig. 1 illustrates the four main types of curves for tumour incidence found experimentally (curves *C*). While the abscissa expresses dose of a radioisotope deposited internally, curves *C* in part also represent tumour incidence induced by external radiations or by radiomimetic chemicals.

In the upper portion of Fig. 1, curve *C* has been split into two hypothetical curves *A* and *B*, which together produce *C*. Curve *A* represents 'pure' carcinogenesis, that is, carcinogenesis in the absence of other radiation effects that may counteract it. Curve *B* represents the combined cytotoxic effects of radiation on tumour cell clones, which prevent the development of all such clones in a percentage of the irradiated animals. At any dose-level, the percentage *C* of animals developing gross cancer during their life-span is the product of the percentage of animals with incipient tumour clones (*A*) and the fraction of animals in which all incipient tumour clones have not been eradicated ($1-B/100$), or:

$$C = A(1-B/100) \qquad (1)$$

Fig. 2 illustrates dissection of the cytotoxic process (curve *B* of Fig. 1) into two components, namely, eradication of tumour cells (curve *D*) and eradication of normal cells (curve *E*), which can cut short the life-span of the host before tumour clones have had time to develop into gross cancer. At any given dose-level:

$$1 - B/100 = (1 - D/100)(1 - E/100) \qquad (2)$$

The need for Fig. 2 can be eliminated by excluding animals which died from causes other than cancer during an experiment. With this correction, curve *B* in Fig. 1 is replaced by curve *D* and represents cytotoxicity against tumour.

Fig. 1 suggests explanations for the carcinogenic properties of a given external radiation, internal emitter or radiomimetic chemical[10]. Thus, production of osteogenic sarcoma in mice[8] places strontium-90 in or close to the highly carcinogenic grade 3, whereas radium falls into the medium carcinogenic grade 2. Apparently the short-range (35μ) and localized dose distribution[11] surrounding microscopic radium deposits in bone is better suited for killing any incipient cancer cells by continuing radiation than the longer range (6,000μ) and more diffuse dose distribution from strontium-90 and its daughter yttrium-09. Production of thyroid carcinomata in rats[9] classifies iodine-131 in the low carcinogenic grade 1, which differs from grade 2 in that cytotoxicity occurs at lower radiation doses than carcinogenicity. Grade 0 illustrates how a strong mutagen

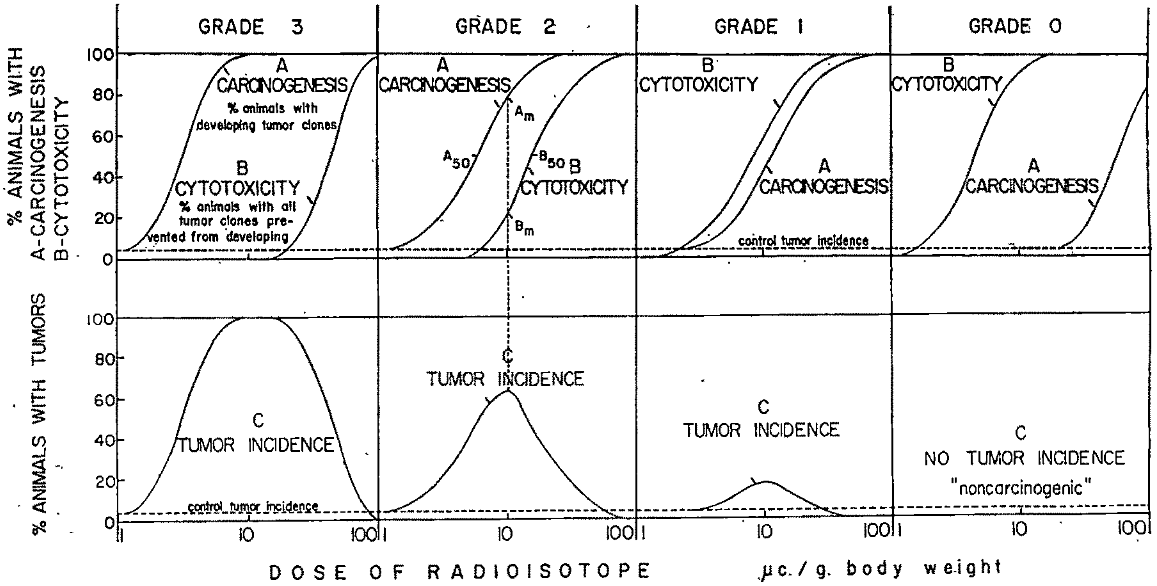

Fig. 1. Idealized curves *C*, of percentage of animals injected with a radioisotope that develop tumours, covering a range from highly carcinogenic (grade 3) to non-carcinogenic (grade 0). The overall shapes of the tumour incidence curves *C* are held to be the resultants of the opposing actions of carcinogenesis (curves *A*) and cytotoxicity (curves *B*)

such as acrolein may be non-carcinogenic[12]: cytotoxicity (in this case, acute lethality at a relatively low dose[13]) may predominate over carcinogenicity to such an extent that no gross tumours can occur. For any radiation, the carcinogenic potency evoked may depend on the mode of administration, the tissue irradiated, and the biological and genetic characteristics of the host.

Looking at Fig. 1 from the point of view of radiation therapy, the most carcinogenic dose-levels may lie just short of those giving complete cytotoxicity and therefore presenting no danger of carcinogenesis. This stresses the well-known importance of irradiating tumour masses with the highest dose-levels that can be tolerated by the patient. Irradiation of fringe areas or irradiation of normal tissues by therapeutic doses of radioisotopes should be minimized, since doses in the optimum carcinogenic range may be delivered. Because of the long latent period of radiation carcinogenesis in most body tissues, these latter considerations may apply only for younger patients.

Quantitative results are obtained by assuming that curves *A* and *B* are similar continuous *S*-shaped curves that are linear and of equal slope over their central portion. This assumption of equal slopes can be eliminated by use of equation (5) below. Curve *A* is identical with the upswing portion of curve *C* for grade 3 tumour incidence (Fig. 1), according to equation (1). While the curves for tumour incidence for strontium-90 and for calcium-45 barely miss[8,14] falling in grade 3, they do have an *S*-shaped upswing portion. For carcinogens other than ionizing radiation, grade 3 incidence curves are obtained with ultra-violet radiation[15] (which is also cytotoxic[16]) and with certain chemical carcinogens[17], and these curves are generally *S*-shaped. Similarly, curve *B* has been judged to be *S*-shaped.

At the optimum tumour dose, with tumour incidence maximal at C_m per cent, the corresponding ordinates A_m and B_m of curves *A* and *B* are related by:

$$B_m = 100 - A_m \tag{3}$$

$$A_m = 10\sqrt{C_m} \tag{4}$$

Curves *A* and *B* can be constructed graphically, provided the experimental curve *C* is known. Initially, curve *C* is drawn on semi-logarithmic paper. Next, the start of curve *A* and the end of curve *B* are traced on a second sheet, to correspond respectively with the start and with the inverted end of curve *C*. Curves *A* and *B* are continued to pass through respectively A_m and B_m, which are evaluated from equations (4) and (3). Finally, the curves are completed and if necessary replotted, such that equation (1) is satisfied by all points.

An additional refinement of questionable value is to eliminate the requirement for equal slopes of the linear portions of curves *A* and *B*. This necessitates replacement of equation (4) by the equation:

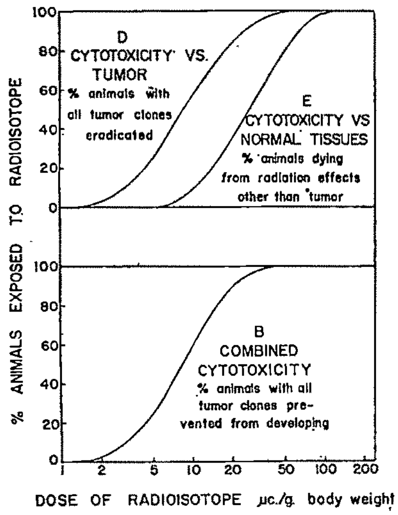

Fig. 2. Idealized curves, illustrating that overall cytotoxicity of an injected radioisotope against development of gross tumours (curve *B*) is held to be the resultant of cytotoxicity both against tumour (curve *D*) and against normal tissues (curve *E*)

$$A_m = 10\sqrt{\left(\frac{a}{b}C_m\right)} \qquad (5)$$

where a and b are the respective slopes read from curves A and B constructed using equation (4), and A_m may lie only between C_m and 100 per cent. The new values of A_m and B_m can be used to re-plot curves A and B.

Points A_{50} and B_{50} are read off curves A and B (see grade 2 curves in Fig. 1) as the LD_{50} doses for carcinogenesis and cytotoxicity, respectively. The inverse dose ratio, B_{50}/A_{50}, is a measure of potency for carcinogenesis relative to cytotoxicity, that is, of the carcinogenic–cytotoxic potency. However, the value of this quantitative result is no greater than the validity of the assumptions on which it is based.

In applying this method to Finkel's results[8,14], integral curves of type C had to be constructed from differential curves of 'tumour-rate' in the case of radium and calcium-45. Using these comparable results resulting from a single injection in mice, the approximate values of the carcinogenic–cytotoxic potencies for radium, strontium-90 and calcium-45 were respectively 1·7, 7·0 and 6·1. When more suitable experimental data are available, better estimates will be possible.

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EFFECTS OF GLUTAMINE AND ASPARAGINE SUPPLEMENTS IN THE DIETARY REGIMEN OF THREE PHENYLKETONURIC PATIENTS

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PHENYLKETONURIA is an inherited condition which is characterized by a biochemical abnormality and usually by extreme mental retardation. The biochemical anomaly is the inability of the body to break down phenylalanine into the forms in which it can be used[1]. Instead, phenylalanine enters the blood stream in an unchanged form, most of it being converted later into phenylpyruvic acid and excreted in the urine[2].

Although many aspects of phenylketonuria are at present incompletely understood, much information has emerged from work carried on over a period of years. Through elimination of phenylalanine from the diets of phenylketonurics, abnormally high concentrations of the decomposition products of phenylalanine have been significantly lowered[3,4]. There is indirect evidence that this diminution of the decomposition products of phenylalanine is accompanied by improvement in mental function, especially in young subjects[5],[6].

The toxicity of some of the metabolites of phenylalanine has been established, but there has been no evidence that phenylalanine *per se* is toxic[7,8]. The process by which phenylalanine is converted to phenylpyruvic acid in phenylketonuria is probably that of transamination. Meister, Udenfriend, and Bessman in 1956 gave evidence of the involvement of transamination in the formation of phenylpyruvic acid when they reported the reduction of this compound in phenylketonuric subjects to whom oral doses of glutamic acid, glutamine and asparagine were administered over a period of 12 hr.[9].

The aims of the present work were to decrease the phenylpyruvic acid–phenylalanine ratio in phenylketonuric subjects through transamination by increasing the concentration of available amino donor and to study the effects of this in the phenylketonuric patients over a period of several months. Three phenylketonuric siblings were fed selected amino-acids under controlled conditions for approximately four months. A fourth affected child received a placebo. Daily urine specimens were collected from each subject for a few weeks before and during the entire period of administration of supplements. In the last weeks of the work one urine specimen was collected from each subject once a week. Urinary phenylpyruvate-levels were determined by a modification of the method of Penrose and Quastel[10]; urinary phenylalanine was determined by paper chromatography[11].

Subject I was a 21-year-old female who had an intelligence quotient (*I.Q.*) score of 18 before the amino-acid administration was begun. After the collection of daily urine specimens from her for a period of 5 weeks, she was given 3 daily doses of 7·3 gm. of L-asparagine over a period of 21 weeks, during which time daily or weekly specimens were also collected. Mental performance was tested once before and twice during the period in which L-asparagine was being given, and an increase of 9 points in *I.Q.* score was observed at the end. Although a significant decrease of phenylpyruvic acid excretion in this subject was not apparent during the treatment, there was a definite decrease in the urinary phenylpyruvic acid–phenylalanine ratio. The *t* value for the significance of the difference between pre-treatment and during treatment means was 2·75, the probability being beyond the 5 per cent level of significance (Table 1).

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Table 1. RATIO OF URINARY PHENYLPYRUVIC ACID TO PHENYLALANINE

Subject	Mean before treatment	Mean during treatment	Difference between means	*t*-Ratio	Degrees of freedom	Probability
I	0·134	0·094	0·404	2·750	5*	> 0·05
II	0·151	0·113	0·179	1·409	23	< 0·05
III	0·102	0·060	0·425	4·618	24	> 0·01
IV	0·119	0·106	0·098	0·351	18	< 0·05

* Degrees of freedom reduced to adjust for lack of homogeneity of variance of the two samples.

Subject II was a 19-year-old phenylketonuric male who had an *I.Q.* score of 12 before the amino-acid administration was begun. Urine specimens were collected daily from him for a period of four weeks, after which time he was also given daily doses of 21 gm. of L-asparagine for a period of 21 weeks. During this time daily or weekly specimens were collected. There was no significant decrease in phenylpyruvic acid excretion in this subject during the treatment, nor was there any change in his mental performance or in the phenylpyruvic acid–phenylalanine ratio. Two possible reasons for the failure of the asparagine to reduce the phenylpyruvic acid–phenylalanine ratio in this subject have been postulated. First, the subject had an average urinary concentration of phenylpyruvic acid which was approximately twice that of any other subject used in this work. Secondly, the attendants were unable to give this subject L-asparagine as regularly as the supplements were given to the other subjects.

Subject III was a 17-year-old phenylketonuric female whose *I.Q.* score before treatment was 21. Daily urine specimens were collected from her 11 weeks before she began receiving 3 daily doses of 3·3 gm. of L-glutamine. She received glutamine for 17 weeks during which time daily or weekly urine specimens were collected. In this subject glutamine feeding was accompanied by a significant decrease in the phenylpyruvic acid–phenylalanine ratio and a significant decrease in phenylpyruvic acid excretion. The *t* value for the decrease in phenylpyruvic acid–phenylalanine ratio was 4·6, the probability being beyond the 1 per cent level of significance (Table 1). An increase of 8 points in *I.Q.* score was noted at the end of the experimental period.

The fourth subject served as a control. He was an 11-year-old phenylketonuric patient who had an *I.Q.* score of 15 before the administration of the placebo. Daily specimens were collected from him 4 weeks before he started receiving the placebo. There were no changes in the *I.Q.* score, the urinary phenylpyruvic acid excretion, or in the phenylpyruvic acid–phenylalanine ratio during the period of administration of the placebo.

The results of this work contribute evidence to the hypothesis that in phenylketonuria phenylalanine is converted to phenylpyruvic acid by means of a transamination process which may be reversed by increasing the available amino donor. The reduction of the phenylpyruvic acid–phenylalanine ratio was accompanied by a slight but significant improvement in each subject's ability. No increase in *I.Q.* score was seen in the two subjects who showed no reduction in the phenylpyruvic acid–phenylalanine ratio. The direct cause of the increase in *I.Q.* scores in the two phenylketonuric subjects is unknown, although the decrease of toxic products formed from phenylpyruvic acid and its derivatives may be one possible influence[12].

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PHAGOCYTIC ACTIVITY OF THE RECTICULO-ENDOTHELIAL SYSTEM

Effect of *B.C.G.*

IT has been reported previously that the active œstrogens are strong stimulants of the phagocytic activity of the reticulo-endothelial system[1-4]. These results were found with both natural and synthetic œstrogens. It would appear that the configuration of the molecule, which confers œstrogenic activity, is also essential for reticulo-endothelial stimulation.

The present investigation was designed to ascertain the effect of *B.C.G.* on the phagocytic activity of the reticulo-endothelial system, to find out whether any non-œstrogenic substances also stimulated the phagocytes and to compare the results with those of other substances reported from this laboratory.

Thirty male white mice (*T.O.* Swiss strain) of 20–25 gm. body-weight were used in these experiments. Twenty mice each received one intravenous injection of 0·05 ml. of an 8-day culture of *B.C.G.* on Dubos medium. At weekly intervals, the phagocytic activity was measured in five animals by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood[5], the procedure used being that described in previous communications[1] and the total body phagocytic activity or phagocytic index being denoted by the symbol *K*. In all cases, after the assessment of phagocytic activity, blood-counts were made and the animals were then killed and the weights of the liver and spleen determined.

Ten of the animals were used as controls and showed a phagocytic index or *K*-value of 16 ± 3·8.

The results are shown in Table 1. It will be noted that at the end of one week there is a slight increase in phagocytic activity. This increase is more marked at the end of the second week and reaches a peak at the end of the third week, when the *K*-value is 78 ± 34. Thereafter the phagocytic index commences to fall and, at the end of the fourth week, the *K*-value is 39 ± 21. The stimulation of phagocytic activity is accompanied by a corresponding enlargement of the liver and spleen, which is most marked at the end of the third week, when the *K*-value is highest and is due to hypertrophy of hepatic and splenic tissue.

Table 1. EFFECT OF *B.C.G.* ON THE PHAGOCYTIC ACTIVITY OF THE RETICULO-ENDOTHELIAL SYSTEM

Duration of treatment	Phagocytic index (*K*-value)	Weight of liver (gm.)	Weight of spleen (gm.)
1 wk.	22 ± 3·6	1·12 ± 0·10	0·09 ± 0·03
2 wk.	41 ± 20	1·37 ± 0·26	0·13 ± 0·03
3 wk.	78 ± 34	1·67 ± 0·19	0·23 ± 0·05
4 wk.	39 ± 21	1·52 ± 0·14	0·17 ± 0·06
Controls	16 ± 3·8	1·20 ± 0·09	0·06 ± 0·01

The total red-cell counts indicated a slight reduction in the number of red cells, but the total white-cell counts remained normal throughout the four weeks of treatment.

The above results are in general agreement with those of Biozzi *et al.*[6,7] and show that the reticulo-endothelial phagocytes can be stimulated by non-œstrogenic substances. It has also been shown by Böhme and Bouvier[8] that killed *M. tuberculosis* strain *B.C.G.* and some of its constituents stimulate the reticulo-endothelial system and fail to induce changes in the percentage distribution of leucocytes in the peripheral circulation.

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Effect of Tri-*p*-anisylchloroethylene, Dienœstrol and Stilbœstrol Diphosphate

IN the case of diethylstilbœstrol, it was shown that the minimum dose required to stimulate the reticulo-endothelial phagocytes is about one hundred times greater than the œstrogenic potential of the substance[2], and that when treatment with diethylstilbœstrol is stopped the phagocytic activity returns to normal level in about 8 days[9]. Other workers, Biozzi *et al.*[10] and Heller *et al.*[11], have confirmed our findings of reticulo-endothelial stimulation by œstrogens.

This communication deals with the effect of tri-*p*-anisylchloroethylene, dienœstrol and stilbœstrol diphosphate on the phagocytic activity of the reticulo-endothelial system and on the blood.

Fifty male white mice (*T.O.* Swiss strain) of 20–25 gm. body-weight were used in these investigations. Groups of five animals were used for testing the various compounds. In the case of tri-*p*-anisylchloroethylene, five animals received one daily-dose of 0·5 mgm. in 0·05 ml. arachis oil subcutaneously for 6 days; five received 1 mgm. in olive oil orally for 6 days and five received a similar dose orally for 12 days. The dienœstrol-treated animals each received one daily dose of 0·5 mgm. in 0·05 ml. arachis oil subcutaneously for 6 days; and those treated with stilbœstrol diphosphate each received one daily dose of 0·5 mgm. in 0·05 ml. water subcutaneously for 6 days. Two days after each course of treatment was completed, the phagocytic activity was measured by the rate of disappearance of a known amount of carbon from the circulating blood[5], the procedure used being that described in refs. 1 and 3, and the total body phagocytic activity or total phagocytic index being denoted by the symbol *K*.

The liver and spleen are known to be the principal reticulo-endothelial organs involved in removing particulate matter from the bloodstream, and, using the combined weights of liver and spleen, Benacerraf *et al.*[12] have applied a corrective factor to the total phagocytic index to give a measure of the phagocytic activity per unit of reticulo-endothelial tissue. This has been called the unit phagocytic index, and has been found to vary for a given dose of carbon according to the following equation:

$$\text{unit phagocytic index} = \frac{\text{total body-weight of animal}}{\text{combined weights of liver and spleen}}\sqrt[3]{K}$$

Generally, when the total phagocytic index *K* is raised, the unit phagocytic index rises also, indicating increased phagocytic activity per unit of reticulo-endothelial tissue.

All the animals used in the present experiments remained in good health during the course of treatment.

Twenty of the animals were used as controls. One group of ten each received 0·05 ml. arachis oil subcutaneously once daily for 6 days, the total phagocytic index *K* and the unit phagocytic index being measured on the eighth day by the carbon method referred to above.

In all the animals after the assessment of phagocytic activity blood counts were made, and the animals were then killed and the weights of the liver and spleen determined.

The results are shown in Table 2, in which, for comparison, the effect of diethylstilbœstrol on the phagocytic activity of the reticulo-endothelial system is also included. It can be seen that all the substances examined are active stimulants of both total and unit phagocytic activity, and that when treatment with tri-*p*-anisylchloroethylene and diethylstilbœstrol was continued for 12 days, the total and unit phagocytic indexes remained high in both cases. The results also show that phagocytic stimulation is accompanied by a corresponding enlargement of the liver in the case of dienœstrol, stilbœstrol diphosphate and diethylstilbœstrol, which is usual with most active œstrogens, but no hepatic enlargement was found in the animals treated with tri-*p*-anisylchloroethylene.

None of the four compounds produced any change in the blood picture, since in all the animals the total

Table 2. EFFECT OF TRI-*p*-ANISYLCHLOROETHYLENE, DIENŒSTROL AND STILBŒSTROL DIPHOSPHATE ON THE PHAGOCYTIC ACTIVITY OF THE RETICULO-ENDOTHELIAL SYSTEM

Compound used	Daily dose	Total phagocytic index (*K*-value)	Unit phagocytic index	Weight of liver (gm.)	Weight of spleen (gm.)
Tri-*p*-anisylchloroethylene	0·5 mgm. in arachis oil subcutaneously for 6 days	54 ± 25	51 ± 8	1·11 ± 0·10	0·13 ± 0·02
	1·0 mgm. in olive oil orally for 6 days	83 ± 31	67 ± 5	1·21 ± 0·13	0·05 ± 0·01
	1·0 mgm. in olive oil orally for 12 days	87 ± 27	66 ± 6	1·18 ± 0·09	0·10 ± 0·01
Dienœstrol	0·5 mgm. in arachis oil subcutaneously for 6 days	40 ± 12	45 ± 5	1·47 ± 0·09	0·13 ± 0·01
Stilbœstrol diphosphate	0·5 mgm. in water subcutaneously for 6 days	40 ± 4	48 ± 4	1·49 ± 0·19	0·13 ± 0·04
Diethylstilbœstrol	0·5 mgm. in arachis oil subcutaneously for 6 days	65 ± 12	45 ± 6	1·79 ± 0·2	0·11 ± 0·02
	0·5 mgm. in arachis oil subcutaneously for 12 days	76 ± 14	65 ± 5	1·59 ± 0·15	0·11 ± 0·02
Untreated controls		15 ± 4	40 ± 4	1·28 ± 0·12	0·09 ± 0·02
Arachis oil controls		12 ± 3	38 ± 3	1·27 ± 0·13	0·14 ± 0·03

red-cell count and the total and differential white-cell counts remained within normal limits.

In the animals given tri-*p*-anisylchloroethylene orally, the high unit phagocytic index, without accompanying enlargement of the liver and spleen, indicates that this œstrogen has produced intense phagocytic activity. This difference in the action of tri-*p*-anisylchloroethylene may be due to the fact that, although it possesses high œstrogenicity and structurally resembles stilbœstrol, it differs significantly from the other synthetic œstrogens at present in clinical use. It is actually a pro-œstrogen which is stored in body fat, released gradually, then converted by the liver into a potent œstrogen, the result closely resembling natural œstrogen secretion.

Unpublished work in this laboratory has shown that an active œstrogen such as diethylstilbœstrol stimulates the reticulo-endothelial phagocytes and increases the amount of gamma globulin in the serum to about twice the normal level, and that this reticulo-endothelial stimulation protects animals against virulent infections. In the case of tri-*p*-anisylchloroethylene, the high total and unit phagocytic indexes found in the present research, together with the known absence of the usual œstrogenic side effects, suggest that tri-*p*-anisylchloroethylene may be the œstrogen of choice for clinical use in stimulating the reticulo-endothelial system and thus increasing the body defence against infection.

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DIFFERENT PERMEABILITY OF CUTICULAR SHEATH IN NORMAL AND GUARD KERATIN FIBRES

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THE property of hair is largely ascribed to the keratin; a protein stabilized by extensive disulphide bridges. In addition, resistant non-keratinous constituents are distributed throughout the fibre, contributing significantly to its overall stability. Those minor components, which at present are not positively identified, can be found in each of three major regions of the hair cuticle, cortex and medulla[1]. Some unequivocal differences in composition and behaviour are known to exist among similar, as well as different, keratin structures in a single species. Even a fibre grown out of a single follicle is heterogeneous in its transverse and axial directions[2–4].

Normal and guard rabbit hairs are known to differ in their pigmentation, diameter and extent of modulation[5], but no information is available concerning their differences in composition and behaviour. The following work establishes the variations in coalescence and composition between normal and guard hairs of rabbit skins, classified organoleptically and physio-chemically to the second class. The differences in coalescence of cuticular membrane between fibres were characterized on the basis of their resistance to the penetration of aniline black and acridine orange.

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In order to protect collagen from the damaging effect of components of the aniline oxidative bath, the samples were pretreated with chromium and fat solutions which introduced 11·2 per cent fat substances and 0·3 per cent chromic oxide. The ash content of the sample was 4·7 per cent and the moisture varied between 9·8 to 10·6 per cent. The hair cover was characterized by a 14·2 E.D.S. (elasticity, density, softness) value, 6·3 per cent abradability and its strength of setting in the corium was 13·2 $kgm./cm.^2$ (ref. 6). The skins were exposed to acridine orange according to the method applied by Jarrett *et al.*[7]. Aniline black was applied to the hair by briefly brushing with a freshly prepared solution containing 7·5 per cent aniline hydrochloride, 2·8 per cent potassium chlorate and 2·8 per cent copper sulphate. The skins were dried for 24 hr. at approximately 45° C. with the initial relative humidity of 65 per cent and an air velocity of 0·5 m./sec. The skins were tumbled in sawdust and combed thoroughly in order to remove aniline oxidation products absorbed on the cuticle. The procedure was repeated 1–4 times. After each operation, 0·5 $cm.^2$ samples were cut from the corium side and used for the cross-sectional examination of the hair cover. The entire amount of hair from each sample was wrapped into a compact bundle, with a thread wound in a tight spiral form. The tight bundle was immersed into acetone–celluloid glue, freed of its excess, dried and cross-sectioned with a Hardy microtome. In order to prevent distortion of the fibres, the specimens were covered with cedar oil and then examined in a drop of glycerine under the microscope. For each sample the ratio of normal to guard hair resisting the formation of nigro- and pernigro-aniline in the cortex was determined. The samples treated with acridine orange were examined in a similar manner by fluorescence microscopy for the presence of red fluorescence in the medulla of the fibres.

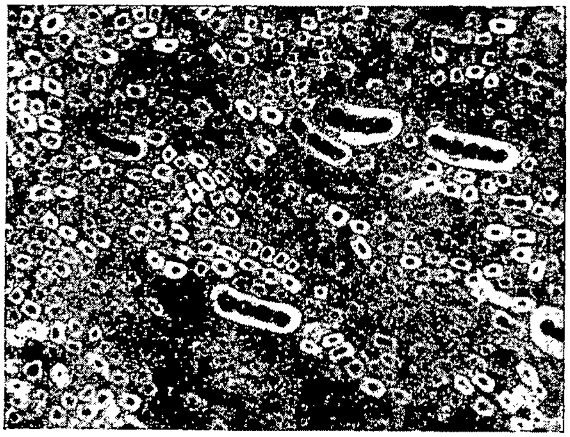

Fig. 1. Cross-section of rabbit hair bundle

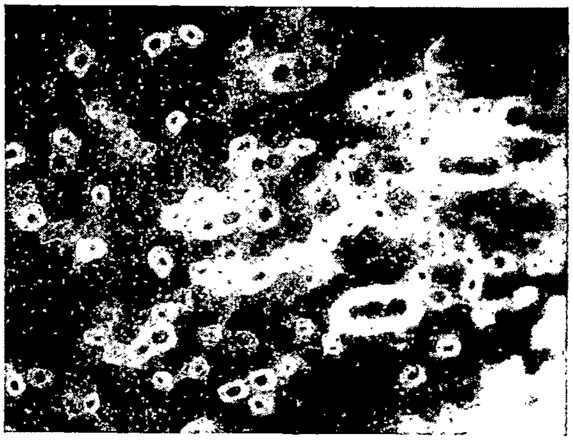

Fig. 2. Hair exposed twice to aniline black

Rabbit skins possess a great excess of normal hair (Fig. 1); its ratio to guard hair lies in the proximity of 1 : 37 bands of square and round shapes, which vary in width from $\frac{1}{8}$ to $\frac{1}{2}$ of the fibre diameter. Less than 10 per cent of total normal hair has no medulla. The guard hair is randomly distributed throughout the area of the skin. Its medullar bands vary from 2 to 8, depending on the cross-sectional region of the fibre. The ends of hair have no medulla. Microscopic examination of the dyed samples revealed that the guard hair is considerably more resistant to the penetration of acridine orange and aniline black than the normal hair (Fig. 2). The impermeable membrane in guard hair is the cuticular sheath. Wherever the cuticle was penetrated by the compound, it was found that the staining or the fluorescence was equally distributed throughout the cortex and medulla. Evidently the subcuticular resistant components which act as a cortical cell membrane[8,9] do not prevent an even distribution of the coloured compounds in this region. Neither acridine orange nor aniline black showed the differences in segmental keratinization of cortex in normal and guard hair[10].

Quantitative evaluation of the results summarized in Table 1 indicates that the cuticle of normal hair has a higher permeability to aniline than to acridine orange. The observed differences can be principally attributed to a smaller steric hindrance of the interchain bonds and non-keratinous substances towards the penetration of lower molecular weight aniline. The incorporation of fluorochrome into the medulla of normal hair is related to the number of exposures to the acridine orange. The cuticular membrane of guard hair is impermeable to this dye regardless of the number of treatments, whereas its resistance to the penetration of aniline decreases gradually.

Table 1. DIFFERENCES IN PERMEABILITY OF THE CUTICLE IN NORMAL AND GUARD HAIR

No. of exposures to dye	Ratio of resistant normal to guard hair		Total percentage of dye-permeable fibres	
	Aniline black	Acridine orange	Aniline black	Acridine orange
0	31·8 : 1	31·8 : 1	0	0
1	15·4 : 1	19·2 : 1	52·2	38·4
2	10·2 : 1	10·9 : 1	80·6	55·6
3	7·7 : 1	4·3 : 1	87·4	78·2
4	4·8 : 1	1·2 : 1	95·7	90·8
5	4·2 : 1	0·2 : 1	99·3	95·2

The rate of changes in the permeability of cuticular sheaths in normal and guard hair exposed to the aniline dye suggests that the treatment modifies progressively the protein structure. After three exposures, the ratio of both fibres resisting the distribution of nigro- and pernigro-aniline remains at the same level. Evidently some component of the dye decreases the structural resistance of the cuticular layer in guard hair to such an extent that its permeability resembles that found in normal hair. In order to establish the nature of this modification, the effect of single components on the dye permeability of guard hair was studied. It was observed that the potassium chloride and the hydrochloric acid evolved during the reaction of aniline influenced the structural resistance of the cuticle. Each pre-treatment with potassium chlorate followed by an introduction of aniline black and acridine orange changed the resistance ratio of normal to guard hair to the values corresponding to the effect of multiple dyeing listed in Table. 1. The observed effect of potassium chlorate on the cuticle of guard hair can be explained by its potential disulphide cleaving action, whereby cystine is converted to cysteic acid. It is known, however, that the action of oxidizing agents like peroxy-acids, H_2O_2, Cl and ClO_2 causes solubilization before cleavage of the disulphide bridges in keratins[11]. In the experimental conditions used in this work, potassium chlorate and hydrochloric acid did not render the protein soluble to any detectable extent. It was observed, however, that the pre-treated fibres solubilized up to 12 per cent on extraction with 0·1 *N* ammonium hydroxide.

High-temperature amino-acid analysis[12] showed that the proline and cystine contents of normal rabbit hair are 5·6 and 12·4 per cent, and in guard hair the corresponding values are 6·8 and 14·1 per cent. The results obtained for cystine were confirmed by the colorimetric method of Zahn *et al.*[13].

The observations reported here indicate that distinct differences exist in the properties of cuticular sheath of normal and guard hair grown from the topographically identical part of the corium. These differences can be explained by the varying extent of keratinization, the principal factor being the degree of disulphide cross-linkage in the cuticle of both fibres. On the other hand, the nature of the action of potassium chlorate and hydrochloric acid does not exclude the possibility that the cleavage of interchain forces occurs at sites other than disulphide bridges and possibly in the structure associated with keratin in the cuticle. It has been observed, for example, that collagen, which is not stabilized by cystine cross-linkages, diminishes rapidly during treatment with potassium chlorate, its typical properties characterized by tensile strength, maximum elongation, hydrothermal stability, etc. Comparative data on this material will be reported elsewhere later.

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EFFECT OF ŒSTROGENS ON ATHEROSCLEROSIS

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Effect of Œstradiol on the Oxygen Consumption of Arteries in Chickens

THE oxygen uptake (Qo_2) of the uterus is enhanced in the rat by the injection of œstrogens[1] and a similar action is shared *in vitro* by endometrium, placenta, mammary gland and adenohypophysis of human origin[2]; the Qo_2 of other organs, though, may be depressed under similar experimental conditions[3-4]. Besides the well-known effects of œstrogens on genital tissues, these hormones can increase atherosclerosis of the great vessels in birds as well as inhibit and reverse coronary lesions in cholesterol-fed cockerels[5]. Since atherogenesis may be modified through a direct hormonal action on the vessels themselves[5], we decided to investigate the effects of œstrogens on the arterial oxygen uptake.

Male white Leghorn chickens raised on a standard laboratory mash and of an approximate weight of 800 gm. were killed; both innominate arterial trunks were rapidly excised and incubated separately at 37° C. on a balanced saline solution[6] also containing penicillin, streptomycin and 0·05 per cent ethanol. In another group of animals, one innominate trunk, chosen at random, was also incubated, but 2 µgm./ml. œstradiol-17β (Δ-1,3,5(10)-œstratriene, 3,17β-diol, melting point 197° C.) was added to the medium, the contralateral artery being taken as control. Oxygen uptake was determined, after incubation for 1 hr., with the usual manometric technique[7] and the results, expressed as $\mu l. O_2$/hr./mgm. of dry tissue, were compared using the *t*-test and tables of Fisher and Yates[8].

Results in Table 1 indicate that Qo_2 is similar in both innominate arterial trunks, although, as shown in Table 2, the oxygen uptake of arteries is increased by œstradiol.

Table 1. OXYGEN UPTAKE OF INNOMINATE TRUNKS IN THE CHICKEN

Artery	No. of birds	Oxygen uptake ($\mu l.\ O_2$/hr./mgm. dry tissue) (mean ± *S.E.*)
Right innominate trunk	6	2·124 ± 0·472
Left innominate trunk	6	2·137 ± 0·463

$t = 0{\cdot}019$; $P > 0{\cdot}90$

Table 2. EFFECT OF ŒSTRADIOL-17β ON OXYGEN UPTAKE OF INNOMINATE TRUNKS IN CHICKENS

Innominate trunk	No. of birds	Oxygen uptake (μl. O_2/hr./mgm. dry tissue) (mean ± *S.E.*)
Control	12	1·584 ± 0·210
Œstradiol-17β (2 μgm./ml.)	12	3·714 ± 0·534

$t = 3·769$; $P < 0·01$

Despite the fact that the present findings in connexion with the problem of atherosclerosis are of questionable significance, they indicate clearly that œstradiol is able to modify arterial metabolism through a direct action on the vessels themselves. Although results obtained *in vitro* may not be related to conditions prevailing in the living organism, it seems appropriate to mention here that local effects of œstrogens on arteries have also been demonstrated as an enhanced activity of alkaline phosphatase in incubated aortas of chickens[9] as well as in connexion with certain aspects of lipid metabolism[10].

Effect of Incubated Arteries on Œstradiol-containing Media

It has already been demonstrated[5,11,12] that œstrogens prophylactically inhibit and therapeutically reverse coronary atherosclerosis in cholesterol-fed cockerels, even in the absence of any influence on aortic atherogenesis. Intimate mechanisms involved in these effects are not known, although recent findings seem to indicate that œstrogens may modify atherogenesis through a direct action on the arteries themselves[5]. Evidence bearing on such a direct action of œstrogens on arteries includes the demonstration of radioactive material within arterial walls after the injection of tritium-labelled œstradiol-6,7 [13], enhancement of alkaline phosphomonoesterase activity in aortæ incubated with œstradiol[9] and increased oxygen uptake of arteries brought about by adding œstradiol *in vitro*. Furthermore, the well-known actions of œstrogens on blood lipids as well as on the endocrine glands, although they cannot be totally discarded in this respect, have been found not to be correlated with the arterial modifications induced by the hormones[14].

In this work, a different approach has been used in order to study the possible relationships of œstrogens and atherogenesis, namely, the determination of the endocrine activity of nutrient media where different arteries from chickens were incubated with or without œstradiol.

Fifteen white Leghorn cockerels fed on commercial mash were used. The aorta, the coronary and the carotid arteries were aseptically removed; a low-power magnifier was used to dissect the coronaries, no effort being made to free them completely from adventitial fat. In groups of 3 chickens, the coronary arteries and aortic or carotid segments of an approximate similar weight were incubated during 72 hr. at 37 ± 1° C. in modified roller tubes as described[9], using approximately 2 ml. of balanced saline medium[6] also containing 30 per cent bovine serum, 0·05 per cent ethanol, penicillin and streptomycin. Incubation was also carried out with similar media containing 2 µgm./ml. of œstradiol-17β (Δ-1,3,5(10)-œstratriene, 3,17β-diol, melting point 197° C.); the media were changed every 24 hr., pooled and kept at −20° C. until used. All media were then similarly diluted in order to attain a theoretical concentration, for those containing the hormone, of 8·33 µgm./100 ml. of medium. They were then injected into groups of 10–12 immature female Swiss albino mice, 7–12 gm. weight, according to the method of Astwood[15] modified as follows: the animals received 2 subcutaneous daily injections of 0·2 ml. during 3 days (a total theoretical dose of 0·1 µgm.), and were killed with ether 18 hr. after the last injection; the mice were then weighed, the internal genitalia dissected under a low-power magnifier (ovaries, uterus, and the vagina as reached through the abdomen) and its weight indicated per 100 gm. of body-weight. Results were compared with Student's *t*-test and its significance evaluated using the tables of Fisher and Yates[8].

The method was standardized by injecting into groups of 10 immature female mice, nutrient media containing total doses of 0, 0·05, 0·1 or 0·2 µgm. œstradiol.

As can be seen in Table 3, the weight of internal genitalia of immature female mice increased proportionally to the injected dose of œstradiol. In Table 4, it is shown that the nutrient media without œstradiol showed no activity, whereas the amount of œstrogens in hormone-containing media incubated without arteries, with the aorta or with carotid arteries, was similar to the theoretical value. The activity of hormone-containing media incubated with coronary arteries, though, was lower than that expected.

Table 3. WEIGHT OF INTERNAL GENITALIA OF IMMATURE FEMALE MICE INJECTED WITH ŒSTRADIOL

Total dose (μgm.)	No. of animals	Internal genitalia (mgm./100 gm. body-weight) (mean ± *S.E.*)
0	7	165·3 ± 10·56
0·01	8	320·3 ± 12·24
0·05	9	492·6 ± 18·85
0·1	8	546·6 ± 24·74
0·2	7	636·9 ± 56·31

The present findings indicate, consequently, that coronary arteries from chickens, when incubated with œstradiol, are able to modify the activity of the hormone in the nutrient medium. Whether this change is secondary to adsorption of the œstrogen to the vessels or to molecular inactivation has not yet been determined. It is interesting that the aorta and the carotid arteries, when similarly incubated, are not able to induce such an inactivation. It is not possible to state at the present time whether this phenomenon is related to the known action of œstrogens which prevent coronary atherosclerosis in chickens without affecting the aorta and the great vessels in these animals[5,11,12]. Nevertheless, the experiments mentioned here, together with those already reported[9,13], indicate that œstrogens may interact with the arteries themselves, and that the action in atherosclerosis may probably be related to metabolic modifications brought about within the

Table 4. WEIGHT OF INTERNAL GENITALIA OF IMMATURE FEMALE MICE INJECTED WITH NUTRIENT MEDIA FROM INCUBATED ARTERIES (MEAN ± *S.E.*)

		Internal genitalia (mgm./100 gm. body-weight)		
Description of media	No. of animals	Without œstradiol	No. of animals	Œstradiol 2 μgm./ml. (theoretical injected dose: 0·1 μgm./animal)
Without arteries	10	181·6 ± 7·10	10	526·8 ± 24·1†
Aorta	10	166·7 ± 16·7	10	636·8 ± 61·7
Carotid arteries	12	155·9 ± 6·20	13	506·8 ± 26·3
Coronary arteries	9	178·1 ± 24·1*	12	416·3 ± 27·0†

* From another set of experiments.
† $P < 0·01$ when compared, as indicated.

arterial walls. Further experiments are being completed and will be discussed in detail elsewhere.

Modification of Aortic Aerobic Metabolism in Rats by Gonadectomy

The importance of the sexual glands on the biology of normal arteries has not been studied thoroughly, although a few facts illustrate that such a relationship may exist. For example, the role of functioning gonads in human atherosclerosis is well known, men being more prone than women to coronary atherosclerosis, this difference tending to disappear after the menopause[16]. Furthermore, œstrogens are able to modify the development of atherosclerosis in several species[5], and a similar action is apparently shared by androgens in birds[17]. The following experiment demonstrates that the aerobic metabolism of the aorta is normally modified in rats through the influence of the gonadal glands.

Adult white albino male and female rats, weighing 150–250 gm., and reared on standard laboratory diet, were killed by brain concussion. The aorta was excised, freed from adventitial fat and the oxygen uptake determined during 1 hr. at 36·5° C. on pools of two arteries with the usual manometric technique[7] using a balanced saline solution[6] also containing streptomycin, penicillin and 0·05 per cent ethanol. Gonadectomy had been performed 30–40 days previously with a clean but not sterile technique under ether anæsthesia.

Table 5. AORTIC OXYGEN UPTAKE IN THE RAT

Groups	No. of determinations*	Oxygen uptake† (μl./hr./mgm. dry tissue)
Intact males	12	0·776 ± 0·055
Castrated males	6	2·546 ± 0·575
Intact females	10	0·680 ± 0·129
Spayed females	6	2·790 ± 0·294
t 1 vs. 2	11·283 $P < 0{\cdot}001$	
1 vs. 3	0·690 $P = 0{\cdot}50$	
3 vs. 4	6·677 $P < 0{\cdot}001$	
2 vs. 4	0·418 $P < 0{\cdot}70$	

* Each determination performed on pools of two animals.
† Mean ± *S.E.*

As can be seen in Table 5, the aortic oxygen uptake, which is similar in both sexes, is greatly accelerated by gonadectomy.

The mechanism through which the aerobic metabolism of the aorta is controlled has not yet been determined, although it is clear that the gonads share in this control. Current experiments performed in our laboratory indicate that it is probably mediated through a direct action of gonadal hormones on the arterial tissue. This work will be reported in detail elsewhere.

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USE OF TRANSPARENT ADHESIVE TAPE IN THE PREPARATION OF MICROSECTIONS

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IN 1954, Palmgren[1] described the use of cellulose adhesive tape as a means of holding together large sections of hard or brittle specimens during the cutting operation. The technique for transferring the section from the tape to a slide was rather elaborate, and in 1959 W. E. Beckel[2] proposed a much simpler method using different adhesive tapes. Scotch tape No. 810 (Minnesota Mining Co.), was used for sections to be fastened to the slide with albumen, while 'Mystik Kylon' tape No. 6400 (Mystik Tape Products Div., Englewood Co., Inc.), was used with 'Celloidin' as an adhesive. The Scotch tape No. 810 was removed in 30 min. or less by treatment with organic solvents such as furan, chloroform and xylene, and we had no difficulty in using the same technique with tape samples supplied by Dr. W. E. Beckel. No sample we were able to buy, however, had a soluble adhesive, and we were informed by the Minnesota Mining Co.[3] that only certain Canadian batches of this tape have soluble adhesive ; usually the adhesive is cross-linked and will not dissolve cleanly in any solvents. We purchased a wide variety of makes and types of adhesive tapes, and the only ones we found with soluble adhesives were 'Tuck' tapes Nos. 200 and 205, made by the Technical Tape Corp., New York 43. Only No. 200 had satisfactory tackiness. Beckel's method for Scotch tape No. 810 may be used with 'Tuck' tape, but it takes 1–10 hr. to remove the tape and adhesive with xylene, because the cellulose backing is not very permeable to the solvent ; the larger the piece of tape the longer it takes.

Previous applications of the tape method have been to whole animals, or heavily sclerotized whole insects. We have applied the method as it stands to the sectioning of textile fibres. Our real modification, however, is to mount the sections without removing them from the tape. In this way, and with a suitable embedding technique, we have been able to section textile fibres and delicate aggregates of polymer particles which are soluble in molten paraffin wax, and in the organic reagents normally used in section preparation. The unaltered method used on paraffin-embedded textile fibres gives very high quality sections down to 1μ, with rare failures.

The following is a typical example of the application of our modified technique to the sectioning of a solvent-sensitive specimen. 'Mystik Kylon' tape No. 6400 is used because of the thinness and flexibility of the backing.

Immerse the sample in polyvinylpyrrolidone solution (see 1 below) in an open dish. Put the dish in the vacuum oven at a temperature of 30–50° C. (depending on what the specimen will stand), and slowly pump down to about 28 in. mercury. Keep the oven in this condition for 2 min., and then bring it up to atmospheric pressure. Lift the sample from the solution with a spoon, taking up about 0·5 c.c. of solution, and pour the whole on to the flat surface of solidified embedding wax (see 2 below) in a Petri dish. The sample should be manipulated into the centre of a strip of the solution about 2 cm. long, and 3–5 mm. wide. Put the Petri dish in a desiccator over 50 per cent w/w sulphuric acid (about 35 per cent relative humidity), and let it stand there for three days. In this time the polyvinylpyrrolidone solution will dry down to a solid with the correct consistency for cutting. With a scalpel make two cuts in the wax, one on each side of the strip, so that the strip lifts away with a wedge of wax attached to it. After suitably trimming the length of the strip, embed it in a block of wax (see 2), and trim the block to a truncated pyramid so that the sample lies just below the upper surface.

Mount the block in the microtome, and cool it to 10–15° C. by blowing a jet of cooled air or nitrogen on it. After cutting down to the desired level, gently press a piece of 'Mystik Kylon' tape No. 6400 on to the exposed face of the specimen and cut the section rather slowly. The piece of tape will have a rectangle of wax on it, in the middle of which is a clear patch of polyvinylpyrrolidone containing the section. Trim the tape around the edges of the wax—it may now be handled with forceps and will not stick to everything that it touches.

Lay the tape on a slide with the wax side up. Put a drop of Farrants' medium (see 3 below) over the section, and drop the cover-slip into place. Stand a 10-gm. weight on the cover-slip, and put the slide into the vacuum oven at the temperature used for the impregnation with polyvinylpyrrolidone. Pump down until air bubbles begin to come out from under the cover-slip. Allow bubbling to go on for 2–3 sec., and then bring the oven rapidly to atmospheric pressure. Excess mountant is removed from the edges of the cover-slip with a wet brush, while the slide is rotated on a turntable. Bell's cement, or 'Murrayite' cement is excellent for sealing the preparation.

When fibres are to be sectioned they are treated as above, except that the polyvinylpyrrolidone embedded bundle is hung up on a wire rack and allowed to dry at 35 per cent relative humidity.

It is often possible to stain sections on the tape before mounting. Thus sections of acid ion-exchange beads may be stained with a variety of aqueous basic dyes, and basic beads may be stained with acid dyes, with little or no colour take-up by the tape. The feasibility of using any particular staining method can only be determined by trial.

If processing in aqueous solutions is not essential the equilibration time for the first embedding can be cut to one day in the open air, at any relative humidity, by impregnating with an alcoholic solution of Dow's 'Devlex *A* 515' resin (see 4 below), instead of polyvinylpyrrolidone. The oven temperature should not exceed 30° C. in this case, and a vacuum of only about 20 in. mercury is needed. The rest of the technique is as described above, except that the section is mounted in Hoyer's gum mastic medium (see 5 below).

The compositions used are as follows (all percentages are by weight):

(1) *Medium for the first embedding.* 43·5 per cent polyvinylpyrrolidone, Grade *K*-30 (av. mol. wt. 40,000) ; 13 per cent diethylene glycol as plasticizer; 43·5 per cent water. Glycerol may be substituted for diethylene glycol, but the film is more sensitive to relative humidity, and has only half the extension at break[4]. The above composition is suitable for sectioning without tape if the room humidity is between 30 and 40 per cent. For other humidities the amount of diethylene glycol may be changed, less being used for higher humidities.

(2) *Wax for the second embedding.* 48 per cent paraffin wax m.p. 58–60° C.; 50 per cent stearic acid; 1 per cent beeswax; 1 per cent carnauba wax.

This composition was found, by a factorial experiment at three levels for each component, to be suitable for direct sectioning of moderately hard materials. It has given excellent results in the above techniques. It was developed from a paraffin-stearic acid mixture, suggested by Preston[5], to give more coherent films on cutting.

(3) *Farrants' medium*[6]. 40 per cent water ; 40 per cent gum arabic; 20 per cent glycerol.

(4) *Alternative first embedding medium.* 43 per cent 'Devlex *A* 515' (a copolymer of N-vinyl-5-methyl-2-oxazolidinone and vinyl acetate, made by the Dow Chemical Co.); about 14 per cent propylene glycol as plasticizer ; 43 per cent absolute ethanol. For sectioning without tape this mixture gives a film which is very insensitive to room humidity. The following plasticizers may also be used : 8 per cent 1-3-butylene glycol, 8 per cent trimethylol ethane, or 8 per cent 1,2,6-tri-hydroxy-hexane, with 46 per cent 'Devlex *A* 515' and 46 per cent ethanol.

(5) *Hoyer's gum mastic mountant*[7]. Gum mastic dissolved in excess 95 per cent ethanol, filtered, and evaporated to the required consistency.

We wish to express our thanks to Dr. John O. VanHook, who suggested and supplied the plasticizers for the 'Devlex *A* 515' resin.

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LETTERS TO THE EDITORS

GEOPHYSICS

A Twilight Method of determining the Vertical Distribution of Ozone

DETERMINATION of vertical distributions of ozone from lunar eclipse observations[1] suggests the practicability of similar ground-based optical methods, provided 'screens' more amenable to experimental control than the Moon can be used. Alternatives are noctilucent clouds, atmospheric layers, balloons, and artificial Earth satellites. Noctilucent clouds are rather rare and beyond experimental control; ionospheric layers give very limited information[2], as do other atmospheric layers such as sodium[3]. Following a suggestion by Dr. V. D. Hopper of this Department, a method analogous to the lunar eclipse method has therefore been developed in Melbourne, for initial application to ground-based filter photometric observations of balloons at twilight.

Rays from the twilight Sun pass tangentially through the ozone layer to a balloon floating above a substantial part of the atmospheric ozone. This gives a very long path-length through the atmosphere, having an effective thickness of ozone which depends critically on solar zenith angle. The effective thickness (reduced to S.T.P.) reaches about 7–10 cm. for the optimum minimum height of the rays, depending on the amount of ozone and its vertical distribution. This gives rise to absorption due to ozone, near the maximum of the Chappuis band, of 30–50 per cent.

A ground-based observer can then measure the intensity of various wave-lengths scattered to ground from the balloon. This has an advantage over lunar eclipse observations, apart from the frequency and control of occurrence, in that direct measurements can be made in the laboratory of the scattering properties of the scattering surface in the absence of an intervening atmosphere.

As the balloon is not above the whole of the atmosphere, it is necessary to modify substantially the lunar eclipse theory. Denoting the total effective thickness of ozone traversed by $X = x_a + x_b$, where x_a is the effective thickness in the path from the Sun to the balloon, and x_b that between the balloon and the ground, then it can be shown that, for two different zenith angles, with effective thicknesses of ozone of X and X', we have:

$$X' - X = (\alpha_2\lambda_1^{-4} - \alpha_1\lambda_2^{-4})^{-1}\left[\lambda_1^{-4}\log\left(\frac{I_2}{I_2'}\cdot\frac{S_2'}{S_2}\right) - \lambda_2^{-4}\log\left(\frac{I_1}{I_1'}\cdot\frac{S_1'}{S_1}\right)\right] \quad (1)$$

for wave-lengths λ_1 and λ_2 having decadic extinction coefficients α_1 and α_2, respectively, where S_i denotes the scattering factor of the balloon (assumed to be a function of wave-length and angle of scattering), and I_i is the intensity at the ground, of wave-length λ_i.

The effects of Rayleigh scattering and non-selective scattering have been eliminated, as well as the effect of differing sensitivities to wave-lengths λ_1 and λ_2. It is assumed that the incident intensities, at the top of the atmosphere, of wave-lengths λ_1 and λ_2, do not change during the course of the observations, without requiring any knowledge of their value or ratio. The elimination of non-selective scattering is based on the assumption that such scattering only occurs in the path from balloon to ground, and that it is constant. This could be invalidated by drifting dust or smoke clouds, or by observations during which the tangential rays pass through layers of non-selective absorbers, as would occur when the depression of the Sun brought the minimum height too low in the atmosphere. In general, this last consideration may well restrict the method to the determination of the vertical distribution above the troposphere.

The right-hand side of equation (1) can be determined by measurement, while the left-hand side can be obtained in terms of the vertical distribution of ozone.

Consider the atmosphere divided into layers of thickness Δh_i km., each having a reduced thickness of ozone per km. of a_i. Then, neglecting the curvature of the Earth for the path from the balloon to the observer, which is reasonable for zenith angles of the balloon of less than about 60°, we have:

$$x_b = \sec Z_b \sum_{h_i = 0}^{h_0} a_i \,.\, \Delta h_i \quad (2)$$

where Z_b is the zenith angle from the observer, of the balloon at a height h_0.

Between the Sun and the balloon the curvature of the Earth must be considered. Thus:

$$x_a = \sum_{h_i = h_{\text{min.}}}^{\infty} a_i \,.\, \Delta l_i + \sum_{h_i = h_{\text{min.}}}^{h_0} a_i \,.\, \Delta l_i \quad (3)$$

where

$$\Delta l_i = [(R + h_2)^2 - (R + h_{\text{min.}})^2]^{1/2} - [(R + h_1)^2 - (R + h_{\text{min.}})^2]^{1/2} \quad (4)$$

R is the radius of the Earth, $h_{\text{min.}}$ is the minimum height of the tangential ray, and h_1 and h_2 are the lower and upper boundaries of the layers considered.

X may therefore be evaluated, for a given set of atmospheric layers, in terms of a linear sum of multiples of the unknown concentrations a_i, where $R + h_{\text{min.}} = (R + h_0) \sin Z_0$ for the Sun at a zenith angle Z_0.

For known pairs of values of h_0, Z_0, and Z_b, it is thus possible to obtain a linear equation containing the unknown concentrations in each layer considered. Hence $(n + 1)$ sets of measurements, each corresponding to a particular position of the Sun and balloon, will give n independent equations of this type, which may then be solved for the unknown concentrations in n layers to give an approximation to the vertical distribution of ozone.

Large meteorological balloons in level flight[4] at 30–40 km. altitude are to be used for the initial observations. Narrow pass-band interference filters centred on 6150 Å. and 4400 Å. have been chosen for the photometer. The choice of 6150 Å. for the wave-length absorbed by ozone is governed by the interfering bands in the visible spectrum[5,6]. Interference is considerably less than in the case discussed by Dufay[5] due to the relatively short path through the troposphere.

The main practical difficulty in this method, using ground-based observation equipment, is that of eliminating the twilight sky background, which changes in a very rapid and complex manner as the Sun sinks below the horizon. This requires that the field of view of the photometer be severely restricted by a diaphragm in the focal plane of an objective mirror or lens. Accurate tracking is necessary to keep a balloon within such a field of view. The accuracy to which the height of the balloon is determined will greatly affect the accuracy of the results. If the balloon is above the bulk of the ozone layer, the uncertainty in height can be regarded as equivalent to a corresponding margin of error in time (or solar zenith angle), since it leads to an uncertainty in the minimum height of the tangential ray.

The minimum height of the tangential ray reaches the troposphere for solar depressions of about 5° or 6°, depending on the height of the balloon. This allows only about 20–25 min. during which to take all the measurements, while the rate of change of atmospheric path makes it necessary to take the readings for a given path within about 10 sec. if an accurate distribution is to be derived.

The method outlined above has a number of limitations. It is restricted to clear sky observations. It determines the vertical distribution smoothed out over large horizontal distances, rather than the distribution above a particular geographical location. It will be largely restricted to stratospheric ozone, and to observations only twice a day (except perhaps in polar regions). Finally, it requires considerable skill and experience.

On the other hand, its great sensitivity to ozone in the upper layers of the atmosphere should enable solar and seasonal variations in the equilibrium region of the ozone layer to be studied with increased accuracy. The method may also be applied to other constituents of the upper atmosphere with comparatively little modification.

I am indebted to Drs. V. D. Hopper and J. E. Laby, and to Mr. K. B. Mather, for their advice and discussions, and to the Dunlop Rubber Co. for financial support.

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Motion of Geomagnetic Field Lines

In a communication with the above title[1], I stated that trapped-particle orbits in the radiation belts follow the motion of the geomagnetic field lines. As this is incorrect, the conclusions reached in that communication cannot be applied to the behaviour of the radiation belts, and my criticism of a hypothesis put forward by Rees and Reid[2] was unfounded. A more satisfactory discussion of this hypothesis has been published by Malville[3].

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PHYSICS

Mercury-206: a New Natural Radionuclide

According to Cameron's mass formula[1], the value of Q_α for lead-210 (radium D) should be 3·566 MeV., which would indicate an alpha half-life of the order of 10^{10} years and an alpha-branching of the order of 10^{-9}. The same source gives 1·361 MeV. for the Q_β of the hitherto unknown mercury-206, which is formed from lead-210 by alpha decay. From a closed cycle of reactions, the sum of Q_α for lead-210 and Q_β of mercury-206 is found to be 5·0 MeV. ; the absence of known alpha-branching in lead-210 further suggests that the Q_α for lead-210 does not appreciably exceed the value mentioned above. (This has been pointed out also by Prof. I. Perlman; private communication by Dr. K. Hyde.)

The ground-state of thallium-206 is reported to be 0^- (refs. 2 and 3), which would indicate a beta half-life of the order of one day for mercury-206 at the energy mentioned above. However, the beta decay of 4·2-min. thallium-206 (Q_β = 1·51 MeV.) exhibits a log (comparative half-life) value of 5·1, which is typical for an allowed transition; if it is assumed that this is the case for mercury-206 also, a half-life of about 10 min. is obtained. On the other hand, mercury-206 contains 126 neutrons which should increase its stability, and several attempts[4] have been made to detect it under the assumption that its half-life is relatively long.

We have detected the alpha-branching of lead-210 by rapidly separating the lead fraction from a radium D,E,F mixture. Preparations from 20–200 microcuries of lead-210 were used and the measurement was carried out in a gridded ion-chamber of the type described by Karras[5]. The measurements were begun about 7 min. after the beginning of the chemical separation and continued for about 1 hr. ; during this time the polonium activity grew from less than one c.p.m. to hundreds of counts per minute. The alpha-branching of bismuth-210 was investigated in an analogous way, with results in agreement with those of Fink and Warren[4a,b] ; results on both nuclides are given in Table 1.

Table 1

Nuclide	Half-life	Measured E_α (MeV.)	Branching
Lead-210	22 years	3·76 ± 0·06	(1·8 ± 0·5) × 10^{-8}
Bismuth-210	5 days	4·80 ± 0·05	(1·9 ± 0·4) × 10^{-6}

In order to investigate the properties of mercury-206, bismuth and polonium were first removed from a solution containing 4 millicuries of lead-210. The solution was then made 1 *N* with nitric acid, whereupon bismuth and thallium holdback carriers and about 40 μgm. of mercury carrier were added. After standing for 60–75 min. the mercury fraction was extracted with an equivalent amount of 0·005 per cent solution of dithizone in chloroform. The extract was scrubbed twice with 1 *N* nitric acid and evaporated on to a small aluminium cup which was counted with an end-window Geiger–Müller counter shielded with 20 cm. of iron.

The time needed for the separation proper was 2·5 min. ; after this period, the thallium-206 daughter was retained and grew during the evaporation and mounting, which took another 2·5 min. A beta-decay graph obtained as an average of five measurements is shown in Fig. 1 (crosses) together with a decay curve calculated using 7·5 min. and 4·2 min. for the half-lives of mercury-206 and thallium-206, and

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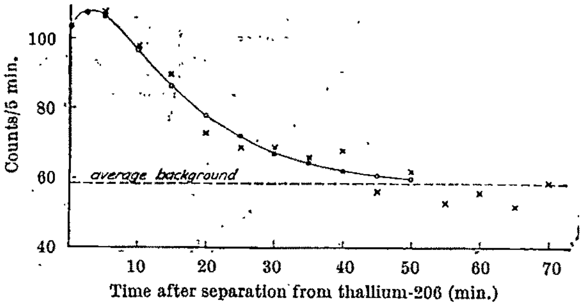

Fig. 1. Observed beta-decay of the composite mercury-206 – thallium-206 activity (crosses ; average of five measurements) compared with a decay graph calculated using 4·2 min. and 7·5 min. for the half-lives of thallium-206 and mercury-206, and assuming equal efficiencies of detection

assuming equal detection efficiencies for both activities. A value of (7·5 ± 1) min. is thus obtained for the half-life of mercury-206, while the closed cycle leads to a value of (1·2 ± 0·1) MeV. for Q_β.

We are indebted to Dr. Richard W. Fink, Dr. Goran Andersson, and Dr. Earl K. Hyde for many valuable suggestions, and to Prof. L. Simons for permission to use the equipment of the Van de Graaff Laboratory in some of the alpha-spectrometry measurements.

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A more detailed report of this work will be published by P. Kauranen in *Ann. Acad. Scient. Fenn.*

Note added in proof. Walen and Bastin-Scoffier (*J. Phys. Radium*, 20, 589; 1959) measured for bismuth-210 E_α = 4·686 and 4·649 MeV.; our result is clearly too high.

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A Theory of Cold-Cathode Arc Movement in a Magnetic Field

WHEN a cold-cathode arc is initiated in the presence of a transverse magnetic field, movement of the cathode root occurs in a direction mutually perpendicular to the arc current axis and the magnetic field lines. At atmospheric pressure the arc moves in the amperian direction; but at low pressure, motion in the reverse sense can occur. Experimental investigations have shown that in both these modes of motion the conditions in the cathode fall region are of primary importance[1,2], and thus an understanding of the processes occurring in this region is necessary to an explanation of arc motion. A mechanism for retrograde motion at low pressure has been described by Ecker[3]; but to date no satisfactory explanation of the normal amperian motion has been put forward.

The cathode fall may conveniently be divided into three zones[3]: (1) a space-charge zone very close to the cathode surface; (2) a transition zone, where the field is less than in the space-charge zone; (3) the contraction zone, where quasi-neutrality exists. It seems probable that for the cold-cathode arc, in which current densities of the order of 10^6 amp./cm.^{2} are generally believed to exist, a field-type of emission process is operative. If either the thermal[4] or individual[5,6] field emission mechanisms are postulated, a background field $\sim 10^7$ V./cm. is likely to be necessary in the space-charge zone. As the voltage across the fall region is always about 10 V. the extent of the space-charge zone must be less than λ^+, the ion mean free-fall distance, even at atmospheric pressure, though the field in this region will decrease somewhat away from the cathode surface. The small depth of the space-charge zone can be explained by assuming a progressive back-diffusion of electrons against the electric field[3].

For convenience, calculations of electron behaviour in the fall region may be made for two different cases with the limits:

$$(a)\ R > \lambda_+ \qquad\qquad (b)\ R < \lambda_+$$

where R is the radius of an emitting area on the cathode. It can be shown that case (*a*) corresponds to the high-(atmospheric) pressure arc, while (*b*) is valid for the low-pressure arc. Only (*a*) will be discussed here since it is proposed to describe a model for amperian motion. As $R > \lambda_+$, and the extension of the space-charge zone, $d_s < \lambda_+$, a one-dimensional electric field results and electron movement in the space-charge zone will be essentially normal to the cathode surface. This is true despite the presence of the transverse magnetic field since the electric field is so large. In passing through the zone an electron will gain about 6–8 eV. energy. Current densities of about 10^6 amp./cm.^{2} have been observed for vacuum arcs as well as those operated at a pressure of several atmospheres, so that it would appear that the background field of about 10^7 V./cm. is affected little by pressure. Since also the cathode voltage remains sensibly constant at about 10 V. for a wide pressure-range, it would seem that both the energy gain in the space charge region and the depth of this region vary very little.

In the transition zone, the electrons will suffer many elastic collisions before they gain the few extra eV. required for an inelastic collision. It is almost certain that in air, which is considered here, ionization occurs by a series of electron impacts which raise the gas particle to successively higher excited levels[7], although thermal energy in this region will to some extent aid this process. In this zone the electrons, prior to excitation collisions, have a high random velocity, by virtue of their energy, but their drift velocity normal to the cathode surface is low, because of the many collisions which occur. The transverse magnetic field is thus able to deflect the electrons in the amperian direction.

By making a few simple assumptions, it can be shown that the extension of the transition zone, d_t, is given by:

$$d_t = \frac{\lambda_-}{2f^{1/2}} \cdot \tanh^{-1} \frac{2f^{1/2}}{E\lambda_-}\,(V_t - V_s) \qquad (1)$$

where λ_- is electron mean free path ; f is fraction of energy lost by electron in elastic collision ; V_t is voltage across both transition and space-charge zones ; V_s is voltage across space-charge zone only; E is field in transition zone, which is assumed approximately constant to aid calculations.

The corresponding deflexion in the amperian direction, y, for an electron moving d_t through the transition zone is given by :

$$y = B\left(\frac{e}{m}\right)^{1/2} \frac{\sqrt{2}}{3} \frac{\lambda_-}{E} (E.d_t + V_s)^{1/2} \qquad (2)$$

where B is transverse magnetic flux density; m is electron mass.

In the physical picture of the model proposed it is implicit that $(E.d_t + V_s)$ must remain approximately constant, in value equal to the first excitation potential of the gas, any variation being merely that required to compensate for variation in the energy loss in elastic collisions.

After the electrons have produced ions at the top of the transition zone by cumulative excitation, the ions drift back to the cathode and condition it so as to give rise to further emission at this new point. Thus the amperian velocity is given by $v = \frac{y}{T}$, where $T = T_1 + T_2$, T_1 being the time for the ions to move from the top of the transition zone to the cathode, and T_2 the time for emission at the new site to become 'established'. Previous experimental results[8] suggest that T_2 is generally greater than T_1. As T_2 would not be expected to vary with magnetic field strength and pressure the arc velocity should be proportional to the deflexion y. Therefore one would expect the velocity to be directly proportional to B, the magnetic flux density, as is in fact observed[8,9]. When the ambient pressure is reduced below atmospheric, Yamamura[9] finds that the velocity decreases only slowly, or not at all, until a critical value ~ 200–400 torrs is attained, following which the velocity falls rapidly to zero on further pressure reduction. Over the range of pressure where v remains almost constant it appears from equation (2) that $\frac{\lambda_-}{E}$ is approximately constant. This is not altogether surprising as the increase in λ_- with decrease of pressure causes $eE\lambda_-$ to increase compared with kT_e (k is Boltzmann's constant, T_e is electron temperature), so that back-diffusion of electrons in the transition region is checked and E correspondingly increases. The process is therefore cumulative. As $(V_s + E.d_t)$ is approximately constant, d_t decreases as E increases, so that the model progressively approaches that given by Ecker for case (*b*) at low pressure, as would be expected.

For satisfactory channelling of the ions to the cathode $eE\lambda_+ > kT_+$. At atmospheric pressure, therefore, E cannot be less than about 5×10^3 V./cm. From equation (2) the value of d_t can thus be estimated, and it may be shown that each electron makes a hundred or so collisions before having an exciting collision, at atmospheric pressure. When the number of elastic collisions per electron in the transition zone becomes small, equations (1) and (2) are no longer valid and the model breaks down. A criterion which loosely defines the point at which the model would fail is :

$$d_s + d_t \simeq \lambda_+ \qquad (3)$$

Calculations indicate that the relation (3) is satisfied at 300–400 torrs, which is the same region as experiments indicate that the velocity tends rapidly to zero.

Finally, substitution of experimental quantities in equation (2) and the use of measured velocities yield values for T. T_1 may be calculated and this shows values of T_2 which are reasonable in the light of previous observations[8]. It would therefore appear that the proposed model offers a reasonable explanation for amperian arc motion.

It is hoped to publish a more detailed account of this mechanism and confirmatory experimental data elsewhere.

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Variation with Temperature of the Photoelastic Constants of Sodium Chloride

Recently, Bhagavantam and Krishnamurty[1] investigated the variation with temperature of the stress-optical constants $(q_{11}-q_{12})$ and q_{44} of a few alkali halide crystals, using Babinet and Rayleigh compensator methods. In continuation of these investigations, we have now undertaken the study of the variation with temperature of the stress- and strain-optical constants of a number of cubic crystals in the temperature range 30–300° C., for the wavelength 5890 Å. This communication gives the outline of the method and the results obtained in the case of sodium chloride.

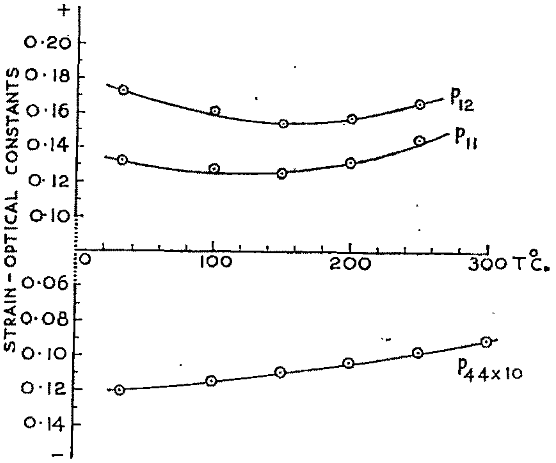

Fig. 1. Variation with temperature of the absolute strain-optical constants of sodium chloride

Table 1

Temperature (° C.)	$-(q_{11}-q_{12})$ (units of 10^{-13} C.G.S.)	$-(p_{11}-p_{12})$	p_{12}/p_{11}	p_{11}	p_{12}	$-q_{44}$ (units of 10^{-13} C.G.S.)	$-p_{44}$
32	1·12	0·041	1·31	0·132	0·172	0·94	0·0120
100	0·99	0·033	1·26	0·128	0·161	0·90	0·0113
150	0·91	0·029	1·23	0·125	0·154	0·87	0·0108
200	0·84	0·025	1·19	0·132	0·157	0·84	0·0103
250	0·78	0·022	1·15	0·145	0·167	0·80	0·0096
300	0·74	0·020	—	—	—	0·76	0·0090

A modified form of Filon's method[2], with a suitable heating arrangement, has been used to measure the stress-optical constants $(q_{11}-q_{12})$ and q_{44} at different temperatures. From these results, using the appropriate elastic constants, the strain-optical constants $(p_{11}-p_{12})$ and p_{44} are evaluated. To determine the absolute strain-optical constants p_{11} and p_{12}, the ratio p_{12}/p_{11} is measured at different temperatures by a modification of the ultrasonic method[3]. Combining the values of p_{12}/p_{11} and $(p_{11}-p_{12})$, the absolute strain-optical constants p_{11} and p_{12} have been evaluated. Table 1 shows the results obtained for sodium chloride. It was possible to measure p_{12}/p_{11} up to only 250° C., as the Hiedemann pattern became very diffuse above this temperature. The variation with temperature of p_{11}, p_{12} and p_{44} is shown graphically in Fig. 1. The elastic constants at different temperatures needed for these calculations are taken from a paper by Hunter and Siegel[4] and the thermo-optical data have been provided by M. A. H. Shareef (private communication). Work with other crystals is in progress and will be published in due course.

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Intensity Aspects as Determinant of $r_e'-r_e''$ in the Bands of the Lanthanum Oxide (LaO)($B\to X$) System

An attempt made by Meggers and Wheeler[1] to investigate the rotational analysis of the yellow system of lanthanum oxide did not prove successful, because of the low resolving power of the concave grating used by them. This band system has not yet been subjected to a successful structure analysis, though some attempts are under way[2]. Tawde and Chandratreya[3] have estimated the difference (Δr_e) in the internuclear separations in the levels B and X of this system to be 0·057 Å., mainly from the study of vibrational transition probabilities in relation to asymmetry of the molecules. A later investigation[4] on the vanadium oxide (VO) (green-yellow) system has revealed the validity of revised rotational constants[5]. This was based purely on consideration of the results of the comparative study of the experimental and theoretical transition probabilities, and not on the study of the asymmetry effect on the transition probabilities made by them for the purpose. It occurred to me to establish the Δr_e-value of the yellow system of lanthanum oxide from considerations similar to those in vanadium oxide, so as to enable those engaged in the rotational analysis of this band system to use the value as a check on their determinations.

Chandratreya[6] has derived the experimental transition probabilities of this band system. They are shown in Table 1. His value of the experimental transition probability of the (0,0) band, which is the intense band of the system, is 0·79. Using this value and the mathematical technique of Manneback[7], Δr_e is calculated for the (0,0) band. It is found to be $0{\cdot}0378_2$ Å. This value was checked from two other bands (0,1) and (1,0) and is about ±0·004 Å. on either side. With this value of Δr_e, the Franck-Condon factors of the other bands have been computed and are shown in Table 1. Hutchisson's method[8] has been used to check these values[9].

Table 1

Band v', v''	Experimental	Theoretical $\Delta r_e = 0{\cdot}0378_2$ Å.	Band v', v''	Experimental	Theoretical $\Delta r_e = 0{\cdot}0378_2$ Å.
0,0	0·79	0·79	2,0	0·05	0·02
0,1	0·21	0·18	2,1	0·14	0·32
0,2	0·05	0·03	2,2	0·31	0·24
			2,3	0·40	0·28
1,0	0·16	0·19	2,4	0·08	0·11
1,1	0·57	0·46	3,1	0·07	0·04
1,2	0·31	0·26	3,2	0·26	0·39
1,3	0·05	0·07	3,3	0·27	0·11
			3,4	0·49	0·25

The molecular constants needed for this computation are taken from Herzberg's treatise[10]. As lanthanum oxide is a molecule of considerable astrophysical importance[11], these two sets of transition probabilities will be useful to the astrophysicists. It is found from Table 1 that there is a good agreement of the experimental transition probabilities with the theoretical set calculated with $\Delta r_e = 0{\cdot}0378_2$ Å., though simple harmonic oscillator wave-functions are used in the theoretical calculations. A similar investigation by Tawde and me on the vanadium oxide (green-yellow) system referred to above lends support to such a method of assigning $\Delta r_e \sim 0{\cdot}038$ Å. in the case of B- and X-levels of lanthanum oxide.

I am grateful to Prof. N. R. Tawde for suggesting the problem. I thank the Council of the National Institute of Sciences of India for the award of a post-doctoral senior research fellowship.

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Two-dimensional Fourier Transform Technique for analysing Random Structures

In the course of a number of analyses of random interstratification of layers, it has been shown that a one-dimensional Fourier transform can be calculated which gives a clue to the correct sequence of layers[1,2].

This method can readily be generalized to two and three dimensions. Suppose we have scattering material, for example, in the form of layers (this is not an essential feature of the method), and that this can be stacked in two or more ways. Suppose that these manners of stacking are mixed together randomly. The result will be an X-ray diagram which consists partly of sharp spots, and partly of diffuse bands. Viewed in three dimensions, the layers will give rise to a series of regularly packed reciprocal rods. These will have a distribution of intensity along them depending on the structure of the layer, and this distribution will further be modified by the nature of the superposition of layers.

Let us consider any plane in reciprocal space, say the ($h0l$) plane, where (001) is the basal plane. The intensity function of such a partially random crystal as we are considering will have a strict periodicity in the h direction, but will be non-periodic in the l direction. It is now possible to generalize the one-dimensional Fourier transform which one of us has described[2] into a two-dimensional transform, consisting of an infinite band, periodic in the a direction, and non-repeating in the c direction. This function will have maxima at points corresponding to unit-cell repeat distances which are present in the random crystal. These unit-cell repeat distances will be shown vectorially. Furthermore, 'combination peaks' will show the degree of randomness in the mixture, as has been described for the one-dimensional case[2].

The result of this will be to give a vector diagram showing the nature of the various combinations of translations which occur. Fig. 1 shows such a diagram for random mixtures of (a) a 15 Å. spacing of $\frac{1}{6}a$ translation, and a $17\frac{1}{2}$ Å. spacing of $\frac{1}{6}a$ translation; (b) a 15 Å. spacing of $\frac{1}{3}a$ translation and a 21 Å. spacing of 0 translation.

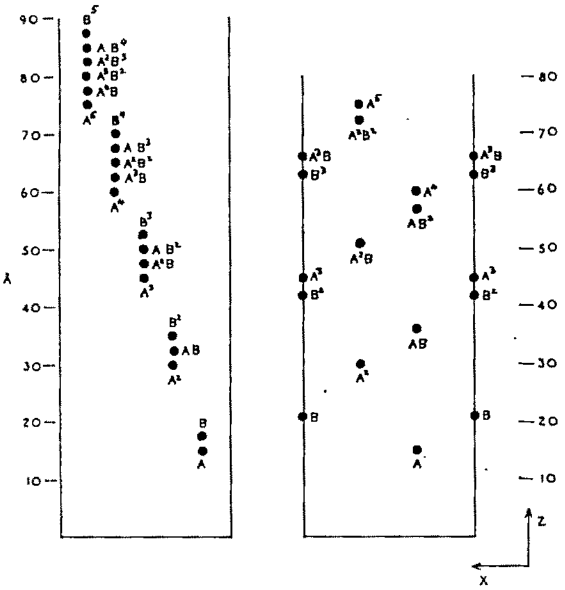

Fig. 1. Theoretical periodograms for two mixtures

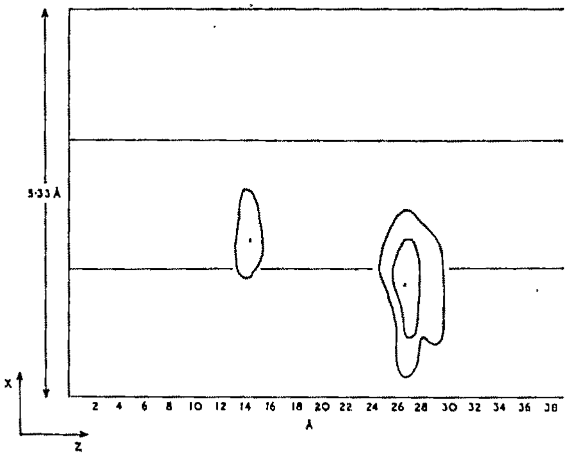

Fig. 2. A periodogram for a $C_6H_{13}NH_2$-vermiculite complex

Fig. 2 shows an actual example of a Fourier transform calculated by this method. It is for a vermiculite complexed with $C_6H_{13}NH_2$. It shows peaks at 14 Å. and 26·5 Å., both corresponding to translation of about $\frac{1}{3}a$.

In contrast to an ordinary Fourier synthesis, a diagram of this sort gives the *periodicities* associated with the crystal, and it may therefore conveniently be called a *periodogram*.

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METALLURGY

Creep of Metals under Simple Shear

In a paper published last year[1], we showed with polycrystalline lead, subjected to a constant simple shear stress, that the $t^{1/3}$ law of creep held very closely within a certain range of strain, the average error being about 0·25 per cent of the range in question. We recorded, however, that for low strains, below about 0·03 with a particular lead, the law was not obeyed, the strain varying more rapidly than was indicated by the law. At high strains, too, the law fails owing to recrystallization, but to comment on that is not the object of the present communication.

Using a more sensitive recording device, we have since found that, for the lower strains in question, the strain under simple shear stress varies precisely as $t^{1/2}$. Thus with a lead for which the $t^{1/3}$ regime begins at a strain of 0·035 in the range of strain from 0·012 to 0·035, the $t^{1/2}$ law, fitted by least squares, holds with an average error of 0·25 per cent of the range, which is about the error of observation.

This $t^{1/2}$ law has been confirmed with leads differing in the small amounts of foreign metal contained, and with cadmium. For the creep under reversed stress, which can be accurately measured by the method in question, the $t^{1/2}$ law also holds within a definite range.

Mr. D. B. Gilding, working under the supervision of the senior of us with the method described in *Nature* last year[2], has confirmed the validity of the $t^{1/2}$ law over a range of small strains with lead and with cadmium.

There is evidence that at still lower strains a different power law, namely $t^{2/3}$, is valid.

Experiments are proceeding with the object of determining the glide mechanism corresponding to the different stages, so as to establish a mechanical explanation of the general creep behaviour.

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Cleavage Fracture Initiation in Notched Impact Tests of Mild Steel

Many experimental observations have confirmed that brittle fracture in steel consists mainly of cleavages on the cube planes of ferrite crystals in the aggregate, with ductile fractures of the bridges formed between them. Important doubts have been expressed as to a cleavage being initiated within a crystal until the intervention of a discrete, inelastic local displacement, which might act as a trigger. Several hypotheses have arisen as to the nature of the trigger mechanism, principally involving slip and twinning. A well-known hypothesis, due to Cottrell[1], involves the intersection of two mutually perpendicular slip bands to form a strain incompatibility giving rise in turn to a void, from which cleavage may nucleate. To the best of our knowledge, the operation of this mechanism has not before been observed in steel.

Our experiments have been undertaken to explore further the nucleation of cleavage fracture in notched specimens, for which we have modified the Charpy impact test. Our objective has been to arrest the fracture as quickly as possible after initiation, in order to study the relative positions of the two fractured surfaces before they separate, using a method made known to us by Kasatkin[2]. In our development of this method, the specimen is deformed by impact at the full speed of the machine, until further bending is stopped by a surrounding rigid frame which accepts the remaining energy of the blow and transmits it to blocks of balsa wood which are permitted to be crushed. In order to secure early arrests, it has been found convenient to notch the specimens to 5 mm. depth, using a standard milling cutter of 0·25 mm. root radius, and to preform the specimens by about 2° of slowly applied bending in the reverse direction to assist crack initiation in the manner demonstrated by Mylonas[3]. We have used specimens of annealed mild-steel, bent by impact to angles between 1° and 5°, and the cracking has been observed on etched microsections taken at mid-thickness. At our testing temperature of 0° C. a completely fractured specimen of standard size shows an energy absorption of 5 ft. lb. and 95 per cent projected area of crystallinity in the fractured surface.

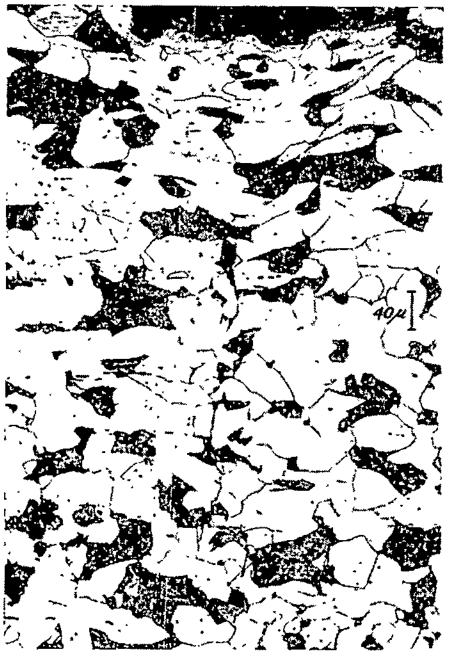

Fig. 1

The accompanying photomicrograph (Fig. 1) shows a fracture arrested at a very early stage (× 125). A significant feature is the void existing at the junction of terminating planes of heavy slip, extending from the flanks of the original notch. The cleavage fracture appears to extend from the void towards the neutral axis of the specimen, and away from the heavily deformed area. Isolated zones of twinning accompany the main cleavage fracture, but the investigation has yet contributed no evidence to distinguish their part in the process. There are isolated cleavage microcracks in advance of the main fracture front.

The initiation mechanism exposed in this specimen appears to correspond with Cottrell's hypothesis, but on a polycrystalline rather than single crystal scale.

The work is to be reported in detail soon.

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CHEMICAL ENGINEERING

Polymers of Carbon Dioxide

Polymeric ions of the form $(CO_2)_2^+$, $(CO_2)_3^+$, ... $(CO_2)_{23}^+$ have been observed in the mass spectrum of carbon dioxide. These may be produced from corresponding molecules in carbon dioxide gas.

The mass spectrometer in which these ions were observed was originally built for investigating free radicals in gases at high pressures. A molecular-beam system is used for transferring the gas from a reservoir to the ion source so that reactive species are not destroyed by wall collisions. Gas molecules pass through a 0·00062-in. diameter orifice and then through a series of larger orifices which define the molecular beam and which are separated by pumping chambers. The molecular beam passes directly into the ion source where ions are formed in the usual way by electron bombardment; the spectrometer itself is a conventional magnetic sector field instrument. Further details of the apparatus are given elsewhere[1].

When carbon dioxide (commercial grade, from a cylinder), at a reservoir pressure of up to 4,000 mm. mercury and at room temperature, is examined with this system, the mass spectrum is found to contain ions corresponding with the groups $(CO_2)_n^+$. Relative intensities of ions CO_2^+, $(CO_2)_2^+ \ldots (CO_2)_{23}^+$ for a pressure of 3,800 mm. mercury (5·0 atm.) are given in Fig. 1.

Ion intensities change very rapidly with reservoir pressure, as shown in Fig. 2 for $(CO_2)_2^+$. In this case the experimental points follow the power law: $I_2 = K_2P^{2.85}$, where I_2 is the $(CO_2)_2^+$ ion intensity, P is the reservoir gas pressure, K_2 is a constant. Similar power laws are followed by ion intensities of larger polymers, namely:

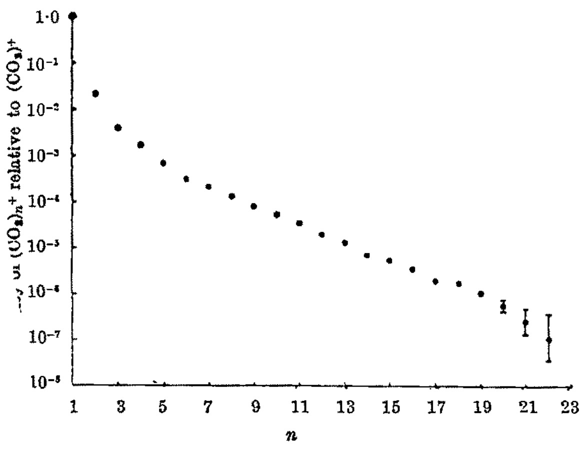
$$I_3 = K_3P^{7.8} \text{ for } (CO_2)_3^+$$
$$\text{and} \quad I_4 = K_4P^{11} \text{ for } (CO_2)_4^+$$

For the polymers larger than $(CO_2)_4^+$, the change in ion intensity with pressure is too rapid to permit even approximate measurements.

There are three possible sources for these polymeric ions: (1) They are formed in the mass spectrometer itself, either by ion-molecule reaction or some other process. (2) Polymeric molecules are formed during the expansion of the gas from the reservoir into the molecular-beam system. (3) Polymeric molecules are present in carbon dioxide gas.

The first possibility has been investigated in detail; although the evidence is not completely conclusive, it seems reasonably certain that the source of the polymers is not in the mass spectrometer. There is no evidence to indicate whether the second possibility is the cause or not; however, it seems unlikely, since an expansion involves (on average at least) the separation of adjacent molecules. Cooling of the bulk gas as a result of the Joule–Thompson effect is less than 6° C.[2] and would therefore seem to be negligible.

The third possibility, that polymeric molecules are always present in carbon dioxide, thus seems to be the most probable in the light of existing evidence.



g. 1. Measured ion intensities for polymeric ions $(CO_2)_2^+$ – $(CO_2)_{23}^+$. Reservoir pressure, 3,800 mm. mercury

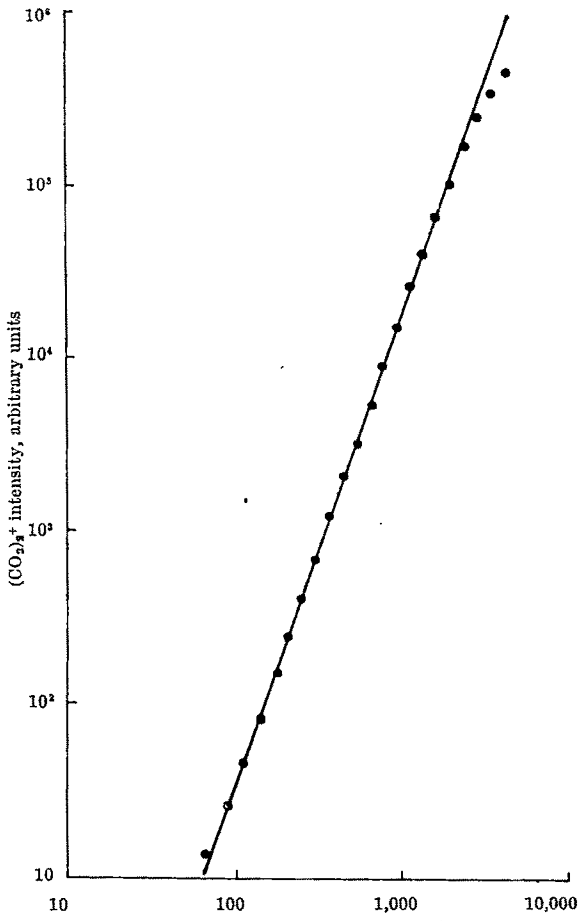


Fig. 2. Variation of $(CO_2)_2^+$ ion intensity with reservoir pressure. ●, Experimental points; —, $I_2 = K_2P^{2.85}$

Measurements at different temperatures might help in locating the source of the polymers; investigation of other gases would also be interesting.

This communication was submitted for publication by permission of Sir William Cook, managing director, and Dr. H. Kronberger, deputy managing director, of the U.K. Atomic Energy Authority (Development and Engineering Group).

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Bubble Formation at an Orifice in a Fluidized Bed

When a gas is passed through a bed of solid particles supported by a porous distributor at a flow-rate sufficient to fluidize the bed, gas bubbles can usually be seen travelling up through the bed and breaking surface at the top. The properties of bubbles in fluidized beds have become the subject of considerable interest recently for the information

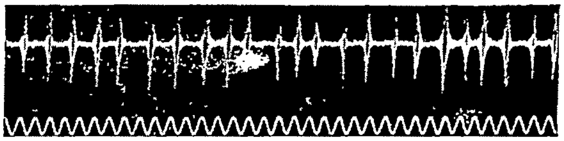

Fig. 1

their study provides on the mechanics of fluidization, and also for the part gas bubbles play in gas-solids coming into contact in fluidized beds. How gas bubbles form in fluidized beds is, however, largely unknown.

It has been shown[1,2] that the properties of gas bubbles in fluidized beds can often be described in terms of the properties of spherical-cap gas bubbles in inviscid liquids of zero surface tension[3]. The bubble and particulate (or dense) phases of a fluidized bed appear to be analogous to the gas and liquid phases in a two-phase system. A study of the formation of a gas bubble at an orifice in an inviscid liquid has recently been published[4] in which it is shown from first principles that the volume of bubble (V ml.) formed at an orifice is given by :

$$V = 1{\cdot}378\ G^{1{\cdot}2}/g^{0{\cdot}6} \qquad (1)$$

where G (ml./sec.) is a constant gas flow-rate through the orifice and g is the acceleration of gravity. In the same investigation the relationship between bubble volume and gas flow-rate through the orifice was investigated experimentally, and the results were found to be in good agreement with equation (1). Some experiments are now described in which a capacitance technique was used to determine the frequency of bubble formation at an orifice in a fluidized bed. The gas flow-rate through the orifice was known and hence the bubble volume could be calculated from the frequency. It has been found that these results may also be interpreted by equation (1), which is new evidence to support the analogy already found between two-phase systems and fluidized beds. It is hoped that these experiments will throw light on the mode of bubble formation in a fluidized bed supported by a distributor made up of orifices ; and also provide information on the initial size of a bubble in a fluidized bed.

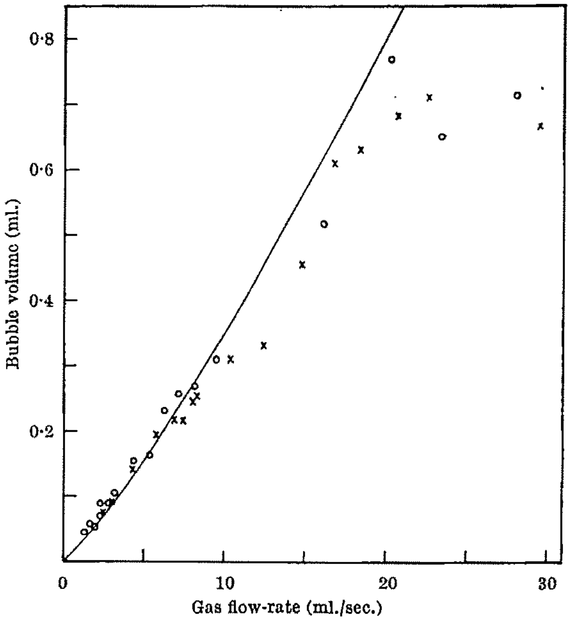

Fig. 2. Graph of bubble volume versus gas flow-rate. O, Sand in air; ×, catalyst in air; — $1{\cdot}378\ G^{1{\cdot}2}/g^{0{\cdot}6}$

The apparatus consisted of a 6-in. diameter tube in which a bed of particles was supported by a filter cloth distributor. Two sets of experiments, using sand (mean diameter 0·01 cm.) and alumina catalyst (mean diameter 0·0057 cm.), were carried out with air as the fluidizing gas. An independent stream of air at a known flow-rate was passed into the bed through a narrow injector tube, the end of which was arranged in the centre of the bed about one-third the way up from the distributor. The technique for measuring the frequency of bubble formation from the orifice at the end of the injector tube was to record the change in capacitance between a probe placed at the orifice and a probe about 3 cm. away from it in the fluidized bed. The diameters of the injector tube and the orifice were identical. A Fielden type *PM*2 proximity meter coupled to an oscilloscope was used to determine the changes in capacitance as bubbles were formed, grew, and detached from the orifice. Fig. 1 shows the output from the proximity meter for an experiment using catalyst particles, together with the 50 c./sec. trace. In this experiment the frequency of bubble formation from an orifice of 0·125 cm. diameter was approximately 32/sec. Fig. 2 shows a plot of the bubble volume against the flow-rate of air through the orifice compiled from about 30 separate experiments using either sand or catalyst particles. The complete line in the figure is a plot of the theoretical relationship between bubble volume and gas flow-rate given by equation (1). It can be seen that there is good agreement between the experimental results and the theoretical curve for flows up to about 10 ml./sec. It is noteworthy that equation (1) was also found to become inaccurate at high flow-rates in the work on air and water[4]. In the fluidized bed experiments it is considered that this inaccuracy is due in part to the formation of 'double bubbles'. Within the range of the experiments carried out so far with various sizes of orifice, the bubble frequency appears to be largely independent of bed height, the main air flow-rate to the fluidized bed, and the nature and size of the particles.

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CHEMISTRY

Radiometric Precipitation in Filter Paper : Rapid Method of Micro-analysis

A radioactive material can usually be detecte and quantitatively measured by simple instrument methods at levels much below those accessible other analytical techniques. In theory, if an inact material reacts quantitatively with the active mate and the compound formed can be separated from unreacted activity, then the inactive material can be analysed at the same low levels. With th mind, several workers[1,2] have reviewed the theo radiometric analysis and considerable progres been made in its application[3-6]. In most cases, however, the difficulty of achieving clean phase separations at µgm.-levels is very great. In addition, to achieve reasonable precision and speed, it has nearly always been necessary to count the excess of unreacted activity rather than the compound formed; a procedure which reduces greatly the sensitivity of the method.

Using filter paper as a supporting medium and extending methods which have been found of value in spot tests[7,8] and paper chromatography[9,10], a rapid and simple method of micro-analysis has been developed which overcomes most of these difficulties. It consists in forming a precipitate from the active and inactive materials in the pores of filter paper and washing the paper to remove unreacted activity. The paper disk is then counted along with disks prepared in an identical manner from calibrating solutions. The method was used as follows for the determination of beryllium with a phosphate solution containing phosphorus-32.

Disks of Whatman 42 filter paper of 2 cm. diameter were numbered in pencil and arranged in rows on a glass sheet. By means of a pipette, the centre of each circle was spotted with 0·05 ml. of different beryllium solutions of known strength. After drying *in situ* at 100° C., the paper disks were transferred to about 100 ml. of 0·3 *M* diammonium hydrogen phosphate buffered at about *p*H 5·5 with sodium acetate and giving about 160,000 c.p.m. of phosphorus-32 per ml. The solution was stirred intermittently for 15 min., following which the active solution was poured off (for re-use). The paper disks were rinsed twice with demineralized water and washed for 30 min. by passing a stream of tap water into the beaker. The disks were then transferred to a large sheet of absorbent paper, dried under an infra-red lamp and counted in aluminium planchets with an end window Geiger counter. Results are given in Table 1.

Table 1

Strength of Be^{2+} solution	0·000 *N*	0·025 *N*	0·050 *N*	0·100 *N*	0·200 *N*
Counts/min./disk	72	280	549	936	1,761

These points plot to a good straight line. Good calibration plots of slightly lower precision were also obtained when 0·10 ml. or 0·01 ml. of the beryllium solutions was transferred to the filter paper instead of the 0·05 ml. used for the results quoted. The precision of a determination in the concentration-range examined would be about 2 per cent but would depend on the nature and amount of impurities and on the care taken to ensure that all the paper disks are treated in an identical manner.

The method was also applicable to the determination of magnesium by precipitation of $MgNH_4{}^{32}PO_4$ instead of $BeNH_4{}^{32}PO_4$. The interference of magnesium in the determination of beryllium could be prevented by the use of ethylenediamine tetraacetic acid in the precipitating solution. There are also indications that it will be possible to operate the method in reverse form using an isotope dilution procedure. Thus, if the centre of the paper disks is spotted with 0·05 ml. of the same inactive diammonium hydrogen phosphate solution and the disks, after drying, are transferred to beryllium solutions containing the isotope beryllium-7 at different specific activities, then the activity taken up by the paper is a reasonable function of the specific activity of the beryllium in solution provided that the total concentrations of beryllium do not vary too widely.

A method to determine chloride ion by a similar technique (by immersing the chloride-impregnated paper disks in a nitric acid solution of ${}^{110m}Ag$) gave a reasonable calibration curve when less than about 30 µgm. of Cl^- was present on the paper, but the results were anomalous at higher levels. These anomalies are thought to be due to a change in the physical form of the precipitate on the paper when certain concentration-levels are exceeded, which results in a larger fraction being removed by the standard washing technique. Further study of the factors affecting the nature of silver precipitation in filter paper will undoubtedly be very profitable since this useful isotope can form precipitates with a large number of organic compounds as well as many inorganic anions.

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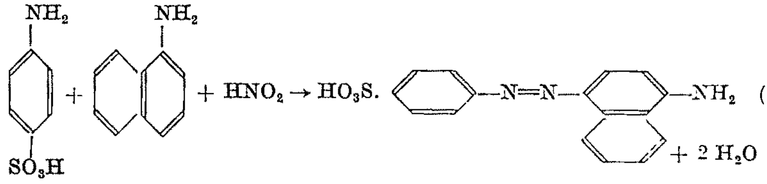
Continuous Determination of Traces of Nitric Oxide

ALL known methods[1] for the determination of traces of nitric oxide in coke oven gas and air make use of the oxidation, both by means of liquid-phase (permanganate) and gas-phase (ozone) oxidation, to nitrogen dioxide and the subsequent absorption and reaction in the Griess-Ilosvay reagent followed by colorimetric measurement of the azo dye. Calibration can be carried out using standard sodium nitrite solution and assuming the following reactions :

$$2\,NO + O_2 \rightarrow 2\,NO_2 \qquad (1)$$

$$2\,NO_2 + H_2O \rightarrow HNO_2 + HNO_3 \qquad (2)$$



(3)

It has been pointed out[1,2], however, that the analysis is affected by the non-stoichiometric character of this reaction sequence. Consequently, the reliability of the determination depends on the standardization procedure.

In order to meet the requirements for a continuous recording of traces of nitric oxide in coke oven gas and air, we have envisaged the possibility of a fast

Table 1. PHOTO-OXIDATION OF NITRIC OXIDE IN A NITROGEN–OXYGEN MIXTURE (0·3 PER CENT OXYGEN) WITH VARIABLE AMOUNTS OF 1,3-BUTADIENE
(H.P.K. mercury lamp (without filter), contact time in quartz tube 5 sec.)

1,3-Butadiene (per cent by vol.)	Nitric oxide introduced* (m.mol.)	Nitrous acid in first spiral washer with Griess-reagent (m.mol.)	Nitrous acid in second spiral washer after preceding oxidation with $KMnO_4$ (m.mol.)
0·0	$2·0 \times 10^{-4}$	$0·0 \times 10^{-4}$	—
0·001	$2·0 \times 10^{-4}$	$0·32 \times 10^{-4}$	$0·47 \times 10^{-4}$
0·01	$2·0 \times 10^{-4}$	$1·06 \times 10^{-4}$	$0·24 \times 10^{-4}$
0·05	$2·0 \times 10^{-4}$	$1·01 \times 10^{-4}$	$0·07 \times 10^{-4}$
0·1	$2·0 \times 10^{-4}$	$1·01 \times 10^{-4}$	$0·04 \times 10^{-4}$

* Concentration about 1 p.p.m.

and reliable determination which approaches as closely as possible the reaction sequence given above.

It has already been pointed out by Fullweiler[3] that the oxidation of nitric oxide to nitrogen dioxide is accelerated by dienes (an instrument based on this principle is marketed by the Rubicon Co.). However, owing to the still relatively slow rate of oxidation, this method cannot be applied for the recording of rapid changes in concentration.

We have found (patent applied for) that a fast (contact time of 5 sec.) oxidation of traces of nitric oxide can be effected by irradiation with a high-pressure mercury vapour lamp. From a preliminary investigation on the reaction mechanism it appeared that the photo-oxidation of nitric oxide occurs only in the presence of dienes. In addition it was found that with 1,3-butadiene the effective wave-length region is below 2300 Å. Therefore it seems likely that the first step in the photo-oxidation of nitric oxide comprises the formation of an excited butadiene molecule, which persists long enough[4] to react with oxygen to a peroxide.

The latter may undergo reaction, through a chain mechanism[5], with nitric oxide and oxygen to nitrogen dioxide:

$$\begin{aligned} &D\ \text{(diene)} + h\nu \rightarrow D^* \\ &D^* + O_2 \rightarrow DO_2 && (4) \\ &DO_2 + 2\,(NO) \rightarrow DO_2\,(NO)_2 && (5) \\ &DO_2\,(NO)_2 + O_2 \rightarrow DO_2 + 2\,NO_2 && (6) \end{aligned}$$

Thus, as a result of the formation of an excited diene molecule, the normal induction period of the oxidation of nitric oxide is decreased appreciably.

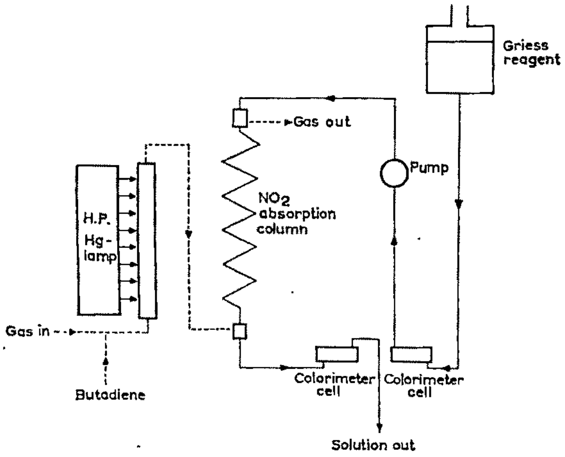


Fig. 1. Schematic diagram of nitric oxide recorder

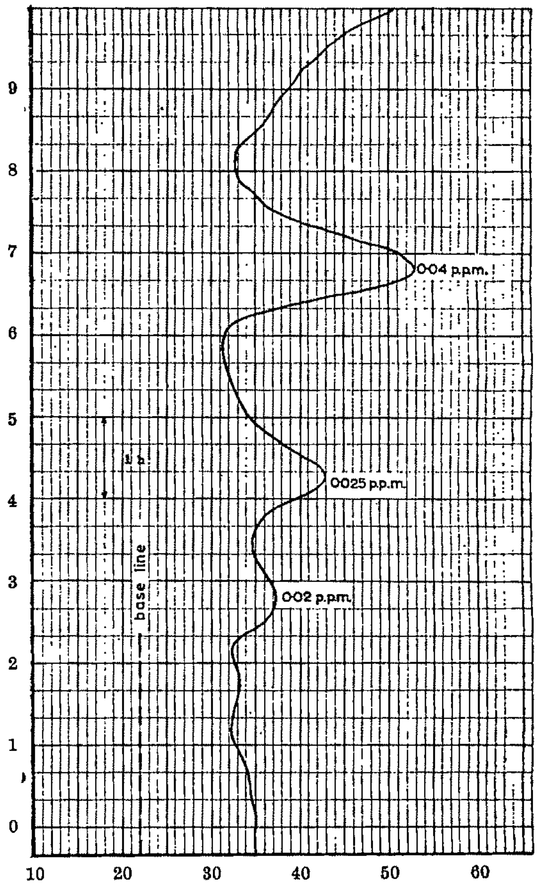


Fig. 2. Record of concentration of nitric oxide in coke oven gas (8 scale divisions = 0·01 p.p.m. oxide)

Some results of the oxidation of nitric oxide in a nitrogen–oxygen mixture with variable amounts of 1,3-butadiene are given in Table 1.

It appears that for butadiene concentrations of 0·05 per cent and higher the stoichiometry of equations 1–3 is satisfied.

A continuous nitric oxide recorder was developed based on the photo-oxidation of nitric oxide and the subsequent absorption and reaction in a spiral washer with Griess-Ilosvay reagent followed by colorimetric measurement by means of a flow type colorimeter[6]. A block diagram of the instrument is given in Fig. 1.

The nitric oxide recorder is applied for the continuous determination of nitric oxide in coke oven gas. A typical record is given in Fig. 2, in which 0·01 p.p.m. corresponds with eight scale divisions.

Finally, the method has also been applied to examination of air pollution. In this case additional 1,3-butadiene, which is already present in sufficient amount in coke oven gas, must be supplied to the air stream.

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Distribution of Oxygen in a Vitrinite and its Extraction Products

A VITRINITE (petrographic purity: 99 per cent; analysis (moisture- and ash-free): 83·9 C; 5·5 H; 8·0 O; 1·4 N per cent; 1·2 per cent S by difference) was extracted with pyridine, the pyridine-soluble part with benzene, and this extract finally with petroleum ether (boiling point 40–60° C.). The ratio of material to solvent was 10 gm./litre in all cases. Each extraction was carried out in boiling solvent of analytical purity, for a period of 24 hr. with efficient stirring and under purified nitrogen. Exhaustive extraction was thus achieved at each step. A 25 per cent yield was obtained for the pyridine-soluble fraction, which could itself be extracted with benzene to the extent of 20 per cent. The petroleum ether extract represented 40 per cent of the benzene-soluble part. The former was washed further with sodium carbonate, sodium hydroxide and hydrochloric acid to yield 96 per cent of a 'neutral oil'.

The content of hydroxyl groups in vitrinite and its extraction products was determined as described previously[1], with the exception of the benzene-soluble fraction and those derived from it, in which it was better to use a method developed for asphaltic bitumens[2]. The amount of quinone oxygen was measured in the pyridine-soluble and derived fractions by means of an analytical adaptation[3] of quinone reduction with copper and hydrogen sulphide[4].

The results are summarized in Table 1. The first column gives the content of oxygen, determined moisture- and ash-free. In the petroleum ether-soluble fraction and the 'neutral oil' there is no ash, but an accurate determination of moisture according to the standard methods used for coal products was not possible on account of the relative volatility of the samples. Therefore, in these cases the analytical data for total oxygen must be considered as maximum values. In the second column (percentage hydroxyl oxygen) the small value reported for the petroleum ether-soluble fraction could not be determined with accuracy, but is certainly correct as an order of magnitude, as can also be ascertained from the near infra-red absorption at about 1·42μ. From the third column (percentage quinone oxygen), it can be seen that the benzene-insoluble fraction is the last in the series to contain quinone oxygen in detectable quantity.

Taking into account that in bituminous coals oxygen is present essentially in the form of hydroxyl groups, quinones, and as 'non-reactive'[5], the results in column 4 show that 'non-reactive' oxygen, calculated by the difference, $O_{\text{total}} - (O_{\text{OH}} + O_{\text{quin.}})$, has a remarkably constant value, of about 2·8 per cent, in all fractions where it follows directly from experimental results. Assuming that this value holds true also for the pyridine-insoluble fraction and for vitrinite itself, their quinone oxygen content can be estimated (see Table 1, column 3, results in square brackets). Considering the experimental errors made, the results obtained fit fairly well in the whole scheme for oxygen distribution in the various fractions.

Table 1. DISTRIBUTION OF OXYGEN IN A VITRINITE (83·9 PER CENT CARBON) AND ITS EXTRACTION PRODUCTS

Sample	Percentage oxygen (moist.-,ash -free)	Percentage hydroxyl oxygen	Percentage quinone oxygen	Percentage non-reactive oxygen (by difference)
Vitrinite	8·0	4·2	[1·0]	[2·8]
Pyridine-insoluble	9·0	4·5	[1·7]	[2·8]
Pyridine-soluble	7·2	3·3	1·0–1·2	2·9–2·7
Benzene-insoluble	8·5	3·7	1·6–1·8	2·8–3·0
Benzene-soluble	4·4	1·8	—	2·6
Petroleum ether-insoluble	5·3	2·5	—	2·8
Petroleum ether-soluble	max. 3·1	(0·5)	—	(2·6)
'Neutral oil'	max. 3·1	—	—	3·1

Trends observed in Table 1 are significant. In each extraction step a disproportionation of hydroxyl groups occurs which favours the insoluble residue. This concentration of polar groups is even more marked with respect to quinones. The above observations can certainly be correlated with the change of polarizability in the solvent series. Further, it may be noted that the ratio of hydroxyl to total oxygen remains remarkably constant down to the petroleum ether-insoluble component. On the other hand, the 'non-reactive' oxygen behaves quite differently, since it remains practically constant in amount from the pyridine-soluble extract down to the neutral oil and should be taken into account as a structural parameter[6].

Experiments have been initiated in this laboratory to investigate the principal nature of this 'non-reactive' oxygen by chemical means. The infra-red spectrum of the neutral oil is sufficiently specific to show clearly a band at 8·35μ (1,198 cm.$^{-1}$) which can be attributed to the antisymmetrical stretching mode of a C—O—C group in which at least one of the carbon atoms is part of an aromatic ring[7]. Thus, making the assumption that the 'non-reactive' oxygen is mainly related to at least partly aromatic ether structures, it seemed worth while to attempt cleavage of these groups with sodium in liquid ammonia[8]. Results so far obtained on the petroleum-insoluble fraction, the neutral oil, and one of its chromatographic fractions (2·5 per cent oxygen) have shown that ether cleavage, indeed, occurs under the conditions of Birch reduction.

The principal change in the infra-red is a new absorption band in the 2·9μ (3,450 cm.$^{-1}$) region due to —OH stretching, accompanied by sharply reduced intensity of the 8·35μ band. After acetylation, the reduced samples show no OH absorption but intense bands at 5·69μ (1,760 cm.$^{-1}$), at 8·31μ (1,205 cm.$^{-1}$) and increased intensity at 7·26μ (1,375 cm.$^{-1}$), which leads to the conclusion that phenolic groups have been acetylated; a further proof that ether oxygen originally present was linked at least on one side to an aromatic system.

The investigation, part of which has been described here, was supported in this laboratory by the Union Carbide Corporation, New York, to which we are grateful for permission to publish the results.

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Rate of Oxidation of Ferrohæmoglobin by Ferricyanide Ions

The iron atom in hæmoglobin is part of an octahedral structure with four bonds in a plane to the nitrogen atoms of the pyrrole rings, the fifth bond directly to the globin (probably through the nitrogen atom of the imidazole ring of histidine) and the sixth bond to a water molecule[1]. Recent X-ray diffraction studies have shown that the four hæm-groups are situated on the surface of the hæmoglobin in pockets formed by folds in the polypeptide chains[2]. St. George and Pauling[3] have suggested that in aqueous solutions the folds in the polypeptide chains interfere with the binding of tertiary butyl *iso*cyanide to the hæm group. In an attempt to obtain further information concerning the effect of the environment of the hæm group on the chemical properties of the iron, we have measured the rate of oxidation of ferrohæmoglobin by ferricyanide ions, and compared this rate with that of oxidation-reduction reactions between various iron compounds.

Oxyhæmoglobin was obtained from freshly drawn human blood. About 30 ml. of blood was added to an anticoagulant medium containing acid citrate and dextrose. This was then centrifuged to separate the red cells, which were washed three times with 0·85 per cent saline solution. The red cells were then hæmolysed by adding 1·2 vol. distilled water and 0·2 vol. toluene and gently shaken. The toluene layer was separated from the aqueous layer, which was then centrifuged. The oxyhæmoglobin in the aqueous layer was estimated spectrophotometrically[4]. The oxyhæmoglobin was converted to ferrohæmoglobin by repeated evacuation and equilibration with nitrogen.

The rate of the ferrohæmoglobin–ferricyanide reaction was measured using the rapid-flow apparatus which has been previously described[5]. The reaction was investigated in a phosphate buffer with a *p*H of 6·0 and an ionic strength of 0·10. There is evidence that, at this *p*H, ferricyanide ions oxidize only the hæm group and leave the globin unchanged[6]. Second-order kinetics were observed. The rate constant for the oxidation of the first hæm group was found to be $7 \cdot 0 \pm 0 \cdot 5 \times 10^4$ l. mole^{-1} sec.$^{-1}$ at 25·0° C. with the concentration of the ferrohæmoglobin solution expressed in terms of its hæm content (mol. wt. = 17,000). Attempts to study the rate of oxidation of ferrohæmoglobin by the *tris*-(2,2′-dipyridine) complexes of osmium(III) and ruthenium(III) were unsuccessful, for these oxidizing agents reacted with the globin in addition to oxidizing the hæm-groups.

The ferrohæmoglobin–ferricyanide reaction is compared with oxidation-reduction reactions between various iron compounds in Table 1. This table also includes the products of the charges of the various reactants and the standard free-energy changes of the reactions calculated from the formal oxidation potentials of the appropriate couples[7,8].

It is apparent from Table 1 that the ferrohæmoglobin–ferricyanide reaction proceeds much slower than reactions (5) and (6). This is somewhat surprising in view of the more favourable standard free-energy change of the ferrohæmoglobin–ferricyanide reaction[7,9]. Moreover, the iron atoms in ferrohæmoglobin are uncharged and unless there are high negative charges on the neighbouring groups, the work required to bring the ferricyanide ion close to an iron atom in ferrohæmoglobin will be less than that required to bring the similarly charged ions together in reactions (5) and (6). Provided we neglect any effects due to the differences in the structures and solvations of the various reactants, we may estimate a lower limit for the rate of reaction (3) from the rates of reactions (2) and (4). Assuming that the rates of reactions (2), (3) and (4) are determined solely by the amount of work required to bring the reactants together, the rate constant of reaction (3) should be somewhat larger than the geometric mean of the rate constants of (2) and (4). This estimate will be further increased if in addition we consider the relative standard free-energy changes of reactions (2), (3) and (4). These considerations lead to an estimate of about 10^6 l. mole^{-1} sec.$^{-1}$ for the lower limit of the rate constant of reaction (3). It is apparent from Table 1 that the rate constant of the ferrohæmoglobin–ferricyanide reaction is smaller than this value.

Table 1. OXIDATION-REDUCTION REACTIONS BETWEEN VARIOUS IRON COMPLEXES AT 25° C.

	Oxidizing agent[a]	Reducing agent[a]	Z_1Z_2	ΔG^0 kcal. mole^{-1}	k l. mole^{-1} sec.$^{-1}$
(1)	$Fe(H_2O)_6^{3+}$	$Fe(H_2O)_6^{2+}$	+ 6	0	4^b
(2)	$Fe(CN)_6^{3-}$	$Fe(CN)_6^{4-}$	+ 12	0	$1 \times 10^{3\ c,d}$
(3)	$Fe(CN)_6^{3-}$	$Hb(H_2O)$	0	− 6·2	7×10^4
(4)	$Fe(phen)_3^{3+}$	$Fe(CN)_6^{4-}$	− 12	− 5·3	$> 10^{8e}$
(5)	$Fe(d\text{-}dipy)_3^{3+}$	$Fe(t\text{-}phen)_3^{2+}$	+ 6	− 3·2	$> 10^{8e}$
(6)	$Fe(phen)_3^{3+}$	$Fe(d\text{-}dipy)_3^{2+}$	+ 6	− 2·5	$> 10^{8e}$

[a] phen = 1,10-phenanthroline; d-dipy = 4,4′-dimethyl-2,2′-dipyridine; t-phen = 3,4,7,8-tetramethyl-1,10-phenanthroline; $Hb(H_2O)$ = ferrohæmoglobin.

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The relative slowness of the ferrohæmoglobin–ferricyanide reaction may be due to a type of steric hindrance in which the polypeptide chains prevent the close approach of the ferricyanide ions to the hæm-groups. In addition any hydrophobic groups on these polypeptide chains will tend to lower the dielectric constant in the vicinity of the hæm groups[10]. This will tend to increase the free energy of activation of the ferrohæmoglobin–ferricyanide reaction by the amount of work required to bring the ferricyanide ion from an environment of higher to one of lower dielectric constant[11]. In this connexion it is important to remember that electron transfer from the hæm group to the ferricyanide ion need not occur directly, but may occur via the polypeptide chains instead.

Another possibility is that the slowness of the ferrohæmoglobin–ferricyanide reaction is due to differences in the structures and solvations of ferro- and ferrihæmoglobin. It is apparent from reactions (1) and (2) of Table 1 that the replacement of cyanide ions in the co-ordination shells of the iron ions by water molecules leads to a marked decrease in the rate of the electron transfer reaction. Such decrease is generally ascribed to the differences in the structures of the inner hydration shells of the ferrous and ferric ions and the energy required to rearrange these shells before electron transfer can occur[11]. In a similar manner, any differences in the iron–oxygen and iron–nitrogen distances in the two oxidation states as well as any differences in the configuration of the surrounding solvent molecules will tend to slow down the rate of oxidation of ferrohæmoglobin. Because of the rigidity of the porphyrin rings, such effects are more likely to be associated with differences in the binding of the imidazole groups and the water molecules co-ordinated to the iron atoms in ferro- and ferrihæmoglobin.

It is interesting to recall that in addition to being relatively resistant to oxidation to the iron(III) state, the iron(II) in ferrohæmoglobin is able to co-ordinate oxygen rapidly and reversibly. This latter property requires that at least one of the ligands attached to the iron(II) be readily replaceable by oxygen[12]. In the case of ferrohæmoglobin this function is fulfilled by the water molecule attached to the iron(II). It is thus possible that this water molecule serves the dual purpose of enabling the ferrohæmoglobin to co-ordinate oxygen molecules rapidly and reversibly, and at the same time renders it relatively resistant to attack by such oxidizing agents as ferricyanide ions.

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Structure and Spectra of $[(\text{Phenanthroline})_2\text{Fe}(\text{CNMe})_2]^{2+}$ and $[(\text{Phenanthroline})_2\text{Fe}(\text{CN})_2\text{H}^2]^{2+}$

THE striking shift in the position of the visible absorption bands of dicyano *bis*(1 : 10 phenanthroline) iron(II) and the corresponding 2-2′-dipyridyl complex caused by the addition of acid was first noted by Schilt[1], who has since shown that these compounds react reversibly to give mono- and diprotonated ions[2]. Schilt suggested that the hydrogen is attached directly to the metal in the protonated species. A second possibility is that the protonated compounds are '*iso*cyanides'.

Treatment of the phenanthroline compound with excess methyl sulphate gave an orange water-soluble product which precipitated from aqueous solution as the perchlorate on addition of excess sodium perchlorate. Analysis showed it to be the perchlorate of the dimethyl derivative of the original complex. It evolved methyl *iso*cyanide on warming in neutral aqueous solution.

The visible absorption spectrum of this compound is strikingly similar to that of the diprotonated form of Schilt's compound in 10 *M* sulphuric acid (Fig. 1). These facts together with the observation that the spectrum of the methyl derivative is essentially unchanged in 10 *M* sulphuric acid suggest strongly that the diprotonated ion should be written as an *iso*cyanide $[(\text{phenanthroline})_2\text{Fe}(\text{C} \equiv \text{NH})_2]^{++}$ analogous to the methyl derivative $[(\text{phenanthroline})_2\text{Fe}(\text{C} \equiv \text{N}.\text{CH}_3)_2]^{++}$.

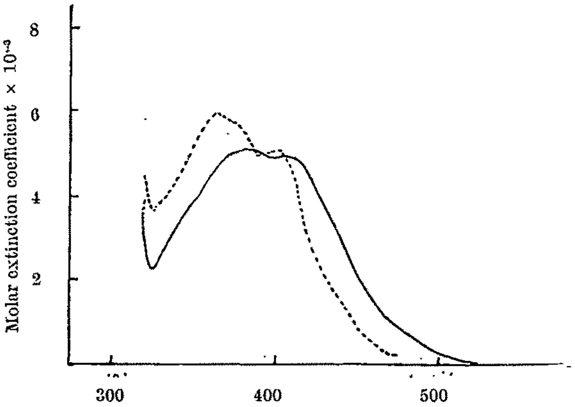

Fig. 1. Molar extinction curves for: (1) $[(\text{phenanthroline})_2\text{Fe}(\text{CNMe})_2](\text{ClO}_4)_2$ in water (—); (2) $[(\text{phenanthroline})_2\text{Fe}(\text{CN})_2]$ in 12 *M* sulphuric acid (Schilt) (...)

In an attempt to decide whether the compounds have the *cis* or *trans* configurations we have measured their infra-red spectra in 'Nujol' mulls. In each case we find evidence of a splitting of the CN frequency into two peaks separated by about 10 cm.⁻¹ (Fig. 2). This suggests, but does not prove, that the compounds have the *cis* configuration.

The intense visible absorption band of the ferrous *tris*-phenanthroline complex has often been classified as a charge-transfer band associated with the photoreduction of the organic ligand by the metal ion[1]. Since there is no permanent reaction this interpretation cannot be confirmed directly by chemical methods. Our results lend strong support to the charge-transfer hypothesis.

We may safely assume that the intense visible absorption of $[\text{Fe}(\text{phenan})_2(\text{CN})_2]$ has the same origin as that of the *tris*-phenanthroline complex. When one CN^- ion is converted to a CNH group by protonation the absorption band moves from 6000 Å. to 5000 Å.

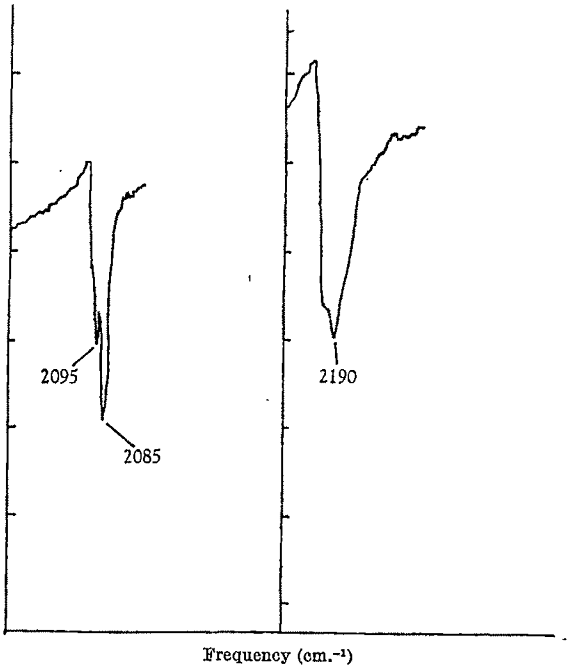

Fig. 2. Infra-red spectra of $\text{C} \equiv \text{N}$ region: (1) $[(\text{phenanthroline})_2\text{Fe}(\text{CNMe})_2](\text{ClO}_4)_2$; (2) $[(\text{phenanthroline})_2\text{Fe}(\text{CN})_2]$

When both CN⁻ groups are protonated or alkylated there is a shift of 3800 Å. We believe that the protonation (or alkylation) of the CN⁻ group transforms it from a moderate to a very strong π electron acceptor, for it is known that the *iso*nitriles resemble CO rather than CN⁻ in their ability to stabilize low valency states. The shift of the absorption spectrum is thus due to the stabilization of the ferrous state with respect to the ferric by the isonitrile group. The magnitude of the effect, about 0·6 eV. or 14 k.cal. per proton, is surprisingly large.

The visible band of the dicyanide is extremely sensitive to the nature of the solvent, moving to shorter wave-lengths as the solvent polarity is increased. This is understandable if we suppose that polar solvent molecules are strongly oriented in the neighbourhood of the cyanide groups by dipole–dipole interaction or by hydrogen bonding. Excitation leads to a quite new charge distribution in the complex which is no longer adjusted to the oriented solvent molecules. It follows that the greater the stabilization of the ion by solvent in the ground-state, the more the excitation energy is increased. Furthermore, since the nitrogen atoms of the cyanide groups lose negative charge during excitation any hydrogen bonds which they form will be weakened. These two factors account for the marked solvent effects.

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Interaction of Model Peptides with Water and Lithium Bromide

Knowledge of the interactions between peptide groups and water or denaturing agents is of major importance in understanding proteins. Klotz and Franzen[1] showed recently by infra-red studies that aqueous N-methylacetamide (NMA) forms few interpeptide hydrogen bonds except at concentrations approaching 12 *M*. Hydrogen bonds between water and NMA are formed more readily. Harrington and Schellman[2] proposed that the increased viscosity of proteins in strong lithium bromide solutions is the result of more extensive bonding and more helical coiling than in water, in contrast to the widely held idea that hydrogen bonds are broken in lithium bromide solution. We present viscometric data relating to the interaction of water solutions of lithium bromide with NMA, dimethylacetamide (DMA), acetone, and polysarcosine. The results show that increased viscosities occur in systems where interpeptide hydrogen bonds cannot form.

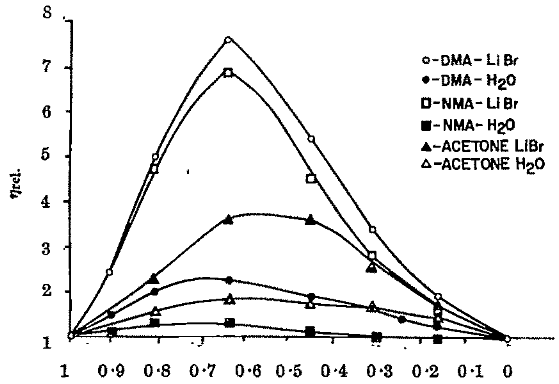


Fig. 1. Viscosities of NMA, DMA and acetone in water and lithium bromide. Solutes were mixed with water or with 10 *M* lithium bromide to obtain the volume fractions indicated

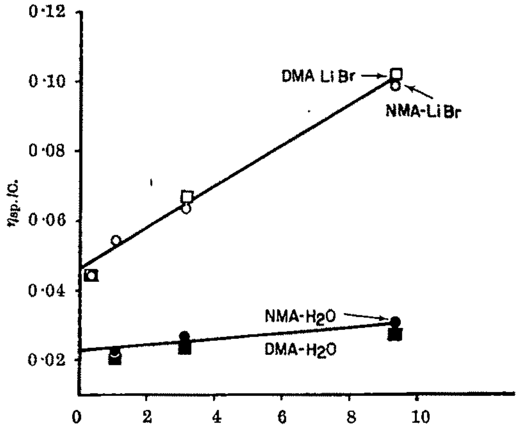


Fig. 2. Intrinsic viscosities of NMA and DMA in water and 9 *M* lithium bromide

Fig. 1 shows that mixtures of NMA, DMA or acetone with water have viscosities higher than those calculated on the assumption of ideal mixing. Mixtures of NMA, DMA or acetone with 10 *M* lithium bromide have still higher viscosities, similar to those reported for the system urea–lithium bromide[3]. The maxima of the lithium bromide curves for DMA, NMA and acetone all occur at a ratio of solute to lithium bromide of nearly 2 : 1. 10 *M* potassium thiocyanate and 9 *M* guanidinium chloride raise the viscosity of acetone or NMA one-third to one-half as much as does lithium bromide.

Fig. 2 shows that the intrinsic viscosities of NMA and DMA are higher in lithium bromide solution than in water. (All the viscosities reported here were measured in Cannon Instrument Co. dilution Ubbelohde viscometers, sizes 50 or 75, at 39° C. The flow time for NMA in the semi-micro, size 50 viscometer was 13 min. and in size 75 was 8 min. for about 1 ml. of liquid.)

In Table 1 are shown viscosities of NMA and DMA in aqueous lithium halides, hydrochloric acid, and carbon tetrachloride. Lithium chloride and bromide give similar results, so that the effect must be largely due to the lithium ion. The similarity between NMA and DMA in Figs. 1 and 2 and Table 1 show that interpeptide hydrogen bonding need not be involved in lithium bromide solutions. In carbon tetrachloride the interpeptide hydrogen bond of NMA is apparent in the elevation of viscosity.

Table 1. Relative Viscosities of Amides in Several Solvents

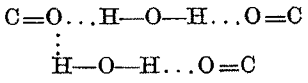
Volume and amide (ml.)		Volume and solvent (ml.)		Relative viscosity
10	NMA	1	10 *M* LiBr	2·14*
10	DMA	1	10 *M* LiBr	1·85*
10	NMA	1	10 *M* LiCl	2·32*
10	DMA	1	10 *M* LiCl	1·79*
10	NMA	1	10 *M* HCl	1·59*
10	DMA	1	10 *M* HCl	1·36*
1	NMA	9	10 *M* HCl	1·35†
1	DMA	9	10 *M* HCl	1·34†
1	NMA	9	CCl_4	1·66‡
1	DMA	9	CCl_4	1·06‡

* Relative to 10 ml. amide plus 1 ml. water.
† Relative to 9 ml. solvent plus 1 ml. water.
‡ Relative to pure carbon tetrachloride.

Of greater importance in relation to proteins is that the intrinsic viscosity of polysarcosine of molecular weight 2,500 (Schwartz Bio-Research, Inc., New York) was 0·086 in 9 *M* lithium bromide and 0·054 in *M* potassium chloride. These results for polysarcosine are contrary to those reported by Steinberg *et al.*[4] for poly-L-proline, which also cannot form interpeptide hydrogen bonds. Polysarcosine of higher molecular weight and copolymers of sarcosine and proline are being prepared for study, as are other copolymers.

$$C{=}O\ldots H{-}O{-}H\ldots O{=}C$$

(I)



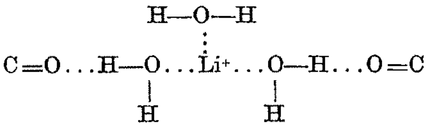
$$\begin{array}{l} C{=}O\ldots H{-}O{-}H\ldots O{=}C \\ \vdots \\ H{-}O{-}H\ldots O{=}C \end{array}$$

(II)

The high viscosities of mixture of NMA, DMA or acetone with water may be due to hydrogen bonding, as in (I) or as in (II). Two hydrogen bonds to one peptide carbonyl have been shown to exist in crystalline acetamide[5]. In the case of NMA and DMA there may also be some hydrogen bonding to the nitrogen. The low viscosity of NMA–water at high water content may result from a breaking down of the water structure. In lithium bromide solutions there may be lithium to carbonyl bonding as in (III), or bonding of carbonyl to water of the lithium hydration sphere as in (IV). The multiplicity of hydrogens in the hydration sphere of lithium would permit formation of larger aggregates than in water. At low peptide concentrations, multiple binding as in (III) or (IV) becomes less probable, and there may be only one peptide group bound for each lithium. The nature of the lithium to peptide bonding is not specified but may be similar to the ion-dipole attraction of lithium to water.

$$C{=}O\ldots Li^{+}\ldots O{=}C$$

(III)



$$\begin{array}{ccccc} & & H{-}O{-}H & & \\ & & \vdots & & \\ C{=}O\ldots H{-} & O & \ldots Li^{+}\ldots & O & {-}H\ldots O{=}C \\ & | & & | & \\ & H & & H & \end{array}$$

(IV)

Experiments were also done with 8 and 12·5 *M* lithium bromide acting on NMA. The maxima of the viscosity curves were 4·5 and 22 and occurred at ratios of amide to lithium of 3 : 1 and 1 : 1, respectively. 8, 10 and 12·5 *M* lithium bromide have water to lithium ratios of about 5, 3·7 and 3 respectively. It appears that lower hydration of lithium results in greater interaction with amides. This supports formulation (III) rather than (IV). However, lower hydrates would be expected to produce stronger hydrogen bonding as the strengthening effect of the lithium charge on the hydrogen bond would be distributed among fewer hydrogens. With 12 *M* lithium bromide the curve was not continuous, a crystalline precipitate appearing at an NMA volume fraction of 0·8. Determination of the structure of this precipitate by X-ray diffraction is planned.

In 10 *M* hydrochloric acid aggregation may be mediated by hydrogen bonding of H_3O^+ or $H_3O(H_2O)_n{}^+$ units to the carbonyl groups. Steinberg *et al.*[4] proposed protonation of peptide groups to explain the high optical rotation of poly-L-proline in strong acid.

The increased viscosity of proteins in strong lithium bromide solution may be due to extension of the polypeptide chain as a result of attaching many hydrated lithium ions in close proximity. The possibility of aggregation is also being investigated. In the case of poly-L-proline special configurational effects may be involved.

We are grateful to Dr. David Harker for suggesting hydrogen bonding of the lithium hydration sphere. This work was supported partly by a grant from the National Institutes of Health.

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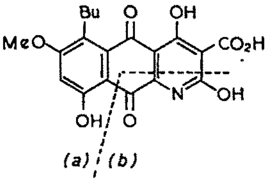
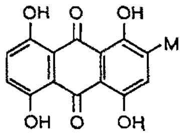
Pigments of *Phoma terrestris* Hansen and their Biosynthesis

Wright and Schofield[1] recently reported the failure of cultures of *Phoma terrestris* Hansen (from the Centralbureau voor Schimmelcultures, Baarn) to produce the pigment phomazarin[2] for which (I) is one of the two suggested structures. In our hands cultures from the same source have given this compound, which agrees well in physical properties with those described by Kögl *et al.*[2]. For reasons which we are unable to define, the same organism grown under the usual conditions[2] does not invariably produce the pigment. Like Wright and Schofield[1] we have observed cyanodontin (II) as a constituent of the mycelium.



(I)



(II)

Radioactivity from ^{14}MeCO$_2$Na and Me^{14}CO$_2$Na is incorporated into cyanodontin. The acetic acid, obtained by Kuhn–Roth oxidation of the ^{14}MeCO$_2$Na-derived material, had one-eighth of the total activity, all on the methyl group. The acetic acid obtained similarly from the Me^{14}CO$_2$Na-derived material had one-seventh of the total activity, all on the carboxyl group. This shows that cyanodontin in common with other natural 2-methylanthraquinones (for example, ref. 3) is biosynthesized through head-to-tail linkage of eight acetic acid units and loss of the carboxyl carbon from the end of the chain.

Radioactivity from H^{14}CO$_2$Na, ^{14}MeCO$_2$Na and Me^{14}CO$_2$Na is incorporated into phomazarin. Degradation has shown that about 75 per cent of the activity of the H^{14}CO$_2$Na-derived material is located on the methoxyl carbon, the remainder being randomly distributed over the carbon skeleton. Therefore the latter is formed probably without the intervention of C_1-units (cf. ref. 4). The acetic acid obtained by Kuhn–Roth oxidation and the carbon dioxide formed by decarboxylation of the $^{14}MeCO_2Na$-derived material each had approximately one-eighth of the total activity. The acetic acid obtained by Kuhn–Roth oxidation of the $Me^{14}CO_2Na$-derived material had approximately one-eighth (12·1 per cent) of the total activity, 98 per cent of it on the carboxyl group. This suggests that the biosynthesis of phomazarin involves at least eight acetic acid units and implies that the nitrogen-containing moiety may be derived from an amino-acid (cf. terramycin[5]), possibly glycine. The derivation of the carboxyl carbon from the methyl carbon of acetic acid favours a structure of type (I) over an alternative which has the terminal benzene ring inverted. If structure (I) is correct then the dotted line dissects the skeleton into portions which could be derived from (*a*) eight acetic acid units linked head-to-tail, and (*b*) glycine. Further degradations are in progress.

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BIOCHEMISTRY

Amino-acid Synthesis from Hydrogen Cyanide under Possible Primitive Earth Conditions

The formation of adenine from hydrogen cyanide has been recently reported from this laboratory[1]. Continuing the work on the synthesis of biochemical compounds under possible primitive Earth conditions[2], we now have investigated the direct formation of amino-acids from hydrogen cyanide.

Gaseous hydrogen cyanide was slowly introduced into 1 litre of 3 N ammonium hydroxide placed in a 2-litre flask provided with a water-cooled condenser. At the end of 1 hr., the flow of gas was stopped and the reaction mixture was analysed for cyanide ion by the method of Schilt[3]. It was found 2·2 M in this ion.

The alkaline aqueous solution was heated at 70 ± 2° for 25 days, after which time the black polymer of hydrogen cyanide[4] was removed by centrifugation. The reddish supernatant solution was fractionated by ion exchange into two main fractions and the fraction containing the amino-acids was analysed by one- and two-dimensional paper chromatography.

For the ion exchange fractionation, 200 ml. of the supernatant were added to the top of a 4 × 40 cm. column of 'Dowex 2' resin (200–400 mesh, X 8, OH^- form). The column was first washed with water until the effluent was neutral and then the acidic and ampholytic components bound to the resin were eluted by means of 4 N acetic acid. A total volume of 1,200 ml. was collected in three sub-fractions of 400 ml. each. The first of these sub-fractions was essentially neutral and corresponded to the water hold-up volume of the column, which emerged just before the acetic acid solvent front. The second and third sub-fractions were, as expected, strongly acidic.

A preliminary paper chromatographic analysis indicated that almost all the amino-acids were present in the second sub-fraction. This solution was concentrated to dryness in a vacuum-rotating evaporator at 60°, then dissolved in 25 ml. of 1 N hydrochloric acid and analysed by paper chromatography.

For this, 25 μl. of the red-yellowish solution was placed on a small sheet of Whatman No. 3 mm. filter paper and chromatographed by ascending development, first in *n*-butanol/acetic acid/water, 4 : 1 : 1, and then, on drying, in *n*-propanol/1 N ammonium hydroxide, 3 : 1. The paper was then sprayed with 0·2 per cent ninhydrin in *n*-butanol and heated in the oven for 5 min.

Fig. 1 shows, on the right side, a two-dimensional chromatogram of the unknown; on the left side, a one-dimensional chromatogram of the standards glycine and alanine, of the unknown, and of a mixture of standards and unknown.

On the basis of this and other chromatographic evidence, the amino-acids alanine, glycine and aspartic acid, which correspond to the top three spots of the two-dimensional chromatogram, were tentatively identified among the products formed by the condensation of hydrogen cyanide in aqueous ammonia. The other spots have not yet been identified. The compound close to the origin, slowly migrating, developed maximum colour intensity only after a few days, indicating that it may be a peptide(s) or a compound of a similar behaviour to a peptide. Total amino-acids were quantitatively determined by the method of Troll and Cannan[5], and found to amount to 10 mM/l. of original reaction

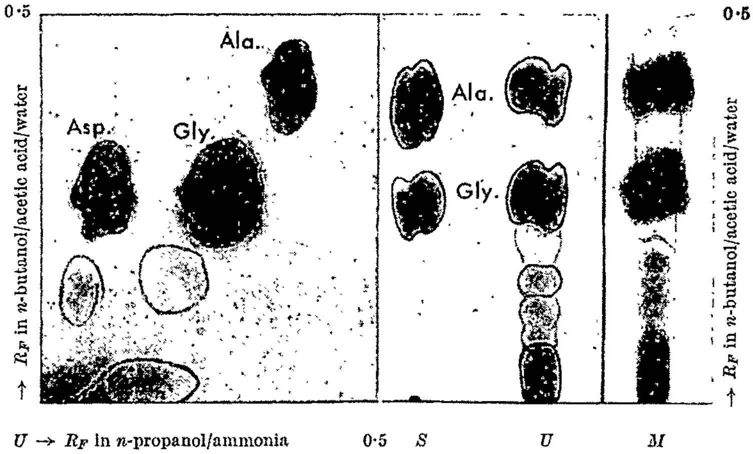
0·5
Ala.
Asp.
Gly.
Ala.
Gly.
0·5
R_F in n-butanol/acetic acid/water →
R_F in n-butanol/acetic acid/water →
U → R_F in n-propanol/ammonia
0·5
S
U
M

Fig. 1. Photographs of two chromatograms of the hydrogen cyanide reaction product after its elution from a 'Dowex 2' column by acid. *S*, Standards, alanine and glycine; *U*, unknown; *M*, mixture of standards and unknown

mixture, measured as glycine. This represents a substantial yield if it is considered that most of the hydrogen cyanide is condensed into polymeric hydrogen cyanide[4].

When 100 µl. aliquots of the test solution were chromatographed, small spots of several other ninhydrin-positive compounds were observed. In addition, by means of a Mineralight lamp (2537 Å.) a number of ultra-violet-light absorbing and fluorescent spots were detected on two-dimensional chromatograms.

In line with the above results, Wippermann[6] and other workers[4] demonstrated many years ago the formation of glycine by mild alkaline hydrolysis of an oligomer of hydrogen cyanide which had been obtained from hydrogen cyanide by base catalysis. It is possible that the well-established tetramer of hydrogen cyanide, di-aminomaleonitrile[7], and its assumed intermediate, aminomalonitrile[8], are the precursors of the C_2-, C_3- and C_4-amino-acids obtained in the present experiments.

Work is in progress for the identification of all the amino-acids, for the elucidation of the mechanism of amino-acid synthesis, and for determination of the nature of the other compounds synthesized from hydrogen cyanide.

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Occurrence of *n*-Odd-Numbered Monoethylenic Fatty Acids in the Liver Oil of the New Zealand School Shark (*Galeorhinus australis* MacLeay)

INVESTIGATIONS on the minor fatty acid constituents of the liver oil of the New Zealand school shark have resulted in the isolation of the saturated *n*-odd-numbered acids as well as the *iso*- and (+)*anteiso* acids of the C_{15} and C_{17} series[1,2]. These acids had not previously been found in fish oils, but had been earlier isolated from the depot[3] and milk fats[4] of ruminants. In addition, the occurrence of *n*-heptadecanoic acid and of (+)-14-methylhexadecanoic acid in tall oil has been established[5]. The occurrence of *n*-odd-numbered unsaturated acids in animal depot fats was first recorded in lamb caul fat, from which Δ^9-heptadecenoic acid was isolated[6] and the presence of pentadecenoic acid indicated: Δ^9-heptadecenoic acid has also been obtained from musk-ox fat[7] and from butterfat[8]. Following the feeding of *n*-odd-numbered saturated fatty acids, Appel, Böhm, Keil and Schiller[9] found that the corresponding Δ^9-mono-unsaturated acids were deposited in the depot and milk fats of goats and sheep. Apart from the above-mentioned instances, however, *n*-odd-numbered unsaturated acids have not been isolated from depot fats.

In shark liver oil, following extensive ester fractionation and low-temperature crystallization, fractions containing concentrates of *n*-mono-unsaturated acids of the C_{15}, C_{17} and C_{19} series have been prepared. These fractions have now made possible the isolation of Δ^9-heptadecenoic acid in pure form, and of Δ^{11}-nonadecenoic acid as a concentrate (73 per cent) with positional isomers mainly Δ^{10}- (13 per cent) and Δ^{12}- (14 per cent) nonadecenoic acids as impurities as judged from the yields of dibasic acids produced by oxidation using the method of Haverkamp Begemann *et al.*[10]. The resolution of these positional isomers is likely to prove difficult in contrast to the Δ^9-heptadecenoic acid, which was relatively easy to crystallize in pure form. From the weights of the concentrates and the results of gas-liquid chromatographic analysis it is estimated that pentadecenoic acid, Δ^9-heptadecenoic acid and Δ^{11}-nonadecenoic acid respectively form 0·018, 0·073 and 0·042 per cent of the shark liver oil fatty acids. These results serve to extend the similarities of ruminant fats and shark liver oils in regard to the occurrence of *n*-odd-numbered saturated and unsaturated fatty acids in addition to *iso*- and (+)-*anteiso*-acids of the C_{15} and C_{17} series earlier reported. Work on the isolation of the *n*-odd-numbered mono-unsaturated fatty acids is being continued.

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Scorpamins: the Toxic Proteins of Scorpion Venoms

FOLLOWING preliminary work[1,2], extensive purification of the toxins secreted by two species of North-African scorpions (*Androctonus australis* and *Buthus occitanus*) was accomplished by aqueous extraction, acetone fractionation in the cold, chromatography on 'Amberlite *CG* 50' and filtration on dextran gel ('Sephadex *G* 25'). Table 1 summarizes the steps of purification from the glands.

Comparative experiments were performed, starting with the organ containing the venom glands (telson) or with the emitted venom obtained by a manual or an electrical method previously described[4]. The

Table 1. Purification of Scorpamins starting with the Telsons of *A. australis* and *B. occitanus*

Steps of purification	*Androctonus australis* (5·29 gm. lyophilized telsons)				*Buthus occitanus* (4·75 gm. lyophilized telsons)			
	A_{280} *	LD_{50}†	*SA* ‡	Yield (per cent)	A_{280}	LD_{50}	*SA*	Yield (per cent)
Extraction by NaCl 0·9 (per cent)	3,180	33,333	10·5	100	4,768	12,308	2·5	100
Acetone fractionation (60–80 per cent)	199	12,545	63	37·6	288	8,182	28·4	66·8
Chromatography on 'Amberlite *CG* 50' ammonium acetate, 0·5 *M*, *p*H = 5·60 §	18·9	4,723	250	14·2	347	2,313	72	18·8
Filtration on 'Sephadex *G* 25' ‖	—	—	—	—	15·7	1,577	100	12·8
Chromatography on 'Amberlite *CG* 50' ammonium acetate, 0·2 *M*, *p*H = 6·80¶	—	—	—	—	0·64	123	192	1

* Absorbance at 280 mμ.

† Number of lethal doses, 50 per cent, determined in a 20-gm. mouse according to Behrens and Karber (ref. 3).

‡ Specific actitivy = LD_{50}/A_{280}

§ Dimensions of the column 4 cm. × 110 cm.

‖ With water as eluant. In this condition the toxin is adsorbed and can be eluted with ammonium acetate 0·05 *M*.

¶ Dimensions of the column 4 cm. × 135 cm.

Table 2. Purification of Scorpamins from *A. australis* starting with Electrically or Manually obtained Venom

Steps of purification	Venom extracted by the electrical method (105·9 mgm.)				Venom extracted by the manual method (114·5 mgm.)			
	A_{280}	LD_{50}	*SA*	Yield (per cent)	A_{280}	LD_{50}	*SA*	Yield (per cent)
Aqueous extraction	92	6,157	66·5	100	89·1	25,396	284·9	100
Filtration on 'Sephadex *G* 25'	34·2	4,046	118·3	65·7	48·7	20,160	413·8	79·4
Chromatography on 'Amberlite *CG* 50' ammonium acetate 0·2 *M*, *p*H = 6·80 *	3·4	1,870	550 †	30·4	14·3	7,961	556 *	31·3

See Table 1 for explanations of abbreviations.

* Column dimension, 4 cm. × 145 cm.

† One LD_{50} corresponds to 1·0 μgm. of pure toxin.

purification procedure is less tedious starting with the desiccated venom according to its higher toxin content (Table 2). The yield in pure toxin is about 30 per cent in these conditions.

The material obtained after the last step on 'Amberlite *CG* 50' was homogeneous with regard to chromatography (constant specific activity) (Fig. 1), electrophoresis and in the ultracentrifuge.

Two proteins, sharing almost equally the total toxic activity, have been separated from the venom of both species (Fig. 1). Their properties are very similar considering specific activity (the two toxins of *A. australis* and one of the two toxins of *B. occitanus* have specific activity of about 500), molar extinction coefficient and symptomatology of the intoxication in mice.

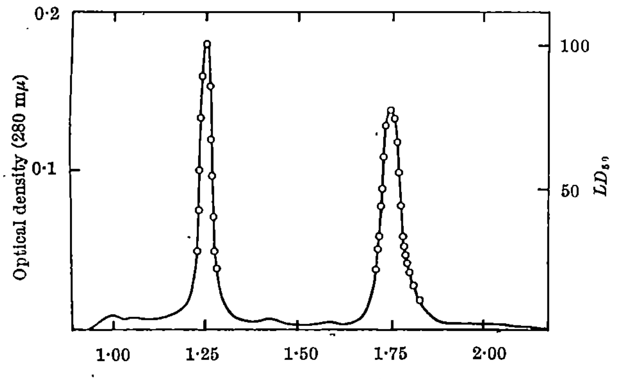

Fig. 1. Chromatographic homogeneity test of the pure toxins of *A. australis* on 'Amberlite *CG* 50' (4 cm. × 145 cm. column) equilibrated with ammonium acetate 0·2 *M*, *p*H = 6·80. Venom obtained by the manual method after filtration on 'Sephadex *G* 25'. The curve in full line corresponds to the optical density at 280 mμ. Open circles express the toxic activity in LD_{50} for the 20-gm. mouse, tested in several tubes of the separated peaks

Preliminary investigations indicate a molecular weight of 12,000 for the toxic proteins of *B. occitanus*, and a pH_i around 8·5 for the toxins of both species. The generic name of scorpamins has been given to the toxins of scorpions[2].

The venom collected by the manual technique is better for obtaining the pure toxins with a good yield (Table 2). The main characteristics of scorpamins are: (1) their pure neurotoxic effect; (2) their low molecular weight; (3) their high resistance to storage and denaturating agents. With regard to these properties, scorpamins constitute a remarkable material for the study of mechanism of action of neurotoxic proteins.

Full details of these experiments will be published elsewhere.

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Reaction of Antimony Pentachloride with Pregnane-3α, 17α, 20α-Triol, Pregnane-3α, 17α, 20α-Triol-11-One and Other Adrenal Cortical Steroids

Antimony pentachloride has been used for detecting a number of steroids on paper chromatograms[1-4]. Pregnane-3α, 17α, 20α-triol (pregnanetriol) and pregnane-3α, 17α, 20α-triol-11-one (pregnanetriolone) can be detected on paper chromatograms by the method of Finkelstein and Cox[5] in which 70 per cent phosphoric acid induces a pink fluorescence in the former and a brilliant blue fluorescence in the latter when viewed with a long-wave (3660 Å.) ultra-violet lamp. However, the phosphoric acid gradually destroys the chromatogram paper, which necessitates delicate manipulation to avoid shredding.

We have found that antimony pentachloride can be used as a detecting agent for both pregnanetriol and pregnanetriolone.

Pregnanetriol (obtained from Mann Research Laboratories, Inc., New York, N.Y., and Sigma Chemical Co., St. Louis, Missouri); pregnanetriolone (obtained from Dr. W. Klyne, Steroid Reference Collection, Postgraduate Medical School, London, and Dr. M. Finkelstein, Hormone Research Laboratory, Hadassah Medical School, Jerusalem); pregnane-3α, 11β, 17α, 20α-tetrol (pregnanetetrol), and pregnane-3α, 17α, 20β-triol-11-one (pregnanetriol β-epimer) (obtained from Dr. D. Fukushima, Sloan-Kettering Institute, New York, N.Y.) were placed on Whatman No. 1 paper in 50-μgm. amounts and sprayed with 20 per cent antimony pentachloride in chloroform (20 gm. in 100 ml.). Immediately the pregnanetriol and pregnanetetrol turned yellow in colour. The pregnanetriol darkened and turned dark brown, while the pregnanetetrol changed to a bright blue colour. Pregnanetriolone in 50-μgm. amounts gave a yellow colour. With less than 50-μgm. amounts the pregnanetriolone and the pregnanetriolone β-epimer gave no visible reaction.

The paper was then heated for 5 min. at 78° C. The pregnanetriol became blue-grey in colour, and the pregnanetetrol became brown-grey. The pregnanetriolone became yellow in colour, while the pregnanetriolone β-epimer became faint yellow. Table 1 summarizes the results. Before heating only 50 μgm. of pregnanetriolone could be detected, while at least 5 μgm. of pregnanetriol gave a colour reaction. After heating, 5 μgm. of pregnanetriolone was faintly visible.

Table 1. REACTION OF ADRENAL CORTICAL STEROIDS WITH ANTIMONY PENTACHLORIDE

Compound	Colour before heating	Colour after heating
Pregnane-3α, 17α, 20α-triol	Yellow → dark brown	Blue-grey
Pregnane-3α, 11β, 17α, 20α-tetrol	Yellow → blue	Brown-grey
Pregnane-3α, 17α, 20α-triol-11-one	No colour	Yellow
Pregnane-3α, 17α, 20β-triol-11-one	No colour	Faint yellow
Hydrocortisone	No colour	Pale yellow
Cortisone	No colour	Very faint yellow
Corticosterone	No colour	Yellow
11-Deoxy-17 hydroxycorticosterone	No colour	Grey
11-Dehydrocorticosterone	No colour	Very faint yellow
11-Deoxycorticosterone	No colour	Blue

Other steroids including hydrocortisone, cortisone corticosterone, 11-deoxy-17 hydroxycorticosterone, and 11-dehydrocorticosterone gave no colour reaction with antimony pentachloride before heating. However, after heating for 5 min. at 84° C. corticosterone gave a yellow colour, 11-deoxycorticosterone a blue colour, 11-deoxy-17-hydroxycorticosterone a grey colour, and hydrocortisone a pale yellow. Cortisone and 11-dehydrocorticosterone gave very faint yellow colours which might not be detected if one did not know exactly where the compounds were located. These results are shown in Table 1.

These reactions with antimony pentachloride have been very useful in locating these various steroids on paper chromatograms because of the simplicity of the method.

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Action Pattern of β-Amylase

CURRENT opinion with respect to the action pattern of β-amylase on amylose is divided into three divergent points of view: (1) single-chain pattern, in which β-amylase, having commenced attack on a given amylose molecule, completely hydrolyses this molecule to maltose before attacking another molecule; (2) multi-chain pattern, in which β-amylase removes a single maltose molecule from the non-reducing terminus of an amylose molecule, then completely separates from the substrate molecule and continues the attack on other amylose molecules in the same way; (3) multiple attack, in which β-amylase remains associated with a given amylose molecule long enough to remove, on the average, several molecules of maltose before attacking another amylose molecule. Substantial evidence exists which can be interpreted in favour of each of these points of view[1-6].

The principal evidence for the single-chain pattern is that at intermediate stages during action of β-amylase on amylose, the residual amylose remains essentially unchanged with respect to such properties as molecular weight, viscosity, and iodine colour[1-4]. With multi-chain or multiple attack it would be expected that the molecular weight of the remaining amylose would gradually decrease during β-amylase action. A critical feature of this interpretation, which has not been adequately considered, is the nature of the chain-length distribution of the original amylose as well as that of the amylose remaining at intermediate stages of hydrolysis. Specifically, if the molecular weight distribution of the original amylose is the 'most probable' distribution[7], any of the three

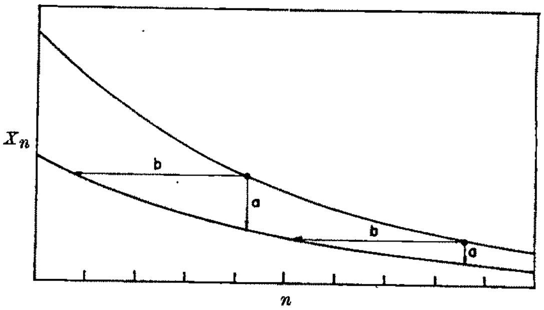

Fig. 1. Attack of 'most probable' amylose by (*a*) single-chain. (*b*) multi-chain action patterns. In (*a*) the number X_n of chains of a given length n is reduced by a given proportion. In (*b*) each chain is shortened by a given amount. The two action patterns are seen to give rise to identical distributions at intermediate stages of degradation. X_n and n are on an arbitrary scale

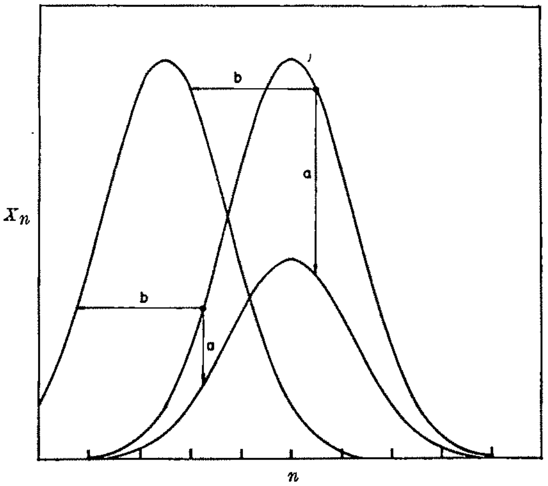

Fig. 2. Attack of 'normal distribution' amylose by (*a*) single-chain, (*b*) multi-chain action patterns. With (*a*) the distribution is unchanged but with (*b*) the average molecular weight is much reduced

suggested action patterns will lead to average molecular weights and size distributions of the residual amylose precisely the same as those of the original amylose. On the other hand, if the distribution of the original amylose is of the Gaussian or Kraemer-Lansing type, multiple attack or multi-chain action patterns would lead to products of substantially lower average molecular weight. These statements, illustrated qualitatively in Figs. 1 and 2, have been proved by exact mathematical analysis. For multiple attack the results are similar to those with the multi-chain pattern.

Most recent evidence is that native amylose corresponds to the 'most probable' distribution[8]. Moreover, while random degradation during preparation of amylose will reduce its average molecular weight, it is expected that the degraded material will still correspond to the 'most probable' distribution[7]. Hence, with native or unfractionated degraded amylose, one cannot distinguish between single-chain, multi-chain, and multiple-attack action patterns.

On the other hand, by synthesis or fractionation, it should be possible to produce high-molecular-weight amylose preparations with molecular size distributions significantly different from the 'most probable' distribution[9], and such materials are the only suitable substrates for definitive studies of the action pattern of β-amylase on high-molecular-weight amylose.

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A Difference between the Modes of Action of Substrate and Hormonal Inducers of Rat Liver Tryptophan Pyrrolase

THE level of tryptophan pyrrolase can be greatly increased in rat liver by the intraperitoneal injection of tryptophan[1], cortisone[2] or hydrocortisone[3]. The administration of adrenocortical hormones is not associated with an increased level of tryptophan in the liver[3]. Moreover, cortisone is a non-specific inducer, in that it affects the levels of several liver enzyme systems[2]. Reserpine[4] has also been shown to increase the level of activity of tryptophan pyrrolase, and since it does not elicit this response in hypophysectomized rats, it has been suggested that its effect is mediated by the pituitary–adrenal system[5]. The present work indicates a qualitative difference in the mode of action of tryptophan on one hand and cortisone and reserpine on the other in the induction of tryptophan pyrrolase.

It has been shown previously[6,7] that rat liver tryptophan pyrrolase is located in the particle-free cell sap and that microsomes, owing to their iron-protoporphyrin content, can activate the enzyme of normal rat liver to a value approximately three times that of unsupplemented cell sap. It can be seen from Fig. 1 that the addition of liver microsomes, from either cortisone-treated or normal rats, causes similar relative increases in the tryptophan pyrrolase activity of liver cell sap preparations from both normal and cortisone-treated rats. Thus, following cortisone treatment, the rise in enzyme-level is not accompanied by a significant increase in the relative amount of available activator. Similar results were obtained when using reserpine as the inducing agent. Table 1 summarizes these results and contrasts them with those obtained following treatment with tryptophan. It can be seen that whereas the ratio of unstimulated to fully stimulated cell sap tryptophan pyrrolase activity has not been changed significantly by treatment with cortisone or reserpine, it has been greatly increased by tryptophan treatment. Although the maximal activity obtainable, presumably reflecting the amount of tryptophan pyrrolase apoprotein, was the same following administration of either tryptophan or cortisone, the effective enzyme activity (in

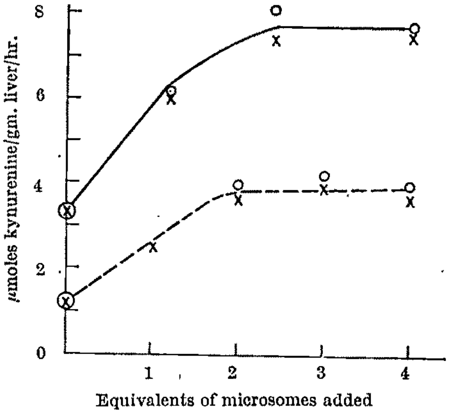

Fig. 1. Effect of microsomes on liver cell sap tryptophan pyrrolase activity of normal and cortisone-treated rats. Liver microsomes and particulate-free cell sap were obtained from normal rats and from rats killed 2 hr. after intraperitoneal administration of cortisone (10 mgm./kgm.). Normal cell sap (– – –) ; cortisone-cell sap (——) ; normal microsomes (×) ; cortisone-microsomes (○). The addition of one equivalent of microsomes restores the microsome–cell sap ratio to that of unfractionated liver homogenate

Table 1. INDUCTION OF TRYPTOPHAN PYRROLASE BY DIFFERENT AGENTS

Cortisone ('Cortisoneacetate', Merck) (0·01 gm./kgm.) and L-tryptophan (1 gm./kgm.) were injected intraperitoneally, and reserpine ('Serpasil', Ciba) (0·001 gm./kgm.) subcutaneously. The four equivalents of microsomes employed gave maximal stimulation

Substance injected	Hours post-injection	Tryptophan pyrrolase activity (μmoles kynurenine/gm. liver/hr.) Unsupplemented cell sap	Cell sap + four equivalent microsomes
—	—	1·6	5·9
Tryptophan	1	3·8	6·0
	2	8·4	8·8
	4	13·0	15·8
Cortisone	1	2·0	5·2
	2	2·8	8·0
	4	4·8	16·0
Reserpine	2	2·6	4·4
	4	3·6	7·8

the absence of added activator) was much greater following treatment with tryptophan.

Previous work with tryptophan[7] suggested that the activation of tryptophan pyrrolase provides the stimulus for induction (defined as increase in total obtainable activity). In the case of cortisone and reserpine, such an activation does not take place, indicating an alternative mechanism for the initiation of enzyme induction.

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Biosynthesis of Citrulline in Plants

IN 1930 citrulline was discovered in water-melons[1]. Later it was shown to be an important participant in the Krebs and Henseleit ornithine cycle in animals and micro-organisms. In recent years several laboratories have worked on the ornithine cycle in plants. In this laboratory citrulline has been shown to play an important part in transporting and storing nitrogen in several families of higher plants[2–4]. Relatively high concentrations of citrulline in exuding sap and citrulline accumulated in artificially rooted leaves of alder trees show the root to have a determining influence in the biosynthesis of citrulline. However, excised branches of alder and hazel could also synthesize citrulline from ammonium nitrate under aseptic conditions. Though ornithine, the precursor of citrulline, is metabolized very quickly, we could not find significant amounts of it, but very high concentrations of the mono-N-acetyl-ornithine were found in all members of the family Fumariaceae, being characteristic of this family[5]. Ornithine reacting with carbamyl phosphate in the presence of carbamyltranspherase is converted into citrulline. My experiments have shown this reaction to occur under physiological conditions even without enzyme, though converting carbamyl phosphate much more slowly to cyanate, which reacts with the amino-groups[6,7]. It was also possible to establish the biosynthesis of citrulline from ornithine and carbamyl phosphate in higher plants[8–11].

To examine the possibility of activity of carbamyl phosphate in seaweeds, different green, brown, and red algae were ground in a mortar with *tris* buffer *p*H 8 at + 4° C. The extract, being usually weakly acid, was brought up to *p*H 8 with sodium bicarbonate, centrifuged (30 min. at 7,000 r.p.m. at 5° C.), and incubated at 30° C. The reaction was stopped by freezing at – 20° C., and the lyophilized reaction mixture re-solved with 70 per cent ethanol immediately before determination. After separation on paper chromatograms the amino-acids were determined colorimetrically with ninhydrin by comparison with standards[4].

The results given in Table 1 show that green and red seaweeds are able to synthesize citrulline from ornithine and carbamyl phosphate. 70–80 per cent of the ornithine was metabolized within the period of reaction. During this time some amino-acids increased also; primarily citrulline and glutamic as well as aspartic acid in small amounts, but not arginine and urea. After 30 min. incubation I found most of the initially produced ornithine was metabolized to citrulline; and there is scarcely a difference between incubation for 30 min. or 6 hr. Before incubation I found a relatively high concentration of citrulline in *Gracilaria*; whereas citrulline synthesis does not occur in *Taonia*. For the present we must leave it undecided whether this lack of citrulline synthesis is dependent on the developmental state of the plant, or whether it is specific only to *Taonia* or for all brown algae, having a special place in the biochemistry of seaweeds.

Table 1. BIOSYNTHESIS OF CITRULLINE IN SEAWEEDS

The complete system contained: 10 μmoles ornithine, 10 μmoles carbamyl phosphate (CP), 5 μmoles magnesium chloride, 1 ml. plant extract (= 1·5 gm. fresh weight of algae extracted and made up to 1 ml. with *tris* buffer *p*H 8)

	μMoles citrulline/1 ml. plant extract			
	Complete system	Complete system	Without CP	Without ornithine
Time of incubation (hr.)	0·5	6	6	6
Green algae				
Cladophora prolifera	6·85	8·60	0	0
Ulva lactuca	3·43	4·00	0·80	0
Red algae				
Neomonospora furcellata	6·29	6·85	1·60	0·69
Gracilaria conferoides	12·00	14·85	8·60	6·58

This work was carried out at the Stazione Zoologica. Napoli.

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A New Preparation of Gastrin: Preliminary Characterization

THIS Laboratory is examining the chemistry of 'gastrin', about which little has been reported. We have recently prepared a gastrin-like secretory stimulant from porcine antral mucosa by a new and somewhat novel method. This is a preliminary report on its chemical properties, and similar data on 'gastrin' as prepared by a more conventional procedure. The quotation marks signify only that it is not known whether gastrin is a single compound, or a group of similar structures with a common unique moiety, or a group of biologically, more or less, specific substances. The facts that gastrin has been reported as dialysable[1], non-dialysable[2], with an isoelectric point near *p*H 5·0 [3], or *p*H 8·0 [4], to name only a few of its properties, leave the question very much open. Recently, Gregory *et al.*[5] described a gastrin-like substance isolated from a pancreatic tumour.

Different from any method we have found in the literature of this substance, we have extracted gastrin from hog antrum with aqueous alkali, *p*H 11–12, at 40° C. Subsequent steps included isoelectric precipitations at *p*H 5·0, and precipitations in sodium chloride solution. At no time was the preparation more acid than *p*H 4·0, or warmer than 40° C. Dialysis of the crude material against running water and several changes of distilled water followed by lyophilization or isoelectric precipitation gave yields of 0·5–2·0 gm. per 20 antral mucosæ.

At this stage we have had consistently good stimulation. Solutions of 75–100 mgm. (as little as 50 mgm. has produced good response) in 50 ml. of sterile saline were injected during $\sim$30 min., using a motor-driven syringe. A sharp rise in secretory flow and of $[H^+]$ resulted, followed by a decline after the injection. For the half-hour of greatest activity, eleven experiments gave us an average rate of secretion of 12·4 ml./hr., and a rate of hydrogen ion production equivalent to 18·0 ml. of 0·01 *N* hydrochloric acid/hr. 80 per cent, or more, of these amounts represented increase over the base line. Our best eight experiments with material prepared by a modification of the Uvnäs method[3] gave comparable figures of 5 ml. (volume) and 10·6 ml. (0·01 *N* hydrochloric acid equivalent.

In our previous work on dialysed gastrin, as prepared by the Uvnäs method, we had often noted vomiting, or an inactive preparation, or acquired tolerance. In the dialysed material from the new method we have not observed such symptoms, and we have had no inactive preparations. Of considerable additional interest are the excellent results obtained with subcutaneous injections. A good flow persisted for 2½–3 hr. after the injection, contrasting with the rapid decline after histamine injection. One such active sample had been kept dry and in the dark at −20° C. for 8 months before the assay.

In the material prepared by acid extraction and trichloracetic acid precipitation[3] we had found a small but consistent content of phosphorus (average 0·05 per cent). We have found 10–15 times as much phosphorus in the new preparation (dialysed), that is, 0·5–0·8 per cent. Treatment with 0·25 *N* alkali at 27° C., isoelectric precipitation and re-dialysis did not lower the phosphorus content appreciably, making the presence of a typical phosphoprotein unlikely[6].

In the ash of the dialysed gastrin, as the only unique constituent in measurable quantity, we have consistently found tin. On standing in dilute stannous chloride and prolonged re-dialysis, the gastrin retained considerable metal ion (ash $\sim$1 per cent, of which $\sim$20 per cent was tin).

We have subjected the dialysed gastrin to diethylaminoethylcellulose ion-exchange treatment, Tiselius electrophoresis (one main component, through the kindness of Dr. P. D. Goldsworthy), and curtain electrophoresis with veronal buffer (*p*H 8·6, ionic strength 0·02) on Whatman 3-*MM* paper at 300–500 volts. The most interesting results have come from the last method. Electrophoresis of a 1 gm. sample of dialysed material led to recovery of 65 per cent (redialysed and lyophilized material). One main protein fraction was observed ($\sim$40 per cent) which migrated (minus to plus) a moderate distance, and gave strong colour with tetrabromophenolphthalein ethyl ester (potassium salt)[7]. Displaced further toward the positive pole, there was a smaller fraction (average 2–3 per cent after dialysis), which gave no protein colour test, and another protein-like material ($\sim$1 per cent). The phosphorus content of the main protein fraction was low (0·05 per cent or less); that of the non-protein fraction was high (4–7 per cent). The main fraction had an ultra-violet spectrum typical for proteins. The ultra-violet spectrum of the non-protein fraction had a sharp intense peak at 260 mμ. Much of the remaining phosphorus, which had not been accounted for, appeared to be on the curtain in the path of the main protein fraction. The latter appears to be bound to the high-phosphorus fraction somewhat tenaciously.

The protein after electrophoresis had considerably less secretory stimulant activity than the material before electrophoresis and appeared to be less stable. The dialysed gastrin and the main protein fraction on electrophoresis have each given a 10 per cent yield, or more, of crystalline material, as long fibrous needles, by an ammonium sulphate procedure. Full details of the extraction procedure, the chemical properties and our experience in tracing the activity will be presented shortly.

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PHYSIOLOGY

Crystallization of Purines in the Vacuole of *Candida utilis*

We have reported previously that *Candida utilis* removes seven purines (adenine, guanine, hypoxanthine, xanthine, uric acid, 2,6-diaminopurine and *iso*guanine) from the medium by an active transport process when these compounds are used as sources of nitrogen for the yeast[1,2]. These compounds and their metabolic products are held within the cell in high concentrations that appear to exceed the solubilities of the compounds in the volume of water available. For example, 5 gm. (wet weight) of yeast that had taken up 450 mgm. of uric acid was dried to constant weight, samples of the dried yeast were heated repeatedly in buffer at 100° C. to extract the uric acid, and the extracts were analysed with purified uricase by the method of Kalckar[3]. It was found that the dried yeast contained 16 per cent uric acid by weight and that 35 per cent of the uric acid administered was recovered in the extracts. It is presumed that the remaining 65 per cent of the uric acid had been metabolized via the yeast uricase. The wet yeast contained 82 per cent water, so that the concentration of uric acid would be about 35 mgm./ml. of water in the wet yeast. The solubility of uric acid is given as 2·53 mgm./100 ml. at 20° C. (ref. 4) and therefore the presence of crystalline uric acid in the yeast cell would be expected with the high concentrations that were observed.

Microscopic examination of yeast cells that had accumulated purines revealed the presence of crystals in the vacuole. These were most evident in the case of *iso*guanine, which crystallized within the cell in needles that usually lay along the long axis of the cell and appeared to extend from wall to wall. To confirm the presence of purine crystals, yeast containing the various purines was treated with dilute silver nitrate to form the silver purines, washed, and treated with dilute sodium sulphide. The crystals became more striking because of blackening due to the formation of silver sulphide on the crystal surfaces; this was not observed with control yeast. Finally, when examined in the crossed field of a polarizing microscope, the crystals were seen as dots or lines of light that exhibited a twinkling effect due to Brownian movement in the case of smaller crystals. Cells that had not taken up purines remained dark in the crossed field.

It was found previously that the accumulated purines with the exception of *iso*guanine were slowly metabolized by the yeast[1,2]. Since it was considered possible that the presence of the crystals within the cell might disrupt the internal structure and interfere with normal growth, cells that contained *iso*guanine crystals or amounts of crystalline uric acid that occupied much of the vacuole were placed on nutrient agar on microscope slides and observed during the course of a day. Normal buds formed and grew from the cells containing either crystalline *iso*guanine or uric acid. Thus, it appears that growth and metabolic processes occur when the crystals are present, and it is evident that the uric acid crystals must slowly re-dissolve and be metabolized, because uric acid gradually disappears from the cells[1]. It is of interest to note that the accumulation of S-adenosylmethionine in the vacuole of *C. utilis* occurs without the appearance of crystals[5].

The crystallization of purines in *C. utilis* provides a useful system for the study of crystallization and crystal growth and dissolution processes within the living cell. The effect of purine crystallization on the active transport of the purines and on the induced formation of enzymes of purine metabolism in yeast is being studied. This investigation was supported by Research Grant *CY*2510 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

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Plasma Clot Dissolution by Urea Derivatives

A method for forming standard human plasma clots has been described in a previous communication[1]. This method allows an accurate measurement of the clot-dissolving activities of various compounds applied in solution to a standardized invariable surface. In this previous work it was found that when a plasminogen activator, such as urokinase, was applied to the preformed clot, it produced a progressive fibrinolysis. Afterwards it was observed that the presence of 'Tween 20' in the activator solution enhanced the activator-induced fibrinolysis. The question then arose as to whether or not denaturation of fibrin by any compound would enhance the fibrinolytic process. The first group of compounds studied, and that reported in this communication, was urea and urea derivatives. Urea, itself, is a well-known denaturing agent. It enhances proteolysis in lesser concentrations[2] and dissolves fibrin in greater concentrations[3].

All compounds studied with the standard clot were dissolved in human defibrinated plasma at various concentrations. These plasma solutions were then applied to the preformed standard clots (obtained by recalcifying of plasma) either in the presence of various concentrations of urokinase or in the absence of urokinase. The progress of the clot lysis at 37° C. was noted at intervals and expressed as percentage of clot lysed.

A number of urea derivatives were found which enhanced the urokinase-induced fibrinolysis of the preformed standard clots. The degree of enhancement of the urokinase-induced fibrinolysis was not the same for each compound. Among the compounds examined were ethyl urethan, methyl urethan, allylthiourea, urethan and urea. They are listed in order of activity with ethyl urethan showing the greatest enhancement and urea showing the least. The presence of ethyl urethan, methyl urethan and allylthiourea, at concentrations as low as 2 per cent, in solution with an ineffective amount of urokinase, still promoted fibrinolysis.

Controls for experiments using concentrations greater than 2 per cent revealed that some of the derivatives were dissolving the preformed plasma clot in the absence of urokinase. The effective concentrations were considerably lower than the

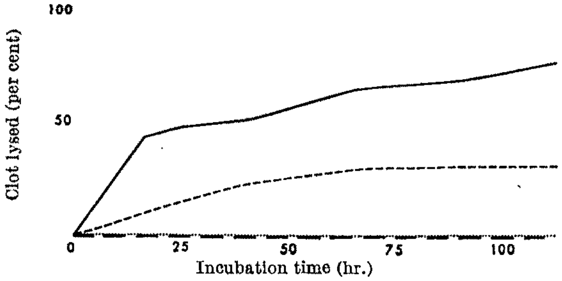

Fig. 1. Rate of dissolution of human plasma standard clots by urea and urea derivatives. ——, 0·4 *M* ethyl urethan; – – –, 0·4 *M* methyl urethan; . . . ., 0·4 *M* urethan; — —, 0·4 *M* urea

urea concentration required to dissolve fibrin clots. Fig. 1 shows a typical example of the dissolution of preformed plasma clots by urea derivatives in the absence of urokinase.

In the experiment represented in Fig. 1, urea and urethan were ineffective. It showed that the introduction of a methyl group into the urethan molecule, however, produces a clot-dissolving substance. Still greater clot-dissolving activity is obtained with the introduction of an ethyl group.

Further work with ethyl urethan revealed that plasma could be clotted after the addition of ethyl urethan in concentrations of 3 per cent or less. As with the preformed clots, it was noted that the fibrinolysis-inducing activity of urokinase is considerably enhanced under these conditions by the presence of ethyl urethan. An example of this is shown in Table 1, where ineffective concentrations of both urokinase and ethyl urethan are combined to produce lysis in a relatively short time.

Table 1. Enhancement of Urokinase Activity by Ethyl Urethan in Human Citrated Plasma

(All clots were formed with thrombin)

Plasma plus urokinase, 15 μgm./ml.	Plasma plus ethyl urethan, 0·5 per cent	Plasma plus urokinase, 15 μgm./ml., and ethyl urethan, 0·5 per cent
No lysis in 24 hr.	No lysis in 24 hr.	Lysis in 90 min.

When 2 or 3 per cent of ethyl urethan was incorporated into plasma clots, in the absence of urokinase, it dissolved the clot within a period of 16–30 hr. This 'chemical' lysis occurred regardless of the clotting technique (spontaneous, recalcification, or thrombin). Representative examples of dissolution of clots spontaneously formed in the presence of ethyl urethan are shown in Table 2. The human plasma was obtained by the silicone technique and afterwards permitted to clot in glass tubes. A firm clot was produced in each case.

Table 2. Dissolution of Human Plasma Clots formed spontaneously in the Presence of 2 per cent Ethyl Urethan

	Clotting time (min.)	Clot dissolution time (hr.)
Plasma *A*	15	23
Plasma *B*	19	22
Plasma *C*	10	30

As a working hypothesis and in analogy with the well-known protein-dissolving properties of higher urea concentrations, it is thought that ethyl urethan and other urea derivatives weaken the fibrin network by denaturation (breaking of hydrogen bonds). Denaturation is known to facilitate enzymatic hydrolysis. It has also been suggested that denaturation is a pre-requisite of any enzymatic degradation of proteins[4]. This would explain the marked enhancement of urokinase-induced fibrinolysis by these urea derivatives. This concept might also hold true for clots formed in the presence of ethyl urethan, but in the absence of urokinase which undergo subsequent dissolution. In this case the fibrin denaturation could have been carried to a point which permitted the endogenous 'masked' fibrinolytic activity of plasma[5] to dissolve the fibrin. We also have suggestive evidence that the urea derivatives described actually activate plasma plasminogen in the presence of the plasma factor (pro-activator ?)[6].

Urea at 30 per cent dissolves fibrogen clotted by thrombin, but does not dissolve clots formed in the presence of a stabilizing serum factor and calcium[7]. Urea was ineffective at the concentrations used in this study of urea derivatives. The urea derivatives found to dissolve plasma clots did so regardless of the method used for clotting and at concentrations of 2 or 3 per cent. This indicates that the mode of action of these compounds is quite different from that of urea.

There were relationships between the chemical structure of the derivatives and their clot-dissolving power. This relationship could be a useful starting point in the search for more powerful 'chemical' clot-dissolving agents. Further work is in progress in our laboratories.

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Clotting-Time of Cold-exposed and Hibernating Hamsters

Prolonged blood-clotting in hibernating hamsters has been reported by Svihla *et al.*[1]. In another experiment with ground squirrels[2], it was concluded that the prothrombin-level decreased during hibernation, but the use of the one-stage prothrombin test for calculating prothrombin concentrations is questionable[3]. Suomalainen and Lehto[4] found that both naturally and artificially dormant hedgehogs had an increased clotting-time and increased heparinocyte counts of the small intestine and bronchioles.

The Syrian hamster (*Mesocricetus a. auratus*) requires several weeks of exposure to cold before entering hibernation. As a result, it has been possible to ascertain whether an increased clotting-time occurs gradually during the weeks of acclimation or is a phenomenon immediately preceding hibernation.

The hamsters were kept in a cold room at 5 ± 1° C. for varying periods of time. Each group contained ten animals, except for the hibernating group in which there were three animals. Each hamster had a separate cage with 'Purina' laboratory chow and water *ad libitum*. The first group lived in the cold

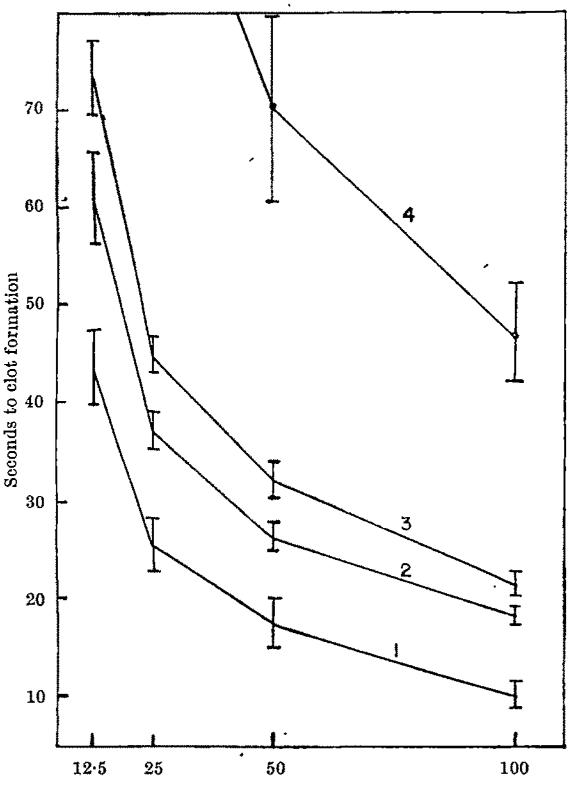

Fig. 1. Clotting-time with Russell's viper venom of various dilutions of plasma from hamsters exposed to cold (5 ± 1° C.) for (1) no weeks, (2) two weeks, (3) eight weeks, and (4) in hibernation

for 2 weeks, the second group for 8 weeks, and the hibernating animals had been in the cold for 12 or more weeks. The control animals were taken from the stock room at 22 ± 1° C. The animals were lightly anæsthetized with nembutal and the blood taken by cardiac puncture into a siliconed needle and syringe. The clotting times of 100, 50, 25 and 12·5 per cent plasma were determined in siliconed glassware at 37° C., using Russell's viper venom for the one-stage prothrombin time test.

In Fig. 1 the means of each group at the various dilutions of plasma are plotted with the vertical lines representing the standard errors. The Russell's viper venom time increased for each dilution as the cold exposure time of the animals increased. The increases in clotting-time from group to group for the various dilutions are relatively similar except for the hibernators. By 8 weeks of cold exposure the clotting-times are approximately between those of the control group and the hibernating group. This indicates that there has been a steady increase or decrease in some component of the plasma which affects the efficiency of Russell's viper venom. According to Fullerton and Anastasopoulos[5], Russell's viper venom is relatively insensitive to alterations in plasma prothrombin. De Beer[6] has shown that viper venom reveals both the intensity and duration of the anticoagulant activity of heparin. Therefore, the increased clotting-time of Fig. 1 cannot be explained as a reduction in prothrombin only and may reveal an increase in heparin.

Table 1. CLOTTING-TIME OF 100 AND 25 PER CENT PLASMA WITH RUSSELL'S VIPER VENOM AND THROMBOPLASTIN AND THE HEPARIN CONCENTRATION OF THE PLASMA OF HAMSTERS EXPOSED TO COLD (5 ± 1° C.)

Weeks in the cold 5 ± 1° C.	No. of animals	100 per cent plasma		25 per cent plasma and thromboplastin	
		Venom clotting-time (sec.)	Thrombo-plastin clotting-time (sec.)	Normal clotting-time (sec.)	Plasma heparin (γ/ml.)
0	8	14·1 ± 1·8	14·5 ± 1·4	23·6 ± 4·0	7·4 ± 1·4
8	7	25·1 ± 2·7	18·9 ± 3·2	22·4 ± 2·5	14·0 ± 1·6
12 (Hibernating)	3	47·3 ± 5·5	30·0 ± 4·5		

The heparin content of the blood from control hamsters and hamsters exposed to cold (5 ± 1° C.) for 8 weeks was determined by a protamine titration technique[7]. The test was modified by adding 5γ rather than 10γ of exogenous heparin (Connaught Laboratories) to the plasma. The results are summarized in Table 1. The concentration of heparin, as measured by this test, was increased twofold in the blood of hamsters exposed to cold for eight weeks. With 100 per cent plasma there was no significant difference in the clotting-time between the venom and brain thromboplastin. After 8 weeks in the cold, the clotting-time was more prolonged with venom but not with thromboplastin. The clotting-time of 100 per cent plasma as determined previously on hibernating hamsters was very significantly increased with both venom and thromboplastin. With 25 per cent plasma and thromboplastin, there was no increase in the clotting-time at 8 weeks, although as seen from Fig. 1 there was a significant increase at this dilution with venom. Some plasma component to which Russell's viper venom, but not thromboplastin, is very sensitive significantly altered in 8 weeks of cold exposure.

Clotting-time with Russell's viper venom is affected by the concentration of plasma lipid, and plasma deprived of lipid will not clot with this venom[5]. In view of this, the present results indicate that the concentration of plasma lipid altered during the prolonged cold exposure. This lengthened the clotting-time with the venom and had only a slight effect on the activity of brain thromboplastin. The accompanying increase in plasma heparin is probably related to the change in plasma lipid since heparin is believed to be involved in fat transport[8]. The highly significant increase in clotting-time during hibernation probably arises from a combination of reduced prothrombin synthesis, resulting from a general lowering of body metabolism, and a high concentration of some heparinoid substance in the plasma.

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Bile Pigment Excretion in Amphibia

In man and in other mammals, bilirubin is excreted in conjugated form, mainly as the glucuronide. Since in mammalian fœtal liver the enzymatic mechanism for glucuronide formation is poorly developed, reaching its full capacity only after birth, transient hyperbilirubinæmia is frequently observed during the neonatal period[1]. In the amphibian genus, *Rana*, hepatic glucuronide formation is absent in the larva, but present in the adult[2]. The larval forms undergo extra-uterine development and hence are readily accessible. These Amphibia would represent an excellent experimental model for studying various aspects of neonatal hyperbilirubinæmia, if it could be established that they, like mammals, excrete bilirubin as glucuronide. Therefore, we have examined amphibian bile pigment excretion to determine, first, whether in the adult *Rana* bilirubin is excreted as a glucuronide; secondly, whether metamorphosis is associated with qualitative or quantitative changes in pigment excretion; and thirdly, whether the excreted pigment is biliverdin, as has been postulated previously[3], bilirubin, or another pigment.

Larval forms of *Rana catesbiana* and adult forms of *Rana pipiens*, *Xenopus laevis*, and *Necturus maculosus* were examined. The last two remain aquatic throughout adult-hood; in addition, *N. maculosus* fails to metamorphose while achieving sexual maturity. As expected[2], hepatic glucuronyl transferase activity was demonstrable only in adult *R. pipiens* but was lacking in the liver of the larvæ, and the two adult aquatic Amphibia (Table 1).

Table 1. ENZYMATIC SYNTHESIS OF *o*-AMINOPHENYL GLUCURONIDE BY LIVER HOMOGENATE FROM AMPHIBIAN SPECIES

Species	Sex	No. of assays and incubation temperature (°C.)	*o*-Aminophenyl glucuronide formed: Average	Range
R. catesbiana, larvæ	M, F (pooled)	2; 21	not demonstrable	
R. pipiens, adults	M	6; 37	0·363	0·279–0·412
		6; 21	0·290	0·260–0·344
X. laevis, adults	M	4; 37	not demonstrable	
		2; 21	not demonstrable	
N. maculosus, adults	M	2; 37	not demonstrable	
		2; 21	not demonstrable	

Liver was homogenized with 5 parts of 0·154 *M* potassium chloride. 1 ml. of homogenate was incubated for 30 min. in air with 30 μM magnesium chloride, 1·0 μM uridine diphosphate glucuronic acid, 0·125 μM *o*-aminophenol and 0·1 ml. 0·5 *M* phosphate buffer *p*H 8·0; final incubation mixture was 1·5 ml. (ref. 6). Results are expressed in μmoles per gm. of liver.

The gall-bladder bile of adult *R. pipiens* invariably contained bilirubin (Table 2) and frequently biliverdin[4], but on paper chromatographic analysis[5] bilirubin glucuronide could not be demonstrated. The bile of *X. laevis* and *N. maculosus* usually exhibited high concentrations of bilirubin (Table 2) and occasionally some biliverdin. The bile of *R. catesbiana* larvæ, though yellow or green in colour, contained only trace amounts of bilirubin and biliverdin and gave a negative Gmelin reaction. Similarly, in adult *R. pipiens*, *X. laevis* and *N. maculosus*, but not in larvæ of *R. catesbiana*, urobilinogen was present in the intestinal tract. The serum of all adult species examined was colourless or only faintly yellow. Larval serum, on the other hand, was intensely yellow with peak absorption at 4600 Å., suggesting the possible presence of bilirubin. The diazo reaction, however, gave only insignificant spectrophotometric readings, and it was not possible to establish that the yellow pigment was a bilirubinoid compound.

Table 2. BILE PIGMENTS IN AMPHIBIAN BILE AND INTESTINAL CONTENT

Species	Gall bladder: Weight (mgm.)	Gall bladder: Bilirubin content* μgm. per gall bladder	Intestinal urobilinogen†
R. catesbiana, larvæ	19·4–33·5	0–0·2	absent
R. pipiens, adults	17·6–42·6	9·3–47·0	present
X. laevis, adults	72·6–75·8	1·6–43·0	present
N. maculosus, adults	198·0–230·0	53·0–54·0	present

* Bilirubin was determined by the diazo reaction (ref. 7).

† Urobilinogen was identified spectroscopically with *p*-dimethylaminobenzaldehyde (ref. 4).

These observations indicate that, in adult Amphibia, biliary excretion of bile pigment is independent of the presence of a functioning mechanism for glucuronide formation. Moreover, in adult *R. pipiens* no evidence was obtained for excretion of bilirubin as a glucuronide, even though glucuronyl transferase is present in the liver. In larvæ, the finding of yellow serum, the virtual absence of bilirubin and biliverdin in the bile, and the absence of urobilinogen from the intestinal tract suggest a mode of bile pigment excretion different from that in adult animals. Finally, in contrast to earlier reports[3], the bile of all adult animals contained bilirubin while biliverdin could not be demonstrated in all instances.

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Fractionation of the Motor Unit during Repetitive Response to Prostigmine

Recent work has demonstrated fractionation of the action potential of the single motor unit of the rat gastrocnemius into anatomically unidentified sub-units[1]. The temporal sequence of firing of these sub-units can be altered by intravenous administration of curare or decamethonium. These drugs, by reducing the end-plate potential, increase the latency of appearance of the propagated spike of some, but not all, of the sub-units which comprise the action potential of the single motor unit. When the time of rise for the end-plate potential of the sub-unit is sufficiently prolonged, the propagated spike of the sub-unit disappears[2], and the total unit potential is reduced. In order to evaluate the effect of prostigmine on the single motor unit, the work reported here was undertaken.

Under intraperitoneal sodium pentobarbital anæsthesia, laminectomy was performed in 18 male albino rats, weighing 250–400 gm. With binocular magnification of × 25, a ventral root innervating the gastroc-

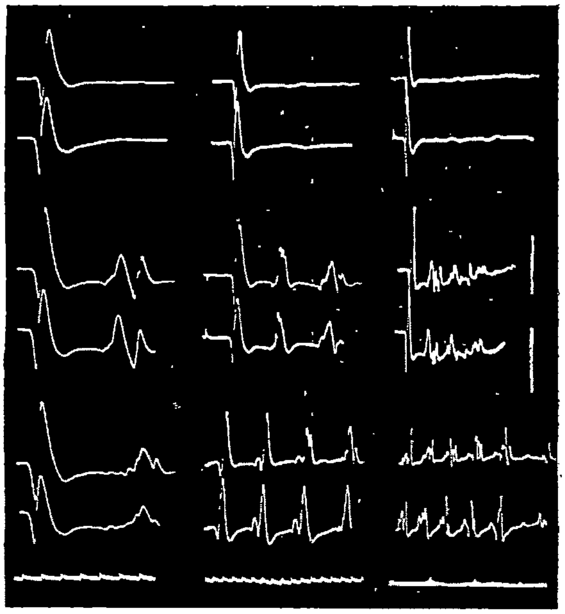

Fig. 1. Response of motor unit to prostigmine. Upper pair of traces, control responses of single motor unit of gastrocnemius to single stimulus delivered to ventral root filament. Middle and lower pairs of traces, random frames following intravenous administration of 0·02 mgm. prostigmine. Rhythmic repetitive responses to a single stimulus delivered to a ventral root filament occur at 4 msec. intervals. The second and subsequent responses reveal decreased amplitude and increased duration secondary to the asynchrony of firing of the 'sub-unit'. Time: cols. 1 and 2, 1 msec.; col. 3, 10 msec. Calibration, 0·1 mV. upper channel; 0·2 mV. lower channel

nemius was separated from the spinal cord and teased into fine filaments, one of which was stimulated with a rectangular pulse of 0·02–0·05 msec. delivered from a Grass 3*C* stimulator. A single motor unit on the surface of the gastrocnemius was identified with two pairs of bipolar recording electrodes made of enamel-insulated copper wire of 140μ diameter, was amplified with a low-level a.c. pre-amplifier and displayed on a dual-beam oscilloscope. Spinal cord, nerve roots and gastrocnemius were maintained at body temperature in small mineral oil pools. The presence of a single unit was determined by a sharp threshold of stimulus strength, below which no response appeared, and above which response form remained unaltered. A series of control responses was photographed at a stimulus strength of at least three times threshold, and intravenous prostigmine methylsulphate was administered in doses of 0·012–0·0375 mgm. The response of the motor unit was followed photographically every 3–6 sec.

Within 38–150 sec. following administration of the drug, multiple responses to the single stimulating shock appeared (Fig. 1). The second and subsequent responses showed fractionation of the action potential of the single unit into component sub-unit potentials, similar to the fractionation that had been demonstrated in the single response to a stimulus following administration of a blocking agent. The duration of the initial response remained at control value following administration of prostigmine; the amplitude of the initial response either remained at control value or increased to a maximum of 27 per cent (Fig. 1, channel *A*) until late in the experiment when a fall of amplitude was ascribed to deterioration of the preparation.

In three animals doses of prostigmine ranging from 0·25 to 1·0 mgm. were used. Repetitive responses failed to appear following this larger dose, and no change in duration or form of the motor unit response occurred. Fasciculation of the gastrocnemius was always visible at the time of onset of repetitive responses, but was not apparent following the larger dose of prostigmine.

Repetitive response of eserinized muscle was described by Brown[3], and has been thought to result from prolonged local potentials at the end-plate[4]. Riker *et al.*[5], and more recently Werner[6], have suggested the repetitive response of muscle to a single impulse in nerve results from the physiological independence of the nerve terminals. Following administration of quaternary ammonium derivatives or physostigmine, single or repetitive post-synaptic orthodromic responses were associated with repetitive antidromic axonal responses. These authors suggest initiation of the repetitive activity at the nerve terminal. The present findings, which demonstrate fractionation of the repetitive responses, would indicate either that the generation of repetitive impulses in the nerve terminals following administration of prostigmine is asynchronous, or that the effect of the drug on the subneural apparatus of a single motor unit permits asynchronous firing of the sub-units. The nature of the sub-unit is still unknown.

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Effect of Artificial Fever in Increasing Susceptibility to Bacterial Endotoxin

THERE is remarkably little information concerning the effect of fever on mechanisms of resistance to infection[1]. Indications of a potentiating effect of high ambient temperatures on mortality in mice challenged with endotoxin or with Gram-negative bacilli have been recorded[2–4]. Recently, Bannister[5] has observed that high ambient temperatures potentiate the febrile response to pyrogenic doses of endotoxin in human volunteers. Burack has observed that the Shwartzman response in the skin of rabbits is also conditioned by the environmental temperature[6]. Work on the effect of environmental temperature on the lethality of endotoxin was therefore undertaken.

Webster–Swiss strain mice, weighing 9–12 gm., were given graded doses of *S. typhosa* endotoxin intraperitoneally. The endotoxin, extracted as previously described[7], was dissolved in sterile saline. No striking sex difference in susceptibility to endotoxin was observed. However, in each experiment mice of a single sex were used. When mice were maintained in an air-conditioned room at 22° C., the LD_{50} of the endotoxin varied between 400 and 800 μgm. When mice were placed in a well-ventilated incubator at 37° C. for 24 hr., and then given endotoxin and placed back at 22° C., the LD_{50} was not significantly altered (Table 1). Mice that were maintained at 22° C., given endotoxin and placed immediately at 37° C., had an LD_{50} of but 21 μgm. (range 11–23 μgm. in repeated experiments). Mice pre-incubated at 37° for 24 hr., given endotoxin and then returned to the 37° C. environment had an LD_{50} of less than 1 μgm. Deaths were not observed in control animals injected with saline and maintained at either temperature. Thus, the effect of raising the ambient temperature was to increase susceptibility to the lethal action of endotoxin.

Table 1. EFFECT OF ENVIRONMENTAL TEMPERATURE ON LETHAL ACTION OF BACTERIAL ENDOTOXIN IN MICE*

Environmental temperature		
Pre-injection	Post-injection	LD_{50} (μgm.)
22° C.	22° C.	500
37	22	732
22	37	21
37	37	$<0·96$

* Female mice weighing 9–12 gm.

The mode of death differed in accordance with the environmental temperature. At 22° C. there was a lag period of several hours, with no apparent evidence of distress, followed by increasing tremulousness and gradual suppression of activity, with death occurring 12–18 hr. after administration of endotoxin. At 37° C., on the other hand, mice appeared quite well for 2–4 hr., then suddenly developed convulsive seizures with collapse and death occurring shortly thereafter.

In order to determine the effect of environmental temperature on the body temperatures of the animals, rectal thermistor probes in fine plastic catheters were inserted, and the mice were restrained from interfering with the probes by immobilization in loose envelopes constructed of ¼-in. open-mesh plastic screening. These envelopes permitted a moderate amount of muscular activity and free exposure to the ambient air. Temperatures were recorded every 15 min. using an electronic recorder, and observations were made for three hours.

Hypothermia was invariably observed in mice kept at 22° C. and given endotoxin, as has been observed by others[8]. Uninjected mice varied in body temperature but consistently increased their body temperatures when placed in an incubator at 37° C. The increased body temperature was sustained during the three hours of incubation, reaching a maximum at about 1 hr. and remaining relatively constant thereafter. However, when mice were given a large dose of endotoxin (1 mgm. per mouse) and placed at 37°, the febrile response was invariably greater than that observed in control animals given saline and kept at the same temperature. Similarly, mice that were given endotoxin after having been kept in the incubator for two hours underwent a still further rise in temperature during the next few hours, whereas animals given saline alone maintained relatively constant temperatures with continued incubation. About 2/3 of the animals receiving 1 gm. of endotoxin died during the 3-hr. period of observation at 37° C., and deaths coincided with rapid elevation of body temperatures to values greater than 41·5° C.

These experiments indicate: (*a*) that elevation of environmental temperature increases susceptibility to the lethal action of endotoxin; (*b*) that careful control of body temperature in experiments employing lethal doses of endotoxin is essential; (*c*) that mice may develop fever when given endotoxin if their body temperatures have initially been elevated; (*d*) that the increased susceptibility to endotoxin and the sudden death seem to be related to the increased body temperature of the host animal.

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HÆMATOLOGY

A New Hæmoglobin Variant (the 'Honolulu Type') in a Chinese

RECENTLY several hæmoglobin variants have been described with electrophoretic mobilities between those of hæmoglobins *G* and *S* in paper electrophoresis, veronal buffer, *p*H 8·6, namely, types *L* (ref. 1), *P* (refs. 2 and 3), and Stanleyville I (refs. 4 and 5). One case of hæmoglobin *G* was found in a Chinese[6]. The present report deals with a new hæmoglobin variant in a Chinese, similar to *L*, *P* and Stanleyville I in paper electrophoresis, but distinguished from them by chromatography or free electrophoresis.

The variant was found in a forty-three-year-old unmarried woman, J. C., a resident of Honolulu, whose condition was termed 'anæmia' by a local physician about three years ago. The initial red blood cell count was 4·5 million per mm.³ and the hæmoglobin 10·4 gm. per cent; after iron therapy the red cell count increased to 6·0 million and the hæmoglobin to 12 gm. per cent. About seven months ago, J. C. consulted another physician for the complaints of

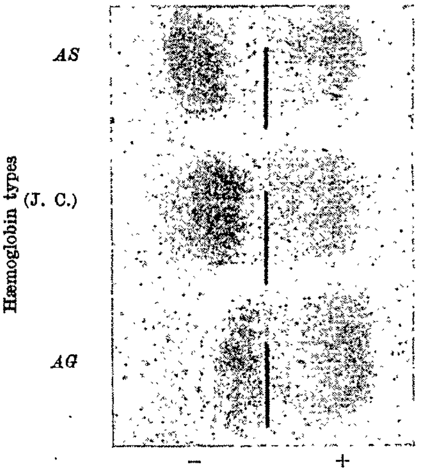

Fig. 1. Paper electrophoresis pattern of J. C.'s hæmoglobin sample compared with *AS* and *AG*, veronal buffer, *p*H 8·6, μ 0·05

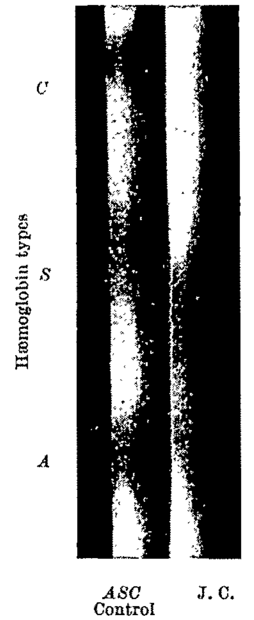

Fig. 2. Chromatography of hæmoglobin of J. C., 'Amberlite *IRC*-50', citrate buffer, *p*H 6·0, compared with *ASC* control

fatigue, pruritus ani, and anxiety ; at that time sternal bone marrow puncture revealed active erythropoiesis, with normally cellular marrow, but deficient in iron stores. Two months later (after iron therapy) she was examined by one of us (R. T. S. J.), who obtained the following laboratory data : red blood cells, 4·8 million, hæmoglobin 12·6 gm. per cent, reticulocytes 1 per cent (Dameshek method), with slight anisocytosis, poikilocytosis, occasional microcytic hypochronic cells and occasional target cells. The serum bilirubin value was 0·3 mgm. per cent total and 0·1 mgm. per cent indirect. Coombs tests, both direct and indirect, were negative. The quantitative osmotic fragility curve showed a shift to the right, beginning at about 0·45 and being completed at about 0·2 per cent sodium chloride. There were no noteworthy findings on physical examination.

In paper electrophoresis, veronal buffer, *p*H 8·6, J. C.'s hæmoglobin sample resolved into two components (Fig. 1), one identical with hæmoglobin *A* and the other moving between hæmoglobins *G* and *S*. In chromatography on 'Amberlite, *IRC* 50', citrate buffer, *p*H 6·0 (ref. 7), J. C.'s hæmoglobin sample slowly separated into two components, one identical with hæmoglobin *A* and the other similar to *S*, but slightly faster, and with considerable 'tailing' towards *A* (Fig. 2). In contrast, hæmoglobin *L* moves between *S* and *C* in this method, and *P* does not separate from *A*, while Stanleyville I is indistinguishable from *S*.

In free electrophoresis, cacodylate buffer, *p*H 6·5, μ 0·1, J. C.'s hæmoglobin sample migrated as one sharp peak. In a 1 : 1 mixture of this sample with one from a patient with sickle-cell anæmia there developed two poorly separated components with 63 per cent in the leading one. This electrophoretic behaviour is similar to that previously observed for hæmoglobin *AP* ; in contrast, Stanleyville I moves like *S* in this method.

In electrophoresis in agar gel, citrate buffer, *p*H 6·0 (ref. 8), J. C.'s hæmoglobin sample moved like hæmoglobin *A* ; however, when large amounts of sample were applied, to the extent of 'overloading', there were two components, like *A* and *G* respectively. In quantitative estimations of this sample in starch block electrophoresis, veronal buffer, *p*H 8·6, the percentage of hæmoglobin *A* and of the variant were approximately equal, and that of hæmoglobin A_2 was 1 per cent or less. In the method of alkali denaturation[9], the *F* hæmoglobin value was 1·5 per cent. Unfortunately, no other members of J. C.'s family were available for study.

In summary, a new hæmoglobin variant, appearing together with hæmoglobin *A*, has been found in a Chinese woman living in Honolulu. It was not accompanied by any distinctive clinical or hæmatological findings. This variant resembles types *L*, *P* and Stanleyville I in zone electrophoresis, veronal buffer, *p*H 8·6. It differs from *L* and Stanleyville I in free electrophoresis, cacodylate buffer, *p*H 6·5, μ 0·1, in which method, like hæmoglobin *P*, it does not separate from *A*. It differs from hæmoglobin *P* (and from *L*) in chromatography on 'Amberlite *IRC* 50', in which it resembles (but is slightly faster than) Stanleyville I. It is suggested that this variant be provisionally designated the 'Honolulu type'.

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The Gm^a Factor in Japanese

A NUMBER of blood group antigens in addition to hair, eye, and skin colours, the shape of teeth, fingerprints and palm-patterns, ear wax, etc., are used as markers in anthropological research.

Grubb[1] showed that certain human sera when added to the serum of certain rheumatoid arthritis patients will inhibit the agglutination of group $O\ Rh_0(D)$ positive cells sensitized with selected incomplete anti-$Rh_0(D)$ antibody. The human sera having a factor inhibiting the agglutinating activity of rheumatoid arthritis serum were named $Gm(a+)$ and others which do not, $Gm(a-)$. It is believed that the *Gm* factor exists in the γ-globulin fraction.

The studies of the Gm^a factor have been made by various workers and the frequency of $Gm(a+)$ individuals among the white population was about 40–60 per cent. However, Grubb *et al.*[2] recognized 95 per cent of 74 Eskimos to be $Gm(a+)$, Moullec *et al.*[3] reported that all of 500 African Negroes were $Gm(a+)$, Steinberg *et al.*[4] found 98 per cent of 98 American Negroes to be $Gm(a+)$, and Steinberg *et al.*[5] observed that all of 89 American Indians were $Gm(a+)$.

Since it was already well known that the blood-group frequencies of Japanese vary from those of other populations[6,7] it seemed possible that the distribution of the Gm^a factor might also manifest a detectable deviation.

We tested for the Gm^a factor employing rheumatoid arthritis serum, $Gm(a+)$ and $Gm(a-)$ control sera obtained through the courtesy of Dr. R. Grubb, Bacteriological Institute, Lund, Sweden, together with two anti-$Rh_0(D)$ sera selected for their suitability from the specimens which had been stored by one of us (M. Y.) at the Blood Typing Laboratory and Rh Center, Tokyo Medical and Dental University. Later, we received a suitable anti-$Rh_0(D)$ serum from Dr. A. Eyquem, Pasteur Institute, France.

The agglutination–inhibition test (Gm^a grouping) was carried out according to the method of Grubb and Laurell. The tests were performed on 816 normal human sera with the following results: Only 2 (1·0 per cent) of 200 random blood donors in Tokyo and 13 (2·1 per cent) out of 616 blood donors in Kumamoto were found to be $Gm(a-)$. Conversely, we found approximately 98–99 per cent of these sera to be $Gm(a+)$ and the serum factor to be inherited as a dominant Mendelian character (Table 1). In addition, a study was carried out on 34 families whose progeny included identical twins. The test was performed only on the sera of the parents and the twins for a total of 136 tests. Five individuals were found to be $Gm(a-)$. This included 1 parent from each of 4 families and 1 twin from one of these 4 families. Gm^a serum grouping together with other genetic investigations are still being conducted on the family in which one of the identical twins and one of the parents were $Gm(a-)$.

Table 1. THE Gm^a FACTOR IN JAPANESE

	Gm^a factor	
No. tested	$Gm(a+)$ (per cent)	$Gm(a-)$ (per cent)
816	801 (98·13)	15 (1·87)
136*	131 (96·32)	5† (3·68)

* From family study on twins (34 families).
† In four families.

As new blood groups are found and their frequencies determined, it seems probable that additional racial differences will be noted in their distribution. The Gm^a factor together with Gm^x, Gm^b and *Gm*-like factors should prove also to be useful tools in anthropological research.

We wish to acknowledge the kind assistance of Dr. R. Grubb and Dr. A. Eyquem in this work.

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The γ-Globulin, Gm^{ab}, in Nigerians

THE γ-globulin (*Gm*) genetic polymorphism, first described by Grubb[1], exhibits considerable geographical variation. Steinberg, Boyer and Stauffer[2] recently observed a striking excess of the phenotype *Gm* $(a + b +)$ among Negroes in the United States. Accordingly it was suggested that an allele, Gm^{ab}, exists in coloured populations. The allele frequency of Gm^{ab} among 593 Negroes from Maryland and Ohio is 0·690. The difference between this value and unity was taken as an estimate of European genetic admixture in American Negroes. Essential to this estimate was the assumption that Gm^{ab} frequency is nearly 1·00 in native West Africans. Moullec[3] had previously reported that 449 residents of Dakar were all $Gm(a+)$; however, typing of *Gm b* was not then possible and has not since been described in a West African population.

In the present work aliquots of serum or whole blood from 409 native Nigerian blood bank donors was forwarded, by air, from Ibadan to the United States. *Gm a* and *Gm b* were detected in the manner previously described[2].

All individuals were clearly *Gm* $(a+\ b+)$. There is a probability of 0·05 that either Gm^a or Gm^b is present with an allele frequency of 0·05 but was, by chance, omitted from the present survey. The assumption that Gm^{ab} has a frequency of near unity in West Africans is consequently confirmed.

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PATHOLOGY

Surface Enamel Magnesium and its Possible Relation to Incidence of Caries

FOLLOWING the semi-empirical administration of a mixture of alkaline phosphate to a group of 200 patients during a period of three years, it has been noticed that the incidence of caries on continuous enamel surfaces has been significantly inhibited at all age-levels. The surface enamel of exfoliated or extracted teeth from these and other patients is being investigated, and the magnesium content has been shown to vary within extremely broad limits.

This magnesium variable has been correlated with caries incidence or inhibition, also with an abrasive or non-abrasive prophylaxis, and finally, with a (possibly) minerally deficient diet or with a minerally enriched diet. The mixed mineral salts supplied were 50 per cent magnesium phosphate, 25 per cent calcium phosphate and a small percentage of potassium, sodium and iron phosphates. They were fluoride-free.

Table 1. Analytical Results, corrected to 0·0050 gm. Sample, Expressed in ml. 0·002 *M* Ethylenediamine Tetraacetic Acid (First Series 29/3/60)

Patient	Age (yr.)	Tooth	Dental status	History	Calcium	Magnesium
P.C. (female)	11	Lower right permanent canine	Non-carious, non-abraded	Magnesium salts, 2 yr., inhibited caries incidence	4·73	0·22
R.R.* (female)	11	Right lower deciduous second molar	Non-carious, non-abraded	Magnesium salts, 8 yr., inhibited caries incidence	4·33†	0·13
L. (male)	30	Left upper first molar	Three enamel surfaces intact	Abraded regular toothpaste, caries-prone, non-salts	4·75	0·03
P.W. (male)	25	Upper permanent canines	Carious distally ; labial, lingual surfaces intact	Abraded regular toothpaste, caries-prone, non-salts	4·6	0·05
A.C. (female)	5	Lower deciduous centrals	Non-carious, non-abraded	Magnesium salts, inhibited caries incidence	3·65†	0·42
D.B. (female)	8	Lower right permanent central	Non-carious	Abraded toothpaste *C* ‡, orthodontic removal, non-salts	4·75	0·19
A.M. (male)	12	Upper deciduous canines	Non-carious, non-abraded	Magnesium salts, inhibited caries incidence	4·19†	0·49
T.G. (male)	10	Upper left deciduous molar	Well filled three intact surfaces collectively non-abraded	Magnesium salts, 2 yr., inhibited caries incidence	4·35	0·30
Mrs. F.	28	Upper right canine	Non-carious, non-abraded	321 123 pyorrhœa alv., intact enamel, otherwise edentulous, toothpaste *C* occasionally, non-salts.	4·70	0·27
J.M. (male)	7	Upper deciduous laterals	One sound, one carious	Exfoliated, abraded, toothpaste, non-salts, caries-prone	4·42	0·08
K.K. (female)	5	Upper left lateral	Non-carious, non-abraded	Magnesium salts, 18 mnth., inhibited caries incidence	4·13†	0·28
R.R.* (female)	11	Left deciduous second molar (lower)	Non-carious, non-abraded	Magnesium salts, 8 yr., inhibited caries incidence	4·31†	0·10
S.M. (male)	12	Lower right permanent canine	Non-carious	Orthodontic removal, not completely erupted, toothpaste non-salts, caries-prone	4·60	0·14
A.C. (female)	5	Lower deciduous laterals	Non-carious, non-abraded	Magnesium salts, 2 yr., inhibited caries incidence	3·50†	0·30
— (male)	56	Lower right second premolar	Carious, buccal surface intact, smooth	Extracted for pulp-degeneration, caries-prone, non-salts	4·50	0·12

* These teeth were re-washed some months after exfoliation. Surface organic degradation may render magnesium more soluble, hence possible loss in washing.

† Organic charring noted in these samples.

‡ Tooth-paste *C* appears to be highly surface reactive, and uniquely compatible with a surface enamel preservation therapy.

Preliminary chemical analyses, carried out in the Chemistry Department, University of Otago, have indicated that an important role can possibly be assigned to magnesium (phosphate) in the stabilization of chemical, physical and electrokinetic states of the surface enamel calcium. As in all biological assays, the method of analysis has been considered of primary importance, and is reported here in detail.

All teeth, immediately after extraction, were washed and rubbed with cotton wool in distilled water, then air dried at 20° C. for several days. Intact enamel surfaces (bucco-labial and lingual) were subjected to a standardized abridement, a new No. 35 *SSW* carbide inverted cone bur, at approximately 50,000 rev./min. in a rigid handpiece, scoring a light line down the incisal (or occlusal) two-thirds of the surface at an approximate depth of 50–100μ. A series of similar, parallel lines was scarified on the area to be sampled, a measurable evenness of depth being obtained with some certainty after considerable experience. The bur was then moved lightly cross-wise against the intervening ridges, reducing the scored area to a uniform level. The very finely powdered enamel thus produced settled (in a still atmosphere) on a foot square of clean 'Polythene' covered card. This was carefully brushed into a prepared, wide-necked dry glass jar, sealed, and labelled ready for micro-sampling.

After each enamel surface was thus sampled, the bur was cleaned and inspected under a microscope for vane damage. If detected, the bur was discarded. Each sample was subjected to a high magnetic drag to remove any ferric contamination possible in this method. Samples were then submitted to water-bath dehydration for 48 hr. A varying weight of sample was micro-weighed and ashed at the minimum temperature with a drop of sulphuric acid in platinum vessels. The mineral ash was dissolved again successfully by dilute acid re-digestion and water elution into 'Polythene' receptacles, and prepared for a 'double blind' series of titrimetric analyses.

The method of Betz and Noll[1] (for total magnesium–calcium estimation) was modified. A 5-ml. sample was pipetted into a white 'Polythene' beaker. 1 ml. standard $MgSO_4.7H_2O$ (0·001 gm./l.) was added and made up to 50 ml. *p*H was adjusted to 7·0 with a glass electrode *p*H meter. 0·3 ml. freshly made standard buffer (A.R. borax, sodium hydroxide) was added, bringing the *p*H to 10·5. 0·1 mgm. 'Solochrome' indicator (standard preparation) was added and swirled until dissolved. Titration was with 0·002 *M* ethylenediamine tetraacetic acid (disodium) under an automatically condensed light from a single Philips' 8-V. 50-W. lamp through a standard blue filter, giving an intense field of beamed white light (approximately *K* 5,500) on to white filter paper. Blue filter is not critical but the titrating light field, when photographed in daylight 'Kodachrome', should give a normal colour balance, indicating approximately light grading *K* 5,500. Light deterioration became evident only after 15 hr. service. All titrations were carried out in a darkroom, at 20° C. Titration end-point was to the definite discharge of the slightest purple tint, the final cold blue being stable over a period of at least 45 min. Continuous vigorous agitation accompanied a minimum titration time of 3 min., a rigorously standardized procedure.

As a trace of iron sometimes gave the blue colour a greyish tint, a preliminary titration of each sample gave a separate standard for colour comparison, being over-titrated 0·1 ml. to a stable blue. All analyses were double- or triple-checked, and consistent figures were obtained throughout.

Calcium estimation was with 'Calcein' indicator, under similar conditions, according to the method of Socolar and Salach[2].

Table 2. COMPARATIVE SUMMARY, ML. OF 0·002 *M* ETHYLENEDIAMINE TETRAACETIC ACID/5 MGM. ENAMEL

Calcium		Magnesium	
Dietary increment of magnesium salts mixture	Non-salts	Dietary increment of magnesium salts mixture	Non-salts
4·73	4·75	0·22	0·03
4·33	4·60	0·13	0·05
3·65	4·75	0·42	0·19
4·19	4·70	0·49	0·08
4·35	4·42	0·30	0·27
4·13	4·60	0·10	
4·31	4·50	0·28	0·14
3·50		0·30	0·12
Average 4·15	4·61	0·28	0·13

This may be regarded as a first-line, expendable and mobile mineral reserve to save calcium demobilization in the carious process. A more extensive series of further analyses is being carried out in the Chemistry Department, University of Otago, and will support a postgraduate thesis, Dental School, University of Otago.

I am indebted to Prof. W. G. Edwards (now of the Chemistry Department, Royal University of Malta) for his assistance and guidance in the analytical work involved. Thanks are due to Mr. Hughes, Physics Department, University of Otago, for micro-measurements of the depths of enamel surface.

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Mechanism of Antigen Transformation of Transplantable Rat Tumour

In earlier investigations we have established that in the case of temporary heterotransplantation of Guérin's rat epithelioma to mice, a change occurs in its antigen structure[1]. The change consists in the appearance of an additional antigen, not found in the original tumour. The presence of this antigen could be demonstrated by various methods as late as 5 years after the modification induced by heterotransplantation, although the tumour had been passaged all this time exclusively on rats[2,3].

The question arose as to the mechanism by which this durable modification of the antigen structure of the rat tumour occurs in the course of temporary passaging on mice. The correctness of various hypotheses was tested experimentally and the mechanism of genetic transformation appeared to be most probable. This is shown by the following experiments carried out on the same principle as the classical experiments of Griffith[4] with pneumococci.

A suspension of rat epithelioma cells was prepared and mixed with homogenated mouse lymphocytes. These cells from mouse lymphatic nodes were mechanically disrupted in a glass mortar and suspended in physiological saline in the ratio 1 : 1. This homogenate was mixed with the rat tumour cell suspension in a volume ratio of 1 : 2, 1 : 3 or 1 : 4 and injected subcutaneously to rats.

At the site of injection, inflammation appeared; this subsided after several days and the tumour developed. From such a tumour a new cell suspension was prepared and mixed with the mouse lymphocyte homogenate as before. In this manner a new sub-line of the tumour was obtained which was further passaged on rats in the routine way. The scheme in Fig. 1 shows the branching sub-lines U_I, U_{II}, U_{III} and W_I, W_{II}, W_{III} of the original line of Guérin epithelioma. In successive passages, the antigen structure of the tumour was tested by the method of antibody absorption and complement fixation and referred to the original tumour as well as to the following control antigens: (1) extract of parenchymal organs of normal rats and mice, (2) rat necrotic tissue obtained by intramuscular injection of 0·1 *N* silver nitrate. Several series of these experiments were carried out. In series W_I, W_{II}, W_{III} the Guérin tumour cell suspension was mixed with homogenated lymphocytes of normal *C57BL* mice; in series U_I, U_{II}, U_{III} the lymphocytes of actively immunized mice *C57BL* with Guérin epithelioma were used.

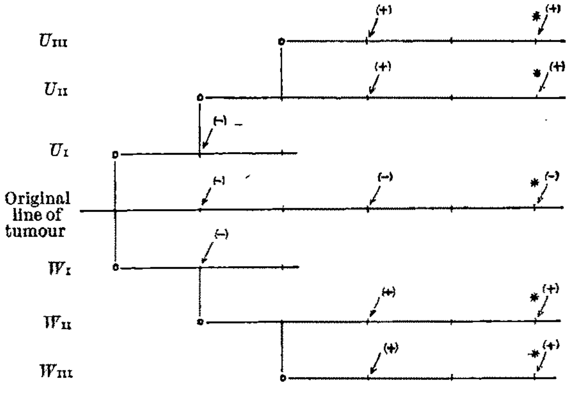

Fig. 1. Scheme of original line and sub-lines of Guérin's epithelioma. Sub-lines W_I, W_{II}, W_{III} produced by mixing the suspension of original Guérin tumour cells with ground lymphocytes of normal mice. In a similar manner the sub-lines U_I, U_{II} U_{III} were obtained using lymphocytes of mice actively immunized against the Guérin tumour. o, Time of mixing the tumour cells with mouse lymphocytes. The arrows indicate the time of serological examination of the tumours. + and −, presence and absence, respectively, of the additional antigen. Asterisks denote complement fixation tests shown in Table 1. Each section of the line marks a monthly period in which the tumour was passaged

In every phase of the experiment the tumour was examined bacteriologically, and only sterile tumour was taken for antigen structure tests. Such control excludes the possibility of contamination of the Guérin tumour by bacterial antigens.

It was established, as a result of these experiments, that in some rat tumours, under the influence of mouse lymphocyte homogenate, an additional antigen appeared. The latter subsisted in the tumour for 3–4 months in spite of repeated passaging. For illustration a record of one of our serological tests is given (Table 1). No difference was observed between the sub-lines obtained with lymphocytes of normal and immunized mice.

Table 1

Antigens	Dilutions of serum				
	1/16	1/32	1/64	1/128	1/256
$T_8\ W_{\rm II}$	+ + +	+ + +	+	±	–
$T_8\ U_{\rm II}$	+ + +	+ + +	+ + +	–	–
$T_8\ W_{\rm III}$	+ + +	+ + +	+ +	+	–
$T_8\ U_{\rm III}$	+ + +	+ + +	+ + +	±	–
$T_8\ C$	+ + +	+ + +	+ + +	+	–
T_8 (original tumour)	+ +	±	–	–	–
From mice (*C57BL*)	–	–	–	–	–
From rat	+ +	–	–	–	–
Necrobiotic antigen	+ +	±	–	–	–

Results of complement fixation with specific antiserum *T8C* adsorbed on original tumour *T8* particles. This antiserum was used in all experiments as standard. Antiserum *T8C* was prepared by immunizing rabbits with the sub-line *T8C* of the Guérin tumour obtained 5 years ago by heterotransplantation on mice (ref. 1).

We believe that in our experiments the disrupted mouse lymphocytes decomposed to smaller particles in the rat organism during inflammation and were possibly incorporated into the genome of the tumour cell.

This enrichment of the tumour cell genome probably brought about the synthesis of an additional cytoplasmic protein manifesting itself as an additional antigen.

The experiments seem to indicate that the mechanism of genetic transformation may be responsible for the antigen modifications occurring in the Guérin tumour. It would be desirable to establish whether a foreign, purified deoxyribonucleic acid molecule will produce a similar transformation. Further experiments in this direction are in progress in our laboratory and the preliminary results seem to confirm this possibility.

In our opinion the mechanism of genetic transformation of somatic cells would be easier to demonstrate if the experiments were performed on anaplastic and poorly differentiated tumour cells and if nucleic acid from normal and highly differentiated cells were used as transforming agent. Under these conditions any property of a highly differentiated cell transmitted to the anaplastic tumour cell can serve as marker.

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ANATOMY

Use of Tritiated Thymidine for the Study of the Origin of the Osteoclast

The origin of the osteoclast has been in question for about a century. So far back as 1873, Kölliker[1] maintained that the osteoclast was formed by the fusion of precursor cells and that these precursor cells were osteoblasts. However, Kölliker's work lacked convincing proof, and as a result, numerous theories appeared concerning the origin of the osteoclast. These theories were based largely on morphological studies of metaphyseal osteoclasts. The presence of numerous cell types at this site makes observation of cellular transitions difficult. In order to circumvent this difficulty, we have examined the distal periosteum of young mouse femora, where numerous osteoclasts appear. The cellular complement of the osteogenic layer of the periosteum generally consists of pre-osteoblasts and osteoblasts.

In a previous communication[2], tritiated thymidine was used to label osteoclasts, which were not seen 1 hr. following administration of the isotope (Fig. 1). Numerous osteogenic cells showed uptake of thymidine, but labelled osteoblasts were found less frequently. The actual number of labelled osteoblasts observed was so few that the chance of finding a labelled osteoclast was exceedingly small. In addition, the observations were made 1 hr. following injection of tritiated thymidine. This period of time is too short to allow labelled pre-osteoblasts in DNA synthesis to pass through the post-synthetic and mitotic phases, undergo transformation into osteoblasts, and in turn fuse to form osteoblasts.

The present work has extended the period of observation to 25 days following isotope administration, in order to allow sufficient time for labelled cells to divide, thereby increasing the actual number of labelled periosteal osteoblasts, which increases the chances of demonstrating labelled osteoclasts.

A subcutaneous injection of 0·5 μc. of tritiated thymidine per gm. body-weight was given to 24 four-

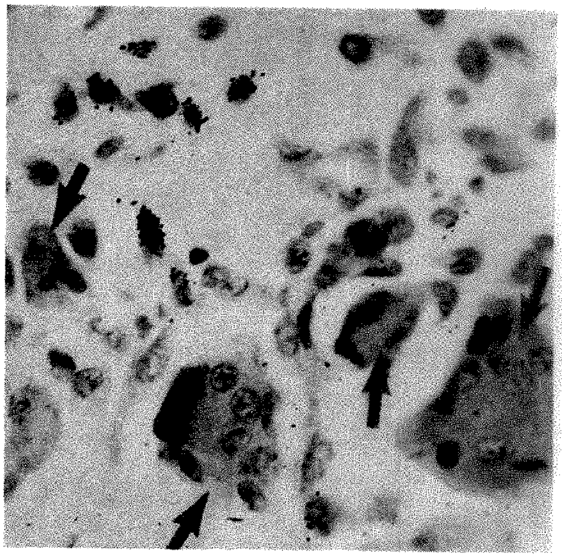

Fig. 1. Autoradiograph of mouse femoral periosteum at the perichondrial region near the distal epiphysis of the femur. Several periosteal cells are labelled with tritiated thymidine 1 hr. after administration of the isotope. The osteoclasts are unlabelled. (Oil immersion, × 470)

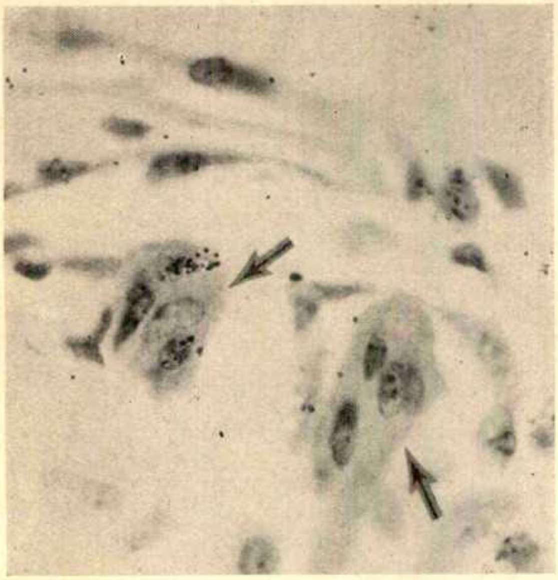

Fig. 2. Autoradiograph of periosteal osteoclasts labelled with tritiated thymidine and observed 36 hr. following the administration of the isotope. (Oil immersion, × 470)

week-old female mice of a *BNL* Swiss albino strain. The animals were killed at various intervals, ranging from 5 min. to 25 days after administration of the isotope. Femora were fixed for 3 hr. in acetic/alcohol (1 : 3) followed by an additional fixation for 24 hr. in formolsaline and 24 hr. of washing. Bone samples were decalcified in a 10 per cent solution of versene. Paraffin blocks were prepared, sections cut at 5μ and autoradiographs prepared with Kodak-NTB_3 liquid emulsion. Preparations were exposed for 20 days, after which time they were developed and stained with hæmatoxylin and eosin.

Autoradiographs revealed labelling of periosteal osteogenic cells throughout the entire period. With increasing time following injection of the isotope, additional osteoblasts were seen to be labelled. Labelled osteoclasts were observed for the first time at 36 hr. post injection (Fig. 2). Labelling was seen in one, and less frequently two, nuclei. The remaining nuclear complement was unlabelled. No osteoclastic labelling was observed 10 days following isotope administration.

A previous report[3] has presented direct cytological and cytochemical evidence for the formation of osteoclasts from osteoblasts. It was also shown that osteoclasts do not arise as a consequence of nuclear division, since none was labelled initially. Considering that osteoclasts are being continually renewed, the frequency of labelled osteoclasts depends on the available number of labelled precursor cells as well as other factors. Labelled osteoclasts were not encountered in the present work until an increase in the labelled population of periosteal osteoblasts had occurred. The results show, therefore, that osteoblasts serve as the precursor cells for osteoclasts, and that their polynucleated character arises from the fusion of osteoblasts and not from the repeated division of the osteoclastic nucleus.

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HISTOLOGY

Influence of Oxygen Concentration and Mechanical Factors on Differentiation of Connective Tissues *in vitro*

MARCHAND[1] postulated that osteoblasts may differentiate from mesenchymal cells. Recently, it was observed that a strain of cells, derived from adult skeletal muscle, elaborated in tissue culture a substance that appeared to be chondro-osteoid[2]. Since the factors responsible for this cell behaviour were unknown, investigation of the effects of varying mechanical and nutritional factors on a reproducible *in vitro* system of osteogenesis was made. Hanging-drop, Maximov double cover-slip cultures were established from 20-day-old chick embryo tibial cortex and were maintained as described by Fell[3].

Effect of compaction and high oxygen. In one experiment, the cultures were excised periodically from the clot; gassed with a mixture of 35 per cent oxygen, 60 per cent nitrogen and 5 per cent carbon dioxide, and transferred to a new clot. As the cell mass was cut free it usually contracted, thus crowding the increasing population into a more compact state. Cultures were killed on the fourteenth day by fixation in cold 80 per cent alcohol, and serial paraffin sections prepared. Bone was found in 100 per cent of these specimens. In most, a dome-shaped plaque of osseous tissue covered the outer portions. However, in some preparations, where contraction of the culture had been uneven, the bony mass was distorted. The tissue contained numerous young osteocytes, embedded in a

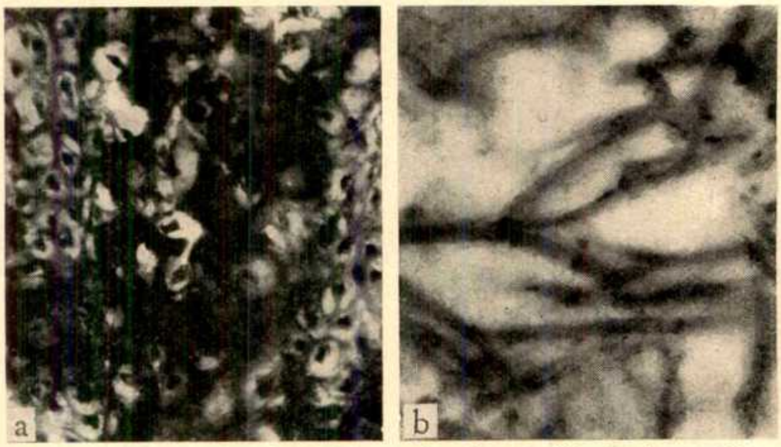
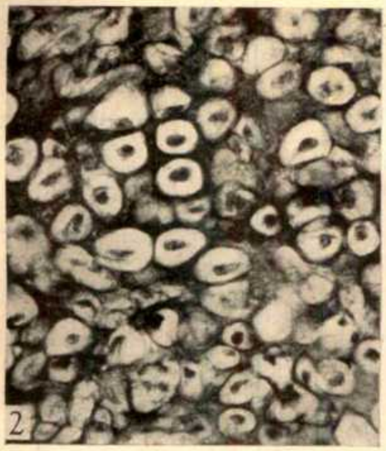


Fig. 1. *a*, Dark areas are heavily calcified, young bone (hæmatoxylin and eosin, × *c*. 350); *b*, electron micrograph of bony area—note 'seeded' collagen (× *c*. 36,000)



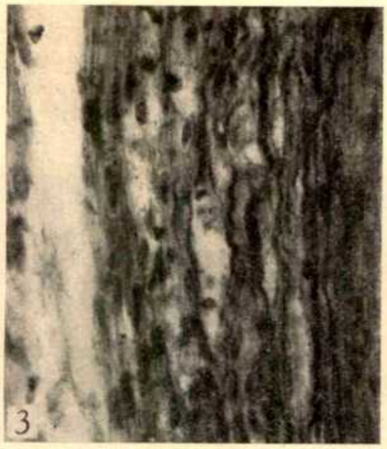


Fig. 2. Dark areas about cells are intensely metachromatic (thionine, × c. 350)

Fig. 3. Oriented wavy collagen fibres with interspersed 'fibrocytes' (Van Gieson, × c. 487)

matrix which appeared to be predominantly collagenous, when stained by hæmatoxylin and eosin, the van Gieson or Masson trichrome techniques (Fig. 1*a*). When this matrix was stained with thionine, alcian blue or toluidine blue at *p*H 2, 4 and 6, metachromasia was not demonstrable. All osseous areas were heavily calcified, when judged by the reaction with hæmatoxylin, alizarin red *S* and von Kossa stains. Electron-microscopic examination of typical bony areas in these cultures was kindly performed by Dr. James Harkin of the Hospital for Special Surgery and showed collagen fibrils. Many of these fibrils were studded with discrete, electron-dense areas which had an average 640 Å. repeat pattern (nucleation centres or crystal seeds) (Fig. 1*b*). In many aspects, the picture resembled that obtained by Fitton-Jackson[4] in her examination of developing, embryonic chick bone. Very dense areas, believed to be similar to calcification fronts described by Robinson[5], were seen. The deeper portions of these cultures appeared to be cartilage when examined by the light and electron microscope.

Effect of compaction and low oxygen. In a second experiment, the cultures were grown as those just described, except that the oxygen concentration in the gassing mixture was lowered from 35 to 5 per cent, while the nitrogen was raised from 60 to 90 per cent. Under these conditions cells proliferated and migrated well, but no bone was observed. However, hyaline and fibrocartilage were found at the periphery and in all cultures the central areas were less well differentiated. Bundles of collagen, interspersed between relatively small chondrocytes in the fibrocartilage, were stained by the van Gieson technique and deep blue with the Masson trichrome technique. In these areas, no metachromasia was observed. However, in areas having the appearance of hyaline cartilage (Fig. 2), the plumper chondrocytes were surrounded by metachromatic, non-fibrillar matrix. No evidence of calcification was seen in these cultures.

Effect of stretching and high oxygen. In the third experiment, the cultures were prevented from contracting by stretching them over progressively larger silicone rods during the growth period. The other conditions were the same as in the first experiment. No cartilage, bone or calcification was found in cultures that were stretched uniformly. The tissue appeared more like young, growing tendon or fascia. Cells, which resembled mature, elongated fibrocytes, were interspersed between thick, parallel bundles of collagen (Fig. 3). No metachromasia was found in these sections. In some cultures uniform tension in the tissue ring could not be maintained and portions of it contracted. Bony masses with cartilagenous centres were found in the compacted areas, while oriented, fibrous tissue was found immediately adjacent in areas where tension was maintained.

These experiments demonstrate that mechanical (stretching and compacting the cell mass) and nutritional (variations in oxygen) factors, acting in concert, can determine the type of connective tissue that differentiates in a culture of cells arising from bone. Although the mechanisms by which the different tissues were produced are not known, at least two interpretations are possible. First, the conditions in culture may favour one cell-type in a mixed population so that it proliferates and produces a specific extracellular matrix. Second, the conditions in culture may determine the secretory pattern of the cell so that it produces different extracellular matrices in response to different environmental factors. In any event, the results appear to agree with the Maximov concept[6] that certain 'fibroblasts' are pleuripotential and may differentiate in a number of ways to produce the various connective tissues.

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BIOLOGY

Efferent Innervation of Mammalian Muscle-spindles

MAMMALIAN intrafusal muscle fibres are innervated by the small γ-efferent fibres of Leksell[1], which have nerve-trunk diameters of 2–8μ. Fibre size histograms of the intramuscular diameters of both intra- and extra-fusal efferents in fresh and gold chloride preparations show that, while both share the same peak at 3·5μ, about 30 per cent of the intrafusal efferents lie outside the extrafusal range at 4–6μ (ref. 2). These larger fibres have been reported in various descriptions of spindle innervation[3–6]; in cat rectus femoris, they take part in the innervation of about one-third of the spindles present (Adal and Barker, unpublished). They could be members of either the 2–8μ γ-group, or the 8–18μ α-group, which have not undergone such extensive branching as the majority of their fellows. The work of Granit, Pompeiano, and Waltman[7] on the α-activation of cat spindles directs attention to the possibility that some members of the α-group may branch so as to innervate both intra- and extrafusal muscle fibres. Such a claim has been made by Weiss and Dutil[8], Cilimbaris[4], Wilkinson[9], Denny-Brown[10], and, more recently, Häggqvist[11]. Wilkinson (echidna spindles) and Häggqvist (monkey spindles) have each published a photograph purporting to show such innervation, but they are unconvincing and open to alternative interpretation. We have examined fifty gold chloride preparations of echidna (*Tachyglossus aculeatus*) spindles without finding instances of collaterals from α-fibres innervating intrafusal muscle fibres, and examination of more than two thousand gold chloride cat spindles, accumulated during work on various aspects of this receptor, has also failed to yield any instance of such innervation. Examination of bat, monkey, opossum, rabbit, and rat spindles has also proved negative in this respect. When apparent examples of such branching have been encountered, closer examination and further tracing has always shown the presence of two discrete fibres; in such instances, the two fibres are usually superimposed one above the other during their course before diverging, so as to give the impression of a single fibre branching into two. Similar instances occur with afferent fibres, and have led to claims[8,12–14] of a single fibre branching so as to terminate in a spindle primary ending and a nearby tendon-organ. If branches of α-efferent fibres do innervate the mammalian spindle, we are of the opinion that the collaterals must originate some distance away from the receptor, either within the muscle nerve or within intramuscular nerve trunks. However, experimental evidence for the innervation of the spindle by α-efferents is conflicting[15], and it seems, on balance, more probable that in mammalian muscle there are discrete intrafusal (γ) and extrafusal (α) efferent systems.

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Glycogen of Mactra

GLYCOGENS, branched chain polymers of glucose containing α-1,4-glycoside bonds in the main chains and α-1,6-glycoside bonds at the branch points, have been identified in the tissues of all animals thus far examined[1]. These polymers are made up of a single kind of sub-unit bonded together by a single type of bond and thus only relatively small differences in the glycogens from different species have been found. The most important difference is in the average chain-length of the molecule, which is inversely related to its degree of branching. This measurement describes quite well the main features of the glycogen molecule, and is therefore of interest to the student of comparative biochemistry. The glycogen of *Mactra* had not been previously characterized; and this communication describes some of the properties of the compound isolated from this pelecypod mollusc.

Specimens of *M. fragilis* obtained from the Supply Department of the Marine Biological Laboratory were used for isolation of glycogen by Pflüger's method: muscle (25·0 gm.) and combined viscera (30·3 gm.) of the animals were digested separately in 50 ml. each of 30 per cent potassium hydroxide on a steam bath. After 30 min. of digestion, homogeneous solutions resulted, and they were cooled and 5 ml. of 0·2 per cent sodium sulphate and 125 ml. of 95 per cent ethanol were added and mixed. The solutions were brought just to the boiling point, and then were chilled and allowed to stand overnight at 3° C. The solutions were centrifuged for 10 min. at 5,000*g* and the resulting supernatant fluid was discarded. The residues were dispersed in 30 ml. distilled water, 1·5 vol. of ethanol were added, and the precipitation was carried out as before. After the muscle glycogen had been precipitated in this way three times and the viscera glycogen four times, the residues were a light tan colour. Following the last precipitation, the glycogen was collected on a sintered glass funnel with gentle suction, and the product was washed on the filter with a little acetone to remove the last traces of a coloured impurity. The resulting white powder was dried *in vacuo* over calcium chloride to constant weight, and was used for the analysis described here.

The ash content of the material was determined by ignition. Samples of the glycogens were hydrolysed in 5 *N* sulphuric acid at 100° C. for 30 min., the solutions were neutralized with dilute sodium hydroxide, and aliquots were taken for reducing sugar analysis and glucose determination by the specific enzymatic reaction with glucose oxidase. The average chain-length was determined exactly as described by Fales[2].

The results of the various analyses are shown in Table 1. In accordance with the experience of other investigators, a significant inorganic residue remains in the glycogen preparations even after repeated alcohol precipitation. The 80 per cent acetic acid precipitation procedure used by Baldwin and Bell for the removal of these salts[3] was not used in the present investigations because of the known lability of the acetal bond in acid. Within the limits of error of the copper reduction method used, all the organic material in the preparations is accounted for as hexose in diglycoside linkage, assuming that these hexoses have a reducing power equivalent to that of glucose. According to the results of the less-precise glucose oxidase analysis, all the hexose is accounted for by glucose itself. Thus the experimental data are consistent with the view that *Mactra* glycogen consists entirely of D-glucose polymerized into chains by glucoside bonds.

Table 1. ANALYSES OF GLYCOGEN FROM *M. fragilis*

	Muscle	Viscera
Yield (gm. per 100 gm. wet tissue)	2·4	2·0
Ash (per cent)	3·1	1·7
Reducing substances (as diglucoside) (per cent)	94·8	96·9
Glucose by glucose oxidase (as diglucoside) (per cent)	108·5	93·3
Average chain-length	13·3	7·3

The result of the average chain-length determination on muscle glycogen is similar to that obtained by Fales[2] with oyster glycogen, and is in the range reported by other investigators who used somewhat different methods of periodate oxidation on glycogen from a variety of species[1,4]. The viscera glycogen of this animal has a considerably shorter average chain-length. The observations do not provide any information about the location in the glucose units of the glycoside bonds, but it may be assumed that they are the customary α-1,4-glycoside bonds with α-1,6-bonds at the branch points.

It is evident that more data on the structure of glycogens from a variety of species must be assembled before any generalizations can be made. Particular emphasis should probably be placed on the examination of material from the most primitive forms available.

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Absence of Chitinase in *Limnoria*

THE question of the presence or absence of a chitinase in *Limnoria* is of interest for two reasons. First, if a chitinase were present, chitin could serve as a principal nitrogen source. Ray and Julian[1] and Ray[2] have shown that *Limnoria*, which eats wood, can break down cellulose to glucose, and can therefore utilize wood as a source of energy. However, it is not yet known what source of nitrogen *Limnoria* utilizes because wood contains so little. One possible nitrogen source is chitin, present in cell walls of fungi growing in wood and often eaten with the wood[3], and present in the animal's exuviæ, which it may eat.

This question is also of interest for another reason. Since both cellulose and chitin are *B*-glucosides, it is possible that the same enzyme could hydrolyse both ; that is, the cellulase found by Ray in *Limnoria* could be a general *B*-glucosidase capable of splitting chitin also.

In an attempt to answer this question, 20, 30, 40 and 60 mgm. of powdered chitin prepared from *Loligo* pens were incubated at 25° and 30° C. for 2–12 days with 0·2 ml. of *Limnoria* enzyme solution and 0–1·0 ml. of McIlvaine's buffer, *p*H 5·6. The enzyme solution was prepared by the method described by Ray[4]. The midgut diverticula of approximately 60 animals were used to make each 0·2 ml. of enzyme solution. At the end of the incubation periods the solutions were tested by paper partition chromatography to determine whether acetylglucosamine or any other reducing sugar had been obtained from the chitin. The tests were all negative, although control solutions of carboxymethyl-cellulose plus enzyme incubated at the same times under the same conditions did contain reducing sugars (mostly glucose) at the end of the incubation periods.

In another experiment, suspensions of chitosan, suspensions of partially hydrolysed chitin, and solutions of carboxymethyl-cellulose were incubated at 30° C. for 7 days with 0·2 ml. of enzyme solution. Each tube contained 20 mgm. of substrate suspended or dissolved in 1 ml. of McIlvaine's buffer at *p*H 5·6. The chitosan was prepared from *Loligo* chitin by treating with hot saturated potassium hydroxide, washing, dissolving in acetic acid, and dialysing against buffer. At the end of the incubation period the solutions were treated as above. No reducing sugar was found, although the control solution of carboxymethyl-cellulose plus enzyme did contain reducing sugar.

Apparently the cellulase present in the midgut diverticula of *Limnoria* is not a general *B*-glucosidase capable of hydrolysing chitin. It is still possible that *Limnoria* produces a chitinase inactive under the conditions tested or that some other portion of the gut produces a chitinase. However, this seems unlikely. Probably, *Limnoria* is not able to utilize chitin as a source of nitrogen.

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Comparative Chemistry and the Relationship of the Hamamelidaceae

IN many of the more recent systems of classification, the Hamamelidaceae have been given a central position, several authors believing that they have been derived from rosalian or magnolialian ancestors, and that they, in turn, have given rise to some of the amentiferous families. Although the family is usually included in the Rosales, a number of systematists have assigned it to the order Hamamelidales.

In investigating the relationships of the Hamamelidaceae and those families which have been included in an order Hamamelidales, as well as those of the Rosaceae and some of the amentiferous families, chemical characters have been used.

The chemical characters of the Hamamelidaceae, based on testing twenty-five species representing twelve genera, are the following[1] : (*a*) they are positive to the hydrochloric acid methanol test ; (*b*) they are positive to the leuco-anthocyanin test ;

(*c*) they give a red reaction to the 'syringin' test ; (*d*) they give a magenta spot with Ehrlich's reagent ; (*e*) they are negative to the hydrogen cyanide test ; (*f*) they are negative to the 'juglone' test ; (*g*) they show no fluorescence in 'juglone' tests ; (*h*) they lack raphides ; (*i*) they lack detectable amounts of glucitol and sedoheptulose ; (*j*) they give the '*Oxalis* reaction' to the cigarette and hot-water tests.

These characters have been compared with those of representatives of the other families tested. Other things being equal, it is assumed that those families which show the closest chemical correspondences are the most closely related.

Of twenty families (Myrothamnaceae, Platanaceae, Cunoniaceae, Eupteleaceae, Stachyuraceae, Eucommiaceae, Cercidiphyllaceae, Trochodendraceae, Buxaceae, Daphniphyllaceae, Coriariaceae, Pittosporaceae, Hydrangeaceae, Saxifragaceae, Bruniaceae, Tetracentraceae, Altingiaceae, Byblidaceae, Podostemaceae and Hydrostachyaceae) which have been included in an order Hamamelidales, representatives of the first fourteen have been tested.

On the basis of results of these tests, one may propose an order Hamamelidales including the Hamamelidaceae, Platanaceae, Myrothamnaceae, and perhaps the Cunoniaceae. This suggestion is based on preliminary work only, but at present there is no chemical evidence against, and much for, such a grouping.

If the sub-family Chrysobalanoideae be excluded, the Rosaceae form a homogeneous group, distinguished by the occurrence of glucitol and cyanogenetic compounds. The chemical evidence indicates that the Rosaceae and the Hamamelidaceae differ sufficiently to make it unlikely that they should be included in the same order ; however, there is no evidence against the view that the Hamamelidaceae are descended from rosalian ancestors.

Representatives of eleven amentiferous families (Garryaceae, Leitneriaceae, Casuarinaceae, Salicaceae, Myricaceae, Juglandaceae, Butulaceae, Fagaceae, Urticaceae, Ulmaceae and Moraceae) have been tested. The chemical evidence indicates that if the Garryaceae, Leitneriaceae and Juglandaceae be excluded, these families form a natural group. There is no evidence against the view that these families have been derived from hamamelidalian ancestors.

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BACTERIOLOGY

Bacterial Polyglycerophosphate

In a previous communication the presence in streptococcal culture supernatants of four serologically distinct red-cell sensitizing antigens was described[1]. One of these antigens, Hickey, which was found to be of wide distribution among the Gram-positive cocci, is almost certainly identical with the non-species specific antigen described by Rantz, Randall and Zuckermann[2]. The distribution of this antigen has been observed to resemble closely that reported by McCarty[3] for a glycerophosphate polymer isolated from various bacterial species, and it seemed possible that we were in fact dealing with the same compound. The polymer as isolated by McCarty had serological properties in that it was precipitated by certain streptococcal antisera—precipitation being inhibited markedly by synthetic polyglycerophosphate prepared by Dr. A. M. Michelson[4]. Glycerophosphate polymers have also been isolated from bacteria by Mitchell and Moyle[5] and by Baddiley *et al.*[6] ; but in neither of these cases were they investigated serologically.

Two samples of synthetic polyglycerophosphate (calcium salts) with average chain-lengths of 6 and 10–15 units respectively have generously been made available by Dr. Michelson, and their capacity to inhibit hæmagglutination by the Hickey serum has now been investigated. These tests were carried out with human Group *O* red cells treated for 2 hr. at 37° C. with a digest broth supernatant of the Hickey strain of *Streptococcus pyogenes*. Both compounds had marked hæmagglutination-inhibiting capacity—in the test system employed showing appreciable inhibitory activity in a concentration of 30 μgm. per ml. No inhibition of hæmagglutination by antisera specific for the other three streptococcal sensitizing antigens described was observed. (These latter tests were carried out with the 6-unit compound). Neither polymer was capable of precipitating with the Hickey antiserum nor of sensitizing red cells to hæmagglutination by it. Inhibition was also demonstrated with a mixture of α- and β-glycerophosphates (calcium salts, approx. 45 per cent α-) and with β-glycerophosphate (sodium salt). The inhibitory activity of the latter compounds was, however, relatively slight—that of the α-glycerophosphate mixture being of the order of one-fortieth, and that of β-glycerophosphate one two-hundredth, of that of the polymers.

These results would indicate, therefore, that the serologically active component of the Hickey sensitizing antigen is, like that of McCarty's precipitating antigen, a glycerophosphate polymer the activity of which is probably due to α-glycerophosphate residues. They suggest also the possibility that the serological specificity of other bacterial red-cell sensitizing antigens might also be due to polyol phosphate components.

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Production of Soluble Melanoid Pigments by *Streptomyces* in Gelatin and Glucose Media

During a taxonomic study of several *Streptomyces* cultures, most of which were isolated from soil, experiments were performed on the production of soluble melanoid pigments on two gelatin media—plain 15 per cent gelatin and 15 per cent gelatin plus 2 per cent glucose.

Table 1. SOLUBLE MELANOID PIGMENT IN MEDIA WITH GELATIN, GLUCOSE AND PEPTONE*

Gelatin	15			15			15			15			15			15			H_2S†
Glucose	—			2			2			—			2			2			
Peptone	—			—			0·5			0·5			—			0·5			
Dist. water	100 S.T.			100 S.T.			100 S.T.			100 S.T.			100 S.S.			100 S.S.			
Days	4	14	30	4	14	30	4	14	30	4	14	30	4	14	30	4	14	30	
Strains 511	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
254	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
328	+	+	+	−	−	−	−	−	+	1	+	+	+	+	+	1	+	+	1
399	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	1	1	1	−	−	−	−	−	1	1		1	1	1	1	1	1	1	−
*B*171	1	1	1	−	1	1	1	1	1	1	1	1	1	1	1	1	1	1	−
64, 135, 361	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* Sterilization at 16–18 lb. for 20 min., stab culture—spore inoculum. † Difco peptone iron agar. S.T., sterilized together; S.S., sterilized separately; + definitely positive; 1, weak; − definitely negative.

Table 2. SOLUBLE MELANOID PIGMENT IN MEDIA WITH GELATIN AND GLUCOSE*

Gelatin	20			20			20			20			20			20			20			20			20		
Glucose	—			0·5			1			2			5			0·5			1			2			5		
Dist. water	100			100 S.T.			100 S.T.			100 S.T.			100 S.T.			100 S.S.			100 S.S.			100 S.S.			100 S.S.		
Days	4	14	30	4	14	30	4	14	30	4	14	30	4	14	30	4	14	30	4	14	30	4	14	30	4	14	30
Strains 511	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
254	+	+	+	+	+	+	1	1	+	−	−	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
328	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+	1	1	+	1	1	+
399	+	+	−	+	+	+	+	+	+	−	1	+	−	−	−	+	+	+	+	+	+	1	+	+	−	−	−
1	1	1	1	1	1	1	−	1	1	−	−	1	−	−	−	1	1	1	−	1	1	−	1	1	−	−	−
*B*171	1	1	1	1	1	1	1	1	1	−	1	1	−	−	−	1	1	1	1	1	1	1	1	1	1	1	1
64, 135, 361	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* Sterilization at 16–18 lb. for 20 min., stab culture-spore inoculum. S.T., sterilized togetheer; S.S., sterilized separately; +, definitely positive; 1, weak; − definitely negative.

Table 3. CHARACTERISTICS AND SYSTEMATIC POSITION OF THE *Streptomyces* STRAINS USED

Strains	Isolated by	From	Aereal mycelium	Substrate mycelium	Systematic position	
					Kutzner key[1]	Waksman key
(4) 399	Capriotti	Italian soil	Light pink	Cream	II/32	*Strept. zoseus*
(1) 511	Capriotti	Italian soil	Rose grey	Grey green	—	*Strept.* sp. *A*
(7) 64	Capriotti	Italian soil	Green	Brown	IX/2	*Strept. viridochro*
(8) 135	Capriotti	Italian soil	Grey	Violet	VII	*Strept. violaceus*
(9) 361	Capriotti	Swedish soil	Grey	Greenish	IV/8	*Strept. griseolus*
(2) 254	Capriotti	Italian soil	Green grey	Ochre	IX/4	*Strept. chartreusis*
(3) 328	Capriotti	Swedish soil	Beige	Maroon	VI/23	*Strept. pyridomiceticu*
(5) 1	Capriotti	Apple	Grey	Grey	—	*Strept.* sp. *B*
(6) *B*171	Kutzner	German soil	Reddish grey	Colourless to grey	III/I	*Strept. lavendulae*

Strept. sp. *A* and *Strep.* sp. *B* are probably new species.

* It treats of lisogenic strain with all the characteristics of *Streptomyces viridochromogenes* except the production of melanine.

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Three groups of strains could be distinguished: strains with abundant deep-brown pigment on both media, strains with pigment production only on plain gelatin, and strains without pigment production on either medium. Because the production of melanoid pigments plays an important part in *Streptomyces* classification, it was decided to study this phenomenon further. This communication deals with some preliminary experiments on this subject. The experimental results are listed in Tables 1 and 2. (The results in Tables 1 and 2 were obtained in two different experiments and are not strictly comparable because the gelatin concentration was slightly changed.) The presence of peptone (0·5 per cent) in the media had no influence on the production of soluble melanoid pigments.

The effect of glucose on pigment production depends to a certain extent on its concentration and whether it is sterilized together with gelatin or separately. It was found that 0·5 per cent glucose has no effect; 1·0 and 2·0 per cent glucose exhibits a pronounced inhibitory effect on pigment production when sterilized together with the gelatin, but no or only slight inhibition when sterilized separately. 5 per cent glucose inhibits pigment production in all 'positive' strains when sterilized together with gelatin, and in some of them when sterilized separately.

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VIROLOGY

An Immunizing Antigen from Rabbit-Pox and Vaccinia Viruses

It is well known that infection with vaccinia or one of the other animal pox viruses produces a high degree of immunity, associated with the appearance of neutralizing antibody in the serum. Recently, Collier, McClean and Vallet[1] have shown that neutralizing antibody can also be elicited by vaccinia virus inactivated by ultra-violet light. But attempts to obtain immunizing material free from virus particles have had little success[2,3]. This communication describes a virus-free immunizing antigen extracted from tissues infected with rabbit-pox or vaccinia viruses. The injection of this soluble material into rabbits resulted in the formation of sera with a neutralizing antibody titre against vaccinia comparable with that found in animals convalescent from rabbit-pox infection.

Rabbit-pox virus (Utrecht strain) was grown for 24 hr. in monolayer cultures of *ERK*1 cells[4]. The infected cells were concentrated to 4×10^7 per ml., and disrupted by ultrasonic vibration. Virus was removed from the suspension by centrifugation and filtration, to give a cell extract which was non-infective when inoculated on to the chorioallantoic membrane of 12-day eggs.

Incubation of the cell extract with a high dilution of hyperimmune rabbit-pox antiserum resulted in disappearance of the neutralizing activity. The substance responsible for this reaction was termed the 'serum-blocking antigen'. A method was developed for the quantitative determination of the serum-blocking activity of a preparation, and this was used to study the properties of the antigen. The serum-blocking antigen was non-dialysable; but it was smaller than the virus, passing a collodion membrane of 330 mμ av. pore diam., and was not sedimented by 20,000*g* for 4 hr. Its titre was reduced about 80 per cent by exposure to trypsin (0·2 per cent crystalline trypsin at 37° and *p*H 8 for 2 hr.), and also by heating (56° at *p*H 7·2 for 1 hr.). It was precipitated by 50 per cent saturated ammonium sulphate, and by dialysis against phosphate-citrate buffer *p*H 4·5. At *p*H 7·0, the antigen was adsorbed to *DEAE*-cellulose and was eluted by 0·2 *M* sodium chloride. It also adsorbed to calcium phosphate, being eluted by 0·2 *M* phosphate *p*H 6·7. These findings indicate that the serum-blocking antigen is a protein. Moreover, since the activity was eluted as a single peak from both *DEAE*-cellulose and calcium phosphate columns, it is likely to be a single substance rather than a mixture.

Rabbit-pox virus was also grown in *L* cells, primary rabbit kidney cultures, primary chick embryo cultures, and egg chorioallantoic membranes. The extracts were tested for serum-blocking activity, and all proved to be active. Vaccinia virus was grown in *ERK*1 cells, *L* cells, primary chick embryo cultures, and rabbit skin. Extracts of these tissues also possessed serum-blocking activity.

Having demonstrated a serum-blocking antigen, attempts were made to immunize rabbits. The extract from *ERK*1 cells infected with rabbit-pox virus was injected subcutaneously with adjuvant. The neutralizing antibody response of the animals was measured against vaccinia by Boulter's method[5]. A single injection produced a neutralizing antibody titre of about 1,000 after 4 weeks. Four injections at weekly intervals increased the titre to about 10,000, a level similar to that found in rabbits after recovery from infection with the living virus. The *ERK*1 cell extract was also fractionated on *DEAE*-cellulose, and the individual fractions used to immunize groups of mice. There was good correlation between the ability of fractions to elicit neutralizing antibody and their serum-blocking activity.

The neutralizing antibody formed in response to soluble antigen differed in some ways from that resulting from infection. The slope of the neutralization curve was less steep, with the result that a relatively high concentration of antibody was needed to produce almost complete neutralization of virus. Also, the neutralization titre against rabbit-pox virus was considerably less than that against vaccinia. In view of these differences, it would be unwise to attempt to predict the protective effect of immunization with soluble antigen. This problem is now being investigated.

It is concluded that the growth of rabbit-pox or vaccinia virus in a variety of tissues yields a soluble antigen which both combines with neutralizing antibody and elicits its formation when injected into rabbits or mice. It is hoped that the further study of this antigen will help to elucidate the mechanism of the infection of cells by pox viruses and its neutralization. The protective action of the antigen is not yet known; if effective, it might have practical application in the immunization of patients for whom a living vaccine is undesirable.

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CYTOLOGY

Chromosome Elimination in Cecidomyidae

Adult members of the gall midges (Cecidomyidae, Diptera) are characterized by possessing a large number of chromosomes in their germ-line cells and a small number in their somatic cells[1]. This difference is established during one or more of the early mitotic divisions when a large but species-specific number of chromosomes is eliminated from all the nuclei destined to form the soma. Elimination is caused by the failure of the majority of the pairs of chromatids to leave the equatorial plate at mitosis.

In *Mayetiola destructor* (Say) there are approximately forty chromosomes in the germ line cells and only eight in the somatic cells of both sexes. Elimination occurs in fourteen nuclei during the fifth mitotic division, when about 80 per cent of the chromosomes are eliminated. The remaining two nuclei, which prior to the fifth mitosis have migrated into the cytoplasm at the posterior end of the egg, do not divide at this time; cell walls surround them to form two pole cells. During subsequent divisions of the pole cells the full number of chromosomes is maintained, and the germ-line is formed.

It has been found that the two nuclei which do not divide during the fifth cleavage become associated at that time with particular granules which lie in the polar cytoplasm, at the extreme posterior end of the egg. Preliminary histochemical investigations indicate that these granules contain ribonucleic acid and are associated with many mitochondria.

In experiments designed to analyse the causes of chromosome elimination the extreme posterior ends of eggs containing the granulated polar cytoplasm were treated with ultra-violet irradiation before the two nuclei had migrated into this area. The remainder of each egg, containing all the nuclei, was shielded from the irradiation. In such eggs the two posterior nuclei took up their normal position near the polar granules and yet divided with chromosome elimination during the fifth mitosis. Eggs treated in this way later gave rise to embryos with the reduced number of chromosomes in all their cells. The presumptive germ cells, in spite of losing about 80 per cent of their chromosomes, still gave rise to the beginning of a germ line.

Similar effects were obtained by: (*a*) preventing the nuclei from migrating into the polar cytoplasm before elimination by constricting the eggs with fine nylon threads; (*b*) by centrifuging the eggs so that the nuclei were displaced and the polar granules were dispersed throughout the cytoplasm, where the granules lost their apparent function of preventing mitosis. In some eggs of both these experiments division with elimination occurred in all nuclei at the fifth mitosis. The constriction and centrifugation results confirm those of Geyer-Duszynska[2], working on another species.

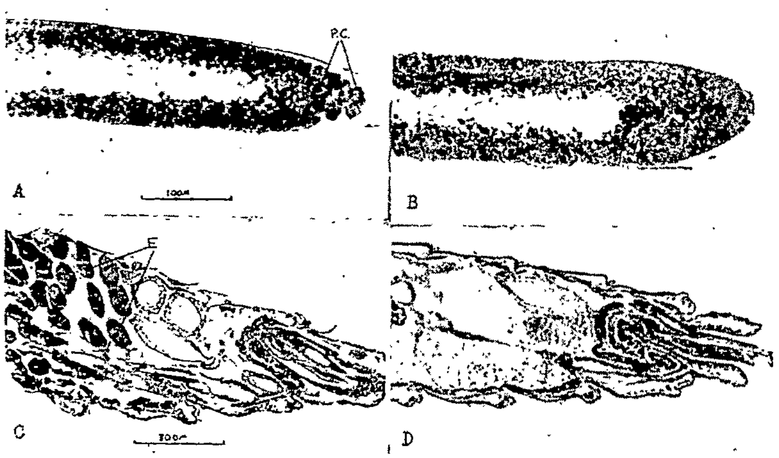

Fig. 1. *A*, Normal embryo at blastoderm stage. The large pole cells (*P.C.*) are at the posterior end; *B*, embryo at blastoderm stage in which the polar cytoplasm had previously been treated with ultra-violet irradiation. Note the absence of large pole cells; *C*, adult female with eggs (*E*); *D*, adult female developed from an embryo treated similarly to *B*. Note the absence of eggs

A few eggs, subjected to the irradiation treatment, were allowed to develop to maturity. So far, only females have emerged and been analysed. It was found that these were sterile and unable to lay eggs, although the secondary sexual characteristics were quite normal. The gonad consisted of a large mass of cytoplasm containing loosely scattered somatic nuclei and degenerating nuclei, apparently belonging to the germ-line. These nuclei were of small size, indicating clearly that they possessed a reduced number of chromosomes.

As a preliminary conclusion it appears that the polar granules prevent chromosome elimination. This might be due to an inhibition of the fifth mitosis in the presumptive germ-line nuclei, or to another mechanism, coinciding in time only with this inhibition. The absence of gametes in pupæ and hatched flies, which had developed from irradiated eggs, indicates that the lost chromosomes are required for gametogenesis.

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Synaptinemal Complexes (Cores) in Primary Spermatocytes of Mouse under Elevated Temperature

WE have observed in the electron microscope and determined statistically the synaptinemal complexes or cores in primary spermatocytes of male mice, *C57 B1*, exposed to an environment of 34·5° C. and 60 per cent humidity. To avoid confusion from the presence of two generations of spermatocytes in the same section we have evaluated only stages I–VI of the spermatogenic cycle.

According to classical findings, elevated temperature reduces the pairing, of homologues[1]. Since our own earlier work[2] and that of others[3–5] has shown that the cores are the chromosomal organelles of pairing, we were interested to observe the effect of elevated temperature on these structures. While the typical complete core is a triplet structure this triplet owes its origin to the coming together of two 'single' structures, one from each homologue; the third structure arises at the line of union[2]. Would elevated temperature result in the presence of the diploid number of unpaired single cores, or would elevated temperature suppress formation of cores proper (triplets)? As shown in Tables 1 and 2 it appears that elevated temperature reduces the total number of cores formed and also interferes with pairing so that the percentage of single cores rises.

A breakdown of the heat-exposed series is given in Table 2.

The time-lag of recovery suggests that the critical biochemical event of core inhibition may occur in the spermatogonia at least 19 days before pairing; but the event can probably also be evoked much more abruptly[6]. Our point of view based on functional and morphological evidence is that the tripartite core, that is, the synaptinemal complex of Moses[3,4] or our core proper, unites two homologous chromosomes in synapsis. This view conforms with those of Fawcett[5], Moses[3,4] Kaufmann[7], and others. In contrast, Sotelo and Trujillo[8] suggest that the tripartite core proper represents a haploid chromosome because they find

Table 1

	Total cells	Total cores	Single cores	Single cores (per cent)	Total cores per cell
Control, 22° C.	60	367	7	2	6·15
Heat-treated, 34·5° C.	56	142	41	29	2·5

Table 2

Treatment	Total cells	Total cores	Single cores	Single cores (per cent)	Total cores per cell
After 28½ hr. heat	19	36	11	30	1·9
After 52½ hr. heat	10	21	8	38	2·1
After 76½ hr. heat, and 14 days recovery	19	60	5	8	3·2
Same, but 19 days recovery	8	25	10	40	3·1

tri-, penta-, and hepta-partite cores in the spermatids of certain insects. While their photographs leave no room for doubt, could not the existence of cores in these insects be a very interesting anomaly? Research on haploid and triploid forms and on strains carrying multiple inversions should settle this point.

The recent work of Charard[9] also agrees with the interpretation of Moses and our interpretation.

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GENETICS

Effect of Induced Awn Mutations on Yield in Wheat

FROM comparisons between sister lines of the same parental derivation segregating in awn character and from experiments using backcrosses, awns have been found to have a positive function as regards grain yield in varieties of bread wheat[1-3]. A spontaneous awned mutant isolated by Mackey[4] in the variety Scandia III outyielded the parent strain in trials carried out over a period of 5 years. Awns have been supposed to influence yield by increasing assimilation and transpiration and thereby the size of the grains. Fully awned mutants frequently occur when varieties belonging to the tipped 1 class of Watkins and Ellerton[5] (that is, awns 1–2 cm. in length and confined to the uppermost spikelets) are treated with both ionizing radiations and chemical mutagens[6]. Several such mutants were found by us in the M_2 progenies of the varieties *N.P.* 799 and *N.P.* 809 treated with phosphorus-32 and sulphur-35 (both 5 µc. per seed), X-rays (16,000 and 22,000 r.) and different dosages of fast and thermal neutrons. Both these varieties have the dominant epistatic gene *B*1 on chromosome 5*A* (nomenclature of Sears[7]) and the established awn mutations behaved as monogenic changes in genetic studies. The mutants had the somatic chromosome number 42 and no prominent change could be detected in their karyotypes.

Twenty-five awned mutants of *N.P.* 799 and six such mutants of *N.P.* 809 were grown in replicated rod-row trials during 1958. All the awned mutants of *N.P.* 799 yielded more than the parent strain, while in *N.P.* 809 some yielded better and some lower. In both the varieties, the mutants found to be most promising in the first trial maintained their superiority in trials conducted during subsequent years. The yield of these awned mutants is indicated in relation to that of the parents in Tables 1*a* and *b*.

Table 1. RELATIVE GRAIN YIELD OF AWNED MUTANTS

(*a*) *N.P.* 799

Trial conducted at:	Awned mutant	Yield (percentage of control)	
		1958–59	1959–60
New Delhi	799–25	120·11*	109·52*
	799–6	110·00*	103·17
Pusa (Bihar)	799–25	110·85*	104·49
	799–6	102·21	104·24

(*b*) *N.P.* 809

Trial conducted at:	Awned mutant	Yield (percentage of control)		
		1957–58	1958–59	1959–60
New Delhi	809–1	126·14*	111·12*	102·14
	809–6	106·43*	106·06*	—
	809–9	104·12	—	—
Simla (Himachal Pradesh)	809–6	—	110·68*	—
	809–9	—	106·10	—
Karnal (Panjab)	809–6	—	—	100·86

* Significantly higher.

The awned mutants of *N.P.* 799 and *N.P.* 809, the performance of which in yield trials is given in Tables 1*a* and *b*, resembled the parents in all other characteristics like maturity period, shape and quality of grain and rust resistance. Thus, the increased yielding ability of some of them could be due to the addition of awns. Vervelde[2] has shown that in warm and dry areas awns play a particularly important part in grain production.

In the plains of India, awned wheats are liked by the farmer since they are damaged less by birds and hence the fully awned mutants of *N.P.* 799 and *N.P.* 809 have a double advantage, that is, they help to satisfy the needs of the farmer and to enhance the yield of the strain. *N.P.* 799–25, an awned mutant which has performed well in the yield trials conducted until now, has been named *N.P.* 836, and it will be tested in larger trials during the coming years.

Of late, there has been much scepticism concerning the possibility of adding through mutation breeding a desirable trait alone to an established variety without simultaneously bringing about some undesirable changes. The agronomic qualities of the fully awned mutants reported in this communication would suggest that in polyploids like bread wheat, which readily withstand whole and part-chromosome deletions, mutation breeding may have considerable scope, particularly if the deletion of a dominant epistatic locus has a favourable effect.

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Transmission of Phosphorus-32 incorporated by Parents into Descendants of *Drosophila melanogaster*

THE quantity and distribution in various phosphorus fractions of phosphorus-32 incorporated into parents by feeding on media containing phosphorus-32 was followed in descendants of *Drosophila melanogaster* strain *Algeria*. The animals were bred at 25° C. in incubators on Belgowsky's culture medium[1] with some drops of a yeast suspension. Reciprocal crosses were made between animals grown on media containing isotopic and normal phosphorus. Repeated passages were carried out in order to eliminate the eventual contamination by phosphorus-32 of the culture media of the descended larvæ by the labelled parents. The fractionation of the phosphorus compounds was carried out according to the Schmit–Tannhauser method[2]. The amount of acid-labile phosphorus in the acid-soluble fraction was assayed by the method of Berenblum[3]. The lipoid-soluble fraction was extracted with a mixture of alcohol/ether (3 : 1). Deoxyribonucleic acid (DNA) was separated after acidification with trichloracetic acid. The protein phosphorus, represented as orthophosphate, was separated from the DNA remaining in the solution by direct phosphorus determination.

The distribution in seven different fractions of phosphorus originating from the culture medium and transmitted to the progeny is shown in Table 1.

Table 1. PHOSPHORUS FRACTIONS IN THE ONTOGENY OF *Drosophila melanogaster* IN γ P/100 LARVÆ

Stage of development	Four-day-old larvæ	Young pupæ	Imagos Female	Imagos Male
Weight per individual (mgm.)	1·82	1·34	1·30	0·92
Fractions:				
Alcohol-soluble	1040	970	1550	380
Acid-soluble	1720	2100	1070	375
Acid-labile	(472)	(1640)	(520)	(54)
Lipoid-soluble	27	23	75	67
DNA fraction	66	102	118	42
Ribonucleic acid fraction	2207	1198	848	532
Protein fraction	111	55	192	333
Total	5171	4448	3853	1729

It may be seen that the intensive morphogenetical processes are preceded by a marked increase in the acid-soluble fraction, mainly at the expense of the alcohol-soluble one. From the pupal stage onwards an increase of lipoid phosphorus was observed. The lowering of ribonucleic acid (RNA) content is significant and might be responsible for the decreasing rate of protein synthesis. A pronounced increase in protein phosphorus was observed in the imago.

Table 2. THE DISTRIBUTION OF PHOSPHORUS ORIGINATING FROM FEMALE AND MALE GAMETES IN THE PHOSPHORUS FRACTIONS OF THE OFFSPRING (γ P/1000 LARVÆ)

Fractions \ Direction of the crosses	Labelled female × normal male	Normal female × labelled male
Alcohol-soluble	4·8	traces
Acid-soluble	8·3	0·130
Acid-labile	(7·4)	(traces)
Lipoid-soluble	1·5	0·042
DNA fraction	1·3	0·054
RNA fraction	6·2	0·099
Protein fraction	3·3	0·150
Total	25·4	0·475

Table 3. THE DISTRIBUTION OF PHOSPHORUS DERIVED FROM THE MEDIUM AND FROM THE GAMETES IN THE F_1 LARVAE OF *Drosophila* (per cent)

Fractions \ Origin of phosphorus	Culture medium (per cent)	Labelled female gametes (per cent)	Labelled male gametes (per cent)
Alcohol soluble	20·2	18·8	—
Acid soluble	33·4	32·9	27·1
Acid labile	(9·1)	(29·4)	(?)
Lipoid soluble	0·7	5·9	8·7
DNA fraction	1·3	5·1	11·8
RNA fraction	42·2	24·5	20·8
Protein fraction	2·2	12·8	31·6
Total	100·0	100·0	100·0

The distribution of phosphorus-32 in the phosphorus fractions of the larvæ orginating from reciprocal crosses is given in Table 2.

It is clear that the quantity of phosphorus transmitted via the ovocyte is fifty times higher than that transmitted by the male gametes. This figure is probably even higher. We must take into consideration the possibility of polyspermy which is a widespread phenomenon in *Drosophila melanogaster*. In the case of a 25-fold polyspermy the transmission by ovocytes surpasses the male transmission 1,250 times. The possibility that the differences are to be attributed to a higher turnover rate of the ovocytes was eliminated by autoradiographic examinations.

The distribution of phosphorus-32 in the phosphorus fractions derived from the medium, from the gametes or the food is shown in Table 3.

It can be seen that the distribution of phosphorus-32 in various phosphorus fractions derived from food, from male or female gametes, is very different. It is obvious that an equal amount of labelled phosphorus is incorporated into the acid-soluble compounds of the alcohol-soluble fraction from both the food and the ovocytes. By contrast, only traces of a similar incorporation were found if the transmission took place via the male germ-cells. There was no remarkable difference in the acid-soluble fraction; however, more phosphorus was incorporated into the acid-labile fraction via the ovocytes than from the food. Relatively less phosphorus was recovered in DNA if incorporated directly from the food than after transmission via the gametes. The differences between the phosphorus content of the RNA and protein fractions might be explained by the different amounts of reserve substances and cytoplasm contained by the ovocytes.

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Hexaploid *Dactylis*

A RECENT intensive survey of *Dactylis* has shown that tetraploids ($2n=28$) are widely distributed throughout the temperate parts of the world[1]. In Europe and Asia, regions where this genus is native, diploids are also found, and each of these has a comparatively limited range of distribution[2].

Table 1. Meiotic Pairing

Plant No.		Metaphase I pairing I	II	III	IV	V	VI	VII	X	Mean chiasmata	Total pollen mother cells
Bc 4556 4	Mean	0·32	9·31	0·96	1·76	0·36	1·0	0·20	0·04	34·28	25
	Range	0–1	7–17	0–2	0–3	0–2	0–4	0–1	0–1		
Bc 4556 13	Mean	—	17·30	—	1·00	—	0·40	—	—	34·05	20
	Range	—	10–21	—	0–4	—	0–2	—	—		
Bc 4556 14	Mean	—	11·40	0·40	3.00	—	1·0	—	—	34·40	5
	Range	—	6–15	0–2	3	—	1–3	—	—		

As a result of screening 175 populations derived from Europe, Western Asia, the Mediterranean and North Africa, wild hexaploids have been discovered in material from Cyrenaica, and these have been compared with wild hexaploids from Egypt, sent to us by Mr. R. Knight, of the Waite Agricultural Research Institute[3].

Taxonomically, these plants are indistinguishable from forms of *Dactylis glomerata* ssp. *hispanica* Domin. Both groups as grown at Aberystwyth have a stout root-stock with bulbous-based tillers, slender, stiff, tough leaves with markedly toothed margins, and slender wiry culms up to 60 cm. tall. The heads are usually compact, the range of forms which occur being similar in both groups. The florets are short, the lemmas having pubescent keels and the lobed apex characteristic of ssp. *hispanica*[4].

The first introduction formed part of a collection made by the Food and Agriculture Organization[5], and originated from the top of Tocra Pass, Cyrenaica, at an altitude of 350–400 m., with a yearly rainfall of about 300 mm. The soil was a stony red clay-loam (Accession No. *Bc* 4556 = F.A.O. 3076). The second population was collected at Rasel Hekma, at a low altitude in the Western Desert of Egypt, with a rainfall of 100 mm. (Accession No. *Bc* 4860).

Chromosome counts were made at meiosis on four plants from Tocra Pass[6]. Twelve of the Rasel Hekma plants were counted at Adelaide, and five of their progeny at Aberystwyth. All were hexaploid. Meiotic behaviour (Fig. 1) was studied by Keith Jones in 50 pollen mother cells of three Cyrenaica plants; the results are given in Table 1, showing some variation in pairing from plant to plant. In plant 13, bivalents predominated, quadrivalents and hexavalents were few and no odd number associations were seen. On the other hand, plant 4 is an interchange heterozygote, and partly as a consequence of this, multivalents were more frequently observed.

It seems probable that, in population plants not heterozygous for interchanges, bivalent pairing will be the general rule. Forty per cent of the cells in plant 13 formed bivalents only.

The size and fertility of the hexaploid pollen was assessed (Table 2). The fertility equals that found for diploid and tetraploid *Dactylis*[7]. The diameter of the pollen of the hexaploid was less than that generally found in the tetraploids, and we consider that this is probably associated with the extreme xeromorphic adaptation of these plants.

Table 2. Pollen Data

Ploidy	Material	Pollen: Diameter (arbitrary units) Mean	Pollen: Diameter (arbitrary units) Range	Pollen: Fertility (per cent)
2 ×	8 populations, including 7 sub-species	7·88	7·13–8·72	78
4 ×	6 populations, including 3 sub-species	9·09	8·67–9·60	82
6 ×	1 genotype, a twin seedling from a tetraploid	8·55	—	84
	Bc 4860, 1 population	8·40	8·00–9·10	88
	Bc 4556, 1 population	—	—	83

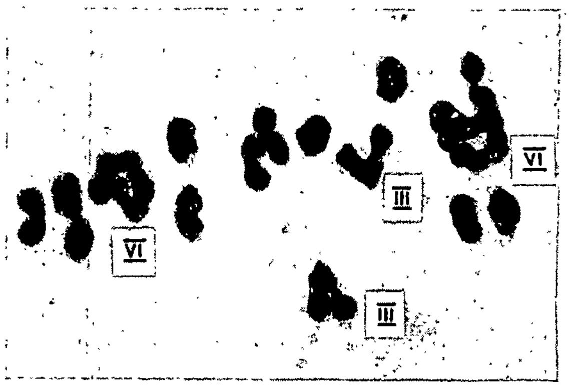

Fig. 1. *Bc*4556, No. 4 $2n = 42$. 2 VI, 2 III, 12 II

The discovery of hexaploid *Dactylis* in two localities in the same geographical area suggests that the type may have a much wider distribution in this region. Ecologically, these forms are adapted to dry conditions in areas of low average rainfall, and long periods of summer drought. These plants may, of course, grow in sites favoured by extra run-off moisture, as described by Boyko[8] for a *Dactylis* ecotype in Israel, which we found to be tetraploid (Accession No. *Bc* 4160). Even so, unequal distribution rather than actual amount of annual rainfall is probably the critical factor in relation to tolerance of drought.

The two most probable modes of origin of the hexaploid are by somatic doubling in a triploid hybrid, or following the occurrence of non-reduction in a tetraploid. Wild triploids have been reported from Israel in areas where diploids and tetraploids meet[9]. We suppose that the hexaploids arose by the doubling of such triploids, which, if they possessed favourable new combinations of genes, might be adapted to survive in the most extreme habitats, and be able to colonize such areas in the event of the chance restoration of fertility by chromosome doubling.

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[1] Borrill, M., *J. Linn. Soc.* (*Bot.*), **56**, 368, 441 (1961).

[2] Stebbins, G. L., and Zohary, D., *Univ. Calif. Pub. Bot.*, **31**, No. 1, 1 (1959).

[3] *Report of the Waite Agricultural Research Institute, S. Aus.*, 1954–1955, 15.

[4] Domin, K., *Acta Bot. Bohem.*, **14**, 3 (1943).

[5] List of Grassland Ecotypes collected by F.A.O. in 1954 and 1955 in *The Natural Vegetation of the Mediterranean Region and the Near East*, p. 11.

[6] Jones, K., and Carroll, C. P., *Cytologia* (in the press).

[7] Borrill, M., *Genetica* (in the press).

[8] Boyko, H., and Tadmor, N., *Bull. Res. Council Israel*, **4**, 241 (1954).

[9] Zohary, D., and Nur, V., *Evolution*, **13**, 311 (1959).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, May 1

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. Graham, Mr. J. W. H. Justice and Mr. J. K. Oxenham: "Progress Report on the Development of a Photo-electric Beam Index Colour Television Tube and System".

Society of Engineers (at the Engineering, Marine, Welding and Nuclear Energy Exhibition, Olympia, London), at 5.30 p.m.—Mr. R. H. Bound: "Jet Propulsion for Boats".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. Tom A. Margerison: "The Presentation of Science". (First of three Cantor Lectures on "The Presentation of Science and the Arts on Television".)

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Mr. E. G. Peppiatt: "Chemistry in the Service of a New Industry".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Capt. R. H. Grant, M.C., R.M. : "The Conquest of Annapurna II".

Monday, May 1—Tuesday, May 2

Electrical Association for Women (in London)—Thirty-sixth Annual Conference.

Tuesday, May 2

Institution of Electrical Engineers, Measurement and Electronics Sections (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. D. W. Pashley: "Structural Properties and Applications of Evaporated Metal Films"; Mr. J. R. Balmer: "Deposition of Evaporated Non-magnetic Films".

Wednesday, May 3

Zoological Society of London (in the Meeting Room, Zoological Gardens, Regent's Park, London, N.W.1), at 10 a.m.—Symposium on "Biological Acoustics".

British Institution of Radio Engineers, Computer and Radar Groups (at University College, Gower Street, London, W.C.1), at 3 p.m.—Symposium on "Computer Control of Air Traffic".

Institute of Physics and the Physical Society (in the Physics Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 5 p.m.—Dr. Scott E. Forbush (Carnegie Institution of Washington): "The Equatorial Electro-Jet in Peru".

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. W. K. B., Marshall and Mr. V. I. Holmes: "Recent Developments in Arc Welding as Applied by the Petroleum Industry".

Society for Analytical Chemistry (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Scientific Papers.

Wednesday, May 3—Thursday, May 4

Institutions of Civil, Mechanical, and Electrical Engineers (at the Institution of Civil Engineers, Great George Street, Westminster, London, S.W.1)—Railway Modernization Conference.

Iron and Steel Institute and the Institute of Metals (at the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 2.30 p.m. on Wednesday and 10 a.m. on Thursday—Symposium on "Structural Processes in Creep".

Iron and Steel Institute (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1)—Annual General Meeting.

Thursday, May 4

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. M. S. Bagguley, Mr. R. A. Stradling and Mr. J. S. S. Whiting: "Cyclotron Resonance Over a Wide Temperature Range. 1: Germanium. 2: Silicon".

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. J. W. L. Beament, Dr. H. E. Hinton, F.R.S., Dr. S. M. Manton, F.R.S., and Dr. John Smart: "On Aspects of the Classification of Insects".

Institute of Refrigeration (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. G. P. Smedley: "Metals for Refrigeration and Other Low Temperature Installations".

University College, London (in the Chemistry Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. Hamilton: "Advances in Elementary Particle Physics".*

University of London (at the Senate House, University of London, Malet Street, London, W.C.1), at 5.30 p.m.—Prof. Roger Y. Stanier (University of California): "Bacterial Photosynthesis".*

Chemical Society (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Prof. A. J. Birch, F.R.S.: "Some Pathways in Biosynthesis" (Simonsen Lecture).

Friday, May 5

Institution of the Rubber Industry and the Plastics Institute (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 9.30 a.m.—Conference on "Foamed Rubber and Plastics".

Institution of Electrical Engineers, Medical Electronics Discussion Group (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Artificial Muscles" opened by Dr. A. B. Kinnear Wilson.

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. R. Hafner: "The Helicopter—The First of the VTOL Aircraft".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. Bart J. Bok (Canberra): "The Southern Milky Way".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or Assistant Lecturer in the Department of Philosophy—The Registrar, The University, Leeds 2 (May 6).

Lecturer (preferably with an interest in physical biochemistry or the study of enzymes and their mechanisms) in Biochemistry—The Registrar, Manchester College of Science and Technology, Manchester 1 (May 6).

Research Assistant (chemist, preferably with two or three years research experience) for work on volatile inorganic compounds, in collaboration with Dr. L. H. Long—The Secretary, The University, Exeter (May 8).

Lecturer in Pharmacology—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (May 9).

Lecturer in the Department of Logic and Metaphysics—The Secretary, The University, Edinburgh (May 10).

Lecturer or Assistant Lecturer in the Department of Inorganic and Structural Chemistry—The Registrar, The University, Leeds 2 (May 12).

Senior Lecturer in Pharmacy in the Science Department, Jamaica College of Arts, Science and Technology, Kingston—Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (May 12).

Assistants (2) in Civil Engineering—The Secretary of University Court, The University, Glasgow (May 13).

Research Fellow in Pure Mathematics—The Registrar, University College, Singleton Park, Swansea (May 13).

Senior Scientific Officer/Scientific Officer Physicists (with a first- or second-class honours degree in physics, chemistry or metallurgy or equivalent) at the National Physical Laboratory, Teddington, Middlesex for (1) development of line gratings and detectors for X-rays in the wave-length region 1 Å. to 100 Å. and (2) application of diffraction gratings to the precise measurement of X-ray wavelengths—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting A.162/A (May 13).

Assistant in the Department of Moral Philosophy—The Secretary, The University, Edinburgh (May 15).

Lecturer in Agricultural Chemistry, with special reference to Animal Biochemistry and Nutrition—The Secretary, Wye College (University of London), near Ashford, Kent (May 15).

Lecturer or Assistant Lecturer (preferably with an interest in the fields of mycology and plant pathology) in the Department of Botany—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (May 15).

Lecturers and Assistants in Experimental Physics, Theoretical Physics and Electronics—The Secretary, The University, Aberdeen (May 15).

Lecturer in Chemical Engineering—The Registrar, University College, Singleton Park, Swansea (May 20).

Lecturer (with a degree in veterinary science from an approved university, or equivalent, and at least three years postgraduate experience, including small animal practice) in Veterinary Medicine at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 20).

Organic Chemist (with a university degree or technical college diploma in organic chemistry, or equivalent qualifications, and preferably experience with biological systems) in the Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T., Australia, to collaborate with a research group investigating the chemical nature and mechanism of action of growth regulators isolated from plants—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 130/469 (May 20).

Lecturer (preferably with a special interest in geomorphology) in Geography—The Registrar (Room 22, O.R.B.), The University, Reading (May 27).

Demonstrator (zoologist, botanist or geneticist) in Biology—The Registrar, The University of Adelaide, Adelaide, South Australia (May 29).

Reader in Chemical Metallurgy at the Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 29).

Senior Lecturers (2) (one with special interests in the field of mycology, and the other in anatomy, ferns or algae) in the Department of Botany, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 30).

Research Fellows (2) in the Department of Anthropology and Sociology, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 31).

Senior Lecturer or Lecturer (with postgraduate experience in research, coupled with a keen interest in teaching) in Physics at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, May 31).

LECTURER IN PHILOSOPHY at the University of Melbourne, Australia —The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 1).

READER (with appropriate academic qualifications and teaching experience) IN PHILOSOPHY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, June 9).

BIOCHEMIST (honours graduate), to assist with research on lipid metabolism in gastric mucosa—Dr. Billing, Department of Medicine, Royal Free Hospital, London, W.C.1.

BIOMETRICIAN (graduate with mathematical and biological interests, and preferably a knowledge of statistics) IN THE SECTION OF STATISTICS, for duties involving consultation and research on design and analysis of experiments—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. 61/12.

CHIEF TECHNICIAN (Grade 1) IN THE DEPARTMENT OF ZOOLOGY, to take charge of the technical staff and services of the Department, and to assist with planning in connexion with a proposed new building —The Assistant Bursar, The University, Reading.

INFORMATION OFFICER (university graduate with at least three years information experience, preferably in science and technology, and the ability to supervise professional staff)—The Director, Aslib, 3 Belgrave Square, London, S.W.1.

LECTURER (graduate in physics or chemical engineering) IN PHYSICS —The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

LECTURER IN BIOLOGY IN THE TRAINING DEPARTMENT, with particular interest in natural history—The Warden, University of London, Goldsmiths' College, New Cross, London, S.E.14.

LECTURER (with qualifications in applied chemistry or chemical engineering, and experience in industry, research or teaching) IN THE DEPARTMENT OF CHEMICAL TECHNOLOGY—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1.

LECTURER (with research experience) IN CHEMISTRY, to teach undergraduates and carry on fundamental research—Prof. J. K. N. Jones, Queen's University, Kingston, Ontario, Canada.

PHYSIOLOGIST or ZOOLOGIST (recent graduate) IN THE PHYSIOLOGY DEPARTMENT, to assist with research on the bio-assay of hormones in blood—The Secretary, National Institute for Research in Dairying, Shinfield, Berks, quoting Ref. 61/11.

RESEARCH ASSISTANTS (2) (with a Ph.D. or suitable taxonomic experience) IN PLANT TAXONOMY, to collaborate in compiling a flora of Turkey—Dr. P. H. Davis, University Department, Royal Botanic Gardens, Edinburgh 3.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Henry Wiggin and Co., Ltd. Publication No. 1941: Machining Wiggin High-Nickel Alloys. (London and Birmingham: Henry Wiggin and Co., Ltd., 1959.) [271

The Organization and Financing of Research in Biochemistry and Allied Sciences in Great Britain. Edited by K. S. Dodgson. Pp. 51. (London: The Biochemical Society, 1960.) 2*s*. 6*d*. [301

National Museum of Wales. Fifty-third Annual Report, 1959–60. Pp. 78+4 plates. (Cardiff: National Museum of Wales, 1960.) [301

Sabouraudia, Vol. 1, Part 1 (January 1961). (Journal of the International Society for Human and Animal Mycology.) Pp. 72+12 plates. Subscription price per volume (four issues) 80*s*.; or 12 dollars. Single parts 20*s*., or 3 dollars. (Edinburgh and London: E. and S. Livingstone, Ltd., 1961.) [301

Regional Advisory Council for Technological Education—London and Home Counties. Twelfth Annual Report, 1959–60. Pp. 48. (London: Regional Advisory Council for Technological Education—London and Home Counties, 1961.) [301

Department of Agriculture and Fisheries for Scotland. Spaced-Plant Trials at East Craigs. VII: Varieties of Cocksfoot *Dactylis glomerata* L., 1956–58. Pp. 6. (Edinburgh: Department of Agriculture and Fisheries for Scotland, 1961.) [301

Ministry of Agriculture, Fisheries and Food. Bulletin No. 154: Grassland Management. Third edition. Pp. v+69. (London: H.M. Stationery Office, 1960.) 4*s*. 6*d*. net. [311

Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1959–60. Republic of the Sudan. Pp. 33. 2*s*. 6*d*. Uganda. Pp. 45. 2*s*. 6*d*. (London: Empire Cotton Growing Corporation, 1960.) [311

Ciba (A.R.L.), Ltd. Technical Notes, No. 217 (January, 1961): "Tecton" Roofing Units. Pp. 6. (Duxford: Ciba (A.R.L.), Ltd., 1961.) [311

Problems of Cybernetics, 1. Chief Editor: A. A. Lyapunov. Translation Editor: Richard Goodman. Consulting Editor: A. D. Booth. Translated from the Russian by M. Nadler, F. J. Griffiths, G. Kiss and S. A. Muit. Pp. 1–214. (London and New York: Pergamon Press, 1960.) 210*s*.; 30 dollars. [311

Scottish Education Department. Technical Education in Scotland: The Pattern for the Future. Pp. 16. (Cmnd. 1245.) (London: H.M. Stationery Office, 1961.) 1*s*. net. [22

Ministry of Agriculture, Fisheries and Food. Agricultural Chemical Approval Scheme (Insecticides, Fungicides and Herbicides): List of Approved Products, 1961. Pp. 93. (London: Ministry of Agriculture, Fisheries and Food, 1961.) [22

Science Masters' Association and the Association of Women Science Teachers. Science and Education: a Policy Statement issued by the Science Masters' Association and the Association of Women Science Teachers. Pp. 12. 1*s*. net. Chemistry for Grammar Schools. Pp. 39. 2*s*. net. Biology for Grammar Schools. Pp. 24. 2*s*. net. Physics for Grammar Schools. Pp. 39. 2*s*. net. (Sections of Part 1 of the Science and Education Report issued by the Science Masters' Association and the Association of Women Science Teachers, to be read in conjunction with the Policy Statement.) (London: John Murray (Publishers), Ltd., 1961.) [22

Department of Scientific and Industrial Research: Warren Spring Laboratory. Mineral Processing Information Notes. No. 1: Supercritical Grinding—a Survey of Current Research. Pp. ii+15. No. 2: Cyclones in Mineral Processing—a Review of Current Information. Pp. iii+29. (London: Department of Scientific and Industrial Research, 1960.) [22

Field Studies Council. Annual Report, 1959–60. Pp. 32+4 plates. (London: Field Studies Council, 9 Devereux Court, W.C.2, 1961.) [32

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 703, Vol. 243 (2 February, 1961): The Terminations of the Afferent Nerve Fibre in the Muscle Spindle of the Frog. By B. Katz. Pp. 221–240+plates 26–61. 60*s*. No. 704, Vol. 243 (2 February, 1961): The Organization of the Atrial Nervous System of Amphioxis (*Branchiostoma lanceolatum* (Pallas)). By Q. Bone. Pp. 241–269+plates 62–65. 16*s*. (London: Royal Society, 1961.) [32

Discovery Reports. Vol. XXXI, pp. 1–222+plates 1–3: Swimbladder Structure of Deep-Sea Fishes in Relation to Their Systematics and Biology. By N. B. Marshall. 55*s*. net. Vol. XXXI, pp. 123–298: The Benguela Current. By T. John Hart and Ronald I. Currie. 75*s*. net. (Cambridge: At the University Press, 1960.) [32

Other Countries

Tanganyika. Records of the Geological Survey of Tanganyika, Vol. 8, 1959. Pp. ii+113+18 plates. Shs. 17.50. Geological Survey of Tanganyika. Memoir No. II: The Rungwe Volcanics at the Northern End of Lake Nyasa. By D. A. Harkin. Pp. ix+172+31 plates. Shs. 25. (Dar-es-Salaam: Government Printer, 1960.) [271

United States Department of the Interior: Geological Survey. Water-Supply Paper 1524: Quality of Surface Waters for Irrigation, Western United States, 1957. Prepared under the direction of S. J. Love. Pp. viii+183+plate 1. (Washington, D.C.: Government Printing Office, 1960.) 1 dollar. [271

Annual Report of the Board of Regents of the Smithsonian Institution showing the Operations, Expenditures, and Condition of the Institution for the year ended June 30, 1959. Pp. x+693+92 plates. (Publication 4392.) (Washington, D.C.: Government Printing Office, 1960.) [271

Astronomical Photographs—Prints—Slides—Transparencies from the collection of the Yerkes Observatory. 1960 edition. Pp. 36. (Chicago: Yerkes Observatory, University of Chicago, 1960.) [301

Food and Agriculture Organization of the United Nations. FAO Agricultural Studies, No. 51: Vibrio Fetus Infection of Cattle. Edited by J. A. Laing. Pp. ix+62. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1960.) 5*s*.; 1 dollar. [301

Smithsonian Institution. Annual Report of the United States National Museum for the year ended June 30, 1960. Pp. vi+175. (Washington, D.C.: Government Printing Office, 1960.) [301

Annals of the New York Academy of Sciences. Vol. 84, Article 17: Culture, Society, and Health. By Dorothy L. Keur, Vera Rubin and 40 other authors. Pp. 783–1060. Vol. 91, Article 1: Fundamentals of Psychology: the Psychology of Thinking. By J. P. Guilford and 10 other authors. Pp. 1–158. (New York: New York Academy of Sciences, 1960.) [301

Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique, No. 88: La Mise en Valeur Agricole du Sud-Est du Kasai: Essai de Géographique Agricole et de Géographie Agraire et ses Possibilités d'Applications Pratiques. Par Dr. H. Beguin. Pp. 289+12 photographies. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1960.) 400 francs. [301

United States Naval Observatory. Circular No. 90: Provisional Ephemeris of Mars 1960–2000. By R. L. Duncombe and G. M. Clemence. Pp. 48. (Washington, D.C.: United States Naval Observatory, 1960.) [301

Transactions of the American Philosophical Society. New Series, Vol. 50, Part 10: Cultural Sequences at the Dalles, Oregon—a Contribution to Pacific Northwest Prehistory. By Prof. L. S. Cressman, in collaboration with David L. Cole, Wilbur A. Davis, Thomas M. Newman and Daniel J. Scheans. Pp. 108. (Philadelphia: American Philosophical Society, 1960.) 3 dollars. [301

East African Meteorological Department. The Weather of East Africa during 1958. Pp. ii+54. (Nairobi: East African Meteorological Department, 1960.) [301

Nautical Almanac Office, U.S. Naval Observatory. Astronomical Papers prepared for the use of the American Ephemeris and Nautical Almanac. Vol. 16, Part 1: Motions of Venus, 1750–1949. Pp. 258. (Washington, D.C.: Government Printing Office, 1958.) 2 dollars. [301

Annals of the New York Academy of Sciences. Vol. 84, Article 16: Deuterium Isotope Effects in Chemistry and Biology. By David Kritchevsky, Joseph J. Katz and 36 other authors. Pp. 573–781. (New York: New York Academy of Sciences, 1960.) 4 dollars. [311

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SUPPORT FOR FUNDAMENTAL RESEARCH

IN reprinting, as part of its tenth anniversary celebrations, *Science: the Endless Frontier*, by Dr. Vannevar Bush, the National Science Foundation included an introduction by its director, Dr. A. T. Waterman, summarizing the extent to which, in the fifteen years since the report was published, its recommendations have been realized. Dr. Waterman recognizes that all the problems relating to the understanding and nature of basic research have not been solved, but he can point to a steady upward trend in Federal funds for basic research and to an increase in the number and diversity of Government sources providing such support. Some progress has also been recorded in research activities within the Government, and the establishment of the President's Science Advisory Committee is in line with the recommendation in the report that a Science Advisory Board should be created. Medical research was another major emphasis in Dr. Bush's report, especially basic research in this field, and here again there has been a rapid increase in available funds; and the organization established—the Division of Biological and Medical Sciences and the National Institutes of Health—meet and even go beyond that visualized in the report. In defence research, however, the situation is rather different from that visualized by Dr. Bush, but the substance of his principal recommendations is probably met by the arrangements with the universities continued by the Office of Scientific Research and Development, the generous support given to basic research by the three Armed Services, and by the number of leading scientists, both within the Department of Defense and in outside institutions, who are dealing with defence problems.

Something has also been done to improve the position with regard to international relations in science, notably by the appointment of scientific attachés, of a Science Adviser to the Secretary of State, and in such matters as visas, attendance at scientific conferences, etc. Notable progress has been made in implementing the recommendations in the chapter discussing the renewal of scientific talent, and, compared with a recommended programme of 29 million dollars by the fifth year, the National Science Foundation by 1960 had obligations for scientific personnel and education totalling more than 65 million dollars.

Recommendations relating to the dissemination of scientific information seem largely to have been fulfilled, and Dr. Waterman's introduction directs particular attention to the development of the National Science Foundation, the establishment of which was put forward as a principal means of implementing other major recommendations in the report. Its structure is substantially that recommended by Dr. Bush, and the Foundation has sought to observe the five basic principles enunciated by him. First, it seeks to ensure the continuity and stability that are needed in basic research; secondly, particular attention has been paid to the calibre of those serving either on its staff or as advisers or consultants; thirdly, it operates no laboratories of its own, but promotes research through grants or contracts; fourthly, it has kept to a minimum any conditions required of academic institutions; finally, it has not found its responsibilities to the President and Congress incompatible with independence and freedom of operation. While, however, Dr. Bush visualized the Foundation as the sole support in the Government of basic research, this has been far from the fact, and of about 200 million dollars for basic research in 1956, some 72 million dollars went through the Department of Defense, 45 million dollars through the Atomic Energy Commission, 26 million dollars through the National Institutes of Health, and the remainder was distributed through other Departments as well as the Foundation. In 1956, its fifth year, its funds totalled only 16 million dollars compared with 122·5 million dollars suggested by Dr. Bush, but in its ninth year, 1960, they had risen to 159·2 million dollars. Dr. Waterman considers that the organization provides the desirable flexibility as well as opportunity for expansion, but points out that there is still insufficient public appreciation of the importance of science and of education as distinct from technology.

This introduction by Dr. Waterman may be appropriately set beside the statement which introduces the Foundation's tenth annual report, covering the year ended June 30, 1960, which has now been published (see p. 494), and the chapter in the report, "Stimulating the National Research Effort", which reviews the Foundation's programmes for promoting basic research and education in the sciences in the broad context of the Federal Government's response to the challenge thrown down by the President's Science Advisory Committee in its report two years ago entitled *Strengthening American Science*. First, the progress of scientific research is considered, including the conduct of basic research, methods of support, facilities for research, participation by the United States in international programmes of research, notably in the International Geophysical Year and the continuing programmes of International Geophysical Co-operation and in the Antarctic, in which United States participation is developed and co-ordinated by the National Science Foundation, with recommendations from the National Academy of Sciences, and the communication of scientific information. Next, steps taken to assist the development of the individual scientists are outlined, such as the provision of fellowships, projects to train more science teachers and measures to improve the curriculum; and thirdly, those factors which determine the most favourable conditions for the growth of institutions for teaching and for research are considered.

On the first, the review emphasizes the extent to which the Federal Government now provides support for basic research; the man-power engaged in such research is to be found in some 450 universities and research institutions. It is interesting to note that, in discussing methods of support, the need for university co-operation is recognized. Like the Advisory Council for Scientific Policy in Britain, the National Science Foundation has come to recognize that desirable projects may cut across departmental boundaries and may even involve inter-university co-operation, and is now making grants of a broader type. As examples, one to the University of Pennsylvania is in support of research applying concepts of chemistry and physics to the biological problem of regulation of metabolism within the cell, while another will permit a concerted attack, at the Massachusetts Institute of Technology, on the problem of the production and nature of plasmas.

Here, and in regard to the provision of adequate facilities for research, the National Science Foundation and the Advisory Council for Scientific Policy appear to be working on similar lines; but the Foundation appears to be much more vigorous in its efforts to promote the communication of scientific information. Divergence is even more marked in regard to efforts to deal with the shortage of science teachers and the improvement of curricula, and it will be noted that in the Director's statement which precedes the review, Dr. Waterman suggests that for the future the main concern of the Federal Government in planning more effectively for science and technology should be in the field of education. Nevertheless, the Foundation's experience confirms that of the Advisory Council on Scientific Policy on the importance of sufficient Government support to research institutions and universities to ensure a proper balance of activities, and on the need to identify the critical areas of basic research and see that they are adequately supported; as well as to ensure that the possibilities of co-operative effort are not neglected, while, on the other hand, the weaknesses of massive, concerted efforts are also recognized and avoided.

Generally, the review suggests that Federal support of basic research and education in the sciences is sufficiently vital and flexible in the United States, though it is suggested that the situation calls for general aid to the universities on some such lines as those provided by the University Grants Committee in Britain. The Federal Government, however, is regarded as now better prepared for the discharge of its responsibilities in science, although stronger support for basic research is still required and the country's potential in science and technology is far from being realized. More effort, too, is needed in training scientists and engineers, and this is seen as a matter of widespread public recognition, understanding and appreciation of the importance of intellectual and scholarly activity and of the pursuit of excellence in all fields of intellectual endeavour.

Some of these points are emphasized even more strongly in Dr. Waterman's prefatory statement already mentioned. The National Science Foundation has a responsibility for the continuing study and analysis of the United States' scientific research and development potential, including research facilities, scientific man-power and education for science. Inquiries initiated seven years ago have now revealed trends that throw light on the present situation as well as assist long-range planning. It was these inquiries that led Dr. Waterman to the conclusion already quoted that the field of education should be the principal concern, and it is clear from his remarks that the United States is well ahead of Great Britain in action taken to remedy a situation that has become equally menacing to both countries. Admittedly, in the United States, little progress has been made in providing adequate salaries and career prospects for secondary school teachers of science, but in the United States standards have been tightened and critical thought devoted to the curricula and to improving the training of teachers and the content of courses. Beginnings of course have been made in Britain, as in the appointment by the University Grants Committee of a committee to study comparatively undergraduate teaching methods and practices; but concerted action is still needed and the situation requires urgent attention, just as it calls for tactful but firm handling.

Nevertheless, Dr. Waterman points out that these inquiries have shown that American institutions of higher learning are in critical need of assistance, especially in their graduate schools and other professional training centres. He comments on the effect of a widened gap between academic and industrial salaries, and on the fact that for several years fewer than 50 per cent on average of competent research workers in colleges and universities have received adequate support for the research they wished to pursue. A source of funds is needed that will enable a university to exercise initiative, judgment and competence in meeting its own needs, and it is this situation presumably that has led the Foundation to suggest, as already noted, a fresh look at the University Grants Committee system of Britain. Dr. Waterman does not wish to see the universities too dependent on the Federal Government for funds for research or training in research, and he stresses the way in which provision of uncommitted funds to universities, while from one point of view less effective than support of research projects, fosters the growth and independence of the institution and its contribution to research and education.

Support of basic research, Dr. Waterman suggests, should be regarded as a form of investment from which the returns in trained man-power are assured and research returns of definite value to the economy are statistically certain, and may more than repay the entire investment. Again, he recognizes the acute problem presented by the high cost of research equipment, more especially in certain critical fields of study, which are by no means always easy to identify, and which may require special scrutiny and decision on more than a scientific basis alone. Nevertheless, so far as the overall progress of science is concerned, he insists that the judgment of the active research worker should be the determining factor, and that the central planning and direction which are inevitable in development would be detrimental to scientific advance.

Generally, Dr. Waterman agrees that in spite of repeated emphasis on the importance of basic research, in the United States support has not been forthcoming on a scale sufficient to enable such research to hold its own in relation to the larger and seemingly more pressing problems of development. Although the position may be somewhat better in Great Britain, the experience of the National Science Foundation during the past decade affords no ground for complacency. The problems confronting the Foundation are similar in many ways to those facing the Advisory Council for Scientific Policy, and it is apparent that there are certain respects in which the Minister for Science might well take steps to see that these problems are tackled with more urgency and determination, and that the necessary co-ordination and financial support are forthcoming, even if it involves raising issues at Cabinet-level. Finally, the reader of this report can scarcely fail to note how much in it supports the argument advanced in the report, *Increasing the Effectiveness of Western Science*, for making the utmost possible use of possibilities of international co-operation in the attack on such major problems as space research, oceanography, polar research and the like.

THE PROCESSES OF DEVELOPMENT

The Biochemistry of Development

By Prof. Jean Brachet. (International Series of Monographs on Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, Vol. 2.) Pp. xii+320. (London and New York: Pergamon Press, 1960.) 65*s*. net.

IN the years since the Second World War, biochemistry, which began with an interest in how living systems maintain themselves, has begun to find itself in a position to tackle the much more difficult question of how they come into being. Chemical embryology may be said to have become a separate branch of study with the publication of J. Needham's great three-volume treatise in 1931. Even that was mainly concerned with the maintenance metabolism of embryos, and it was only with the publication of the same author's *Biochemistry and Morphogenesis* in 1942, and J. Brachet's *Embryologie Chimique* in 1944, that the study of the chemical processes by which development itself takes place acquired a massive impetus. Brachet's classical book was revised and translated into English in 1950, and since then he has published another extensive survey of similar ground, in his *Biochemical Cytology* of 1957. In the present book, he returns to embryology. The work is, as he points out in the foreword, to be considered as a summary of the advances that have been made since the last edition of his *Chemical Embryology*. In general, it follows the plan of that book, with successive chapters on gametogenesis, fertilization, cleavage, two chapters on the chemical embryology of invertebrate and of vertebrate eggs, nucleo-cytoplasmic interactions during morphogenesis, and finally, the biochemistry of differentiation.

Prof. Brachet modestly discounts any claim to treat these subjects in a complete and exhaustive manner, emphasizing that he is concerned only with reviewing the recent advances. However, as we have learnt to expect from this author, the subjects which he chooses to treat include nearly all the topics which have attracted the attention of embryologists in recent years. Moreover, they are discussed on the basis of his well-known combination of extremely wide reading and considerable, original, and largely unpublished, observations by himself and his collaborators. The result is a book which has, on one hand, the merits of a very valuable work of reference, but which is also something much more, in that it presents a most stimulating general synthesis of the field as it is seen by one of the most active contributors to it. The recent period in embryology has been remarkable for the number and power of the new technical methods which have been made available for the investigation of developing systems. We have seen, in particular, the introduction of refined biochemical methods for studying the nucleic acids, and such technical advances as the use of tracers, electrophoresis, immunological methods, the electron microscope, nuclear transplantation, and so on. Brachet and workers in his laboratory have themselves used many of these methods, and he writes with real authority about them.

Perhaps Brachet's most characteristic contribution to our general theories of embryonic development is his emphasis on the importance of gradients of ribonucleic acid content in various regions of the egg and embryo. In this book also, the evidence on this subject is fully discussed, and a number of as yet unpublished observations are described. To me it seems that, although there is undoubtedly a great deal of importance in Brachet's concept of nucleic acid gradients, the conception does not do adequate justice to the undoubted existence of an enormously elaborate system of specific types of ribonucleic acid and protein. Indeed, biochemistry is probably not yet equipped to tackle this aspect of the matter. It is primarily the study of genetics which leads us to realize that any given cell probably contains hundreds, if not thousands, of different species of ribonucleic acid and protein. If any criticism is to be offered of Brachet's book, it is that one would like to have seen more genetics in it. Perhaps, however, it is unreasonable to ask for so much. A satisfactory synthesis between genetics and biochemical embryology is what we are all seeking, and one can scarcely demand of an author that he should provide something that no one has yet been able to formulate. We have enough to be grateful for in a work which deals with the whole biochemical side of development in a manner which is both thorough and also controversial enough to be stimulating.

C. H. WADDINGTON

POLAROGRAPHY

Advances in Polarography

Proceedings of the Second International Congress held at Cambridge, 1959. Edited by Ian S. Longmuir. Vol. 1: Pp. xv+1–407. Vol. 2: Pp. ix+408–803. Vol. 3: Pp. vii+804–1204. (London and New York: Pergamon Press, 1960.) £15 per set of three volumes.

THESE three volumes constitute the collected papers of the second International Congress on Polarography, held at Cambridge in 1959, in honour of the seventieth birthday of Prof. Jaroslav Heyrovsky, Nobel laureate. Many hundreds of investigators have published papers in this field of science since Heyrovsky's first research paper on this subject in 1924, and the passing years have brought not only a tremendous increase in our knowledge of the general theoretical principles governing polarography but also many important practical advances in the design and sensitivity of the related instrumentation. The applications of polarography have been outstandingly successful in the analytical and industrial fields, and have been extended and diversified to include investigatory work of the greatest importance to the biological, agricultural and medical sciences.

These proceedings of the Congress are published almost entirely in English, although the three volumes do contain a few papers where the text is in French or German, a circumstance which serves as a useful reminder to the more insular among us that science is, indeed, international, and that a modicum of linguistic ability has many advantages to the up-to-date scientist.

The volumes abound in theoretical excursions by international experts which must prove of the greatest value to the academic teacher or pure researcher. Ilkovic discusses his celebrated equation for the mean diffusion current in d.c. polarography, and detailed comments on the equations of Matsuda and Koutecky are also included in papers by Koutecky and other authors. H. Irving provides an authoritative paper on the stability of metal complexes and gives a detailed thermodynamic treatment of step-equilibria in solution: P. Delahay relates electrode process phenomena and polarographic theory: R. Brdicka presents an extensive theoretical treatment of kinetic currents and J. Heyrovsky gives a mathematical treatment of the principles of oscillographic polarography.

Recent advances in the instrumental techniques of polarography are dealt with in numerous papers by acknowledged experts. G. C. Barker and G. W. C. Milner describe the development for trace analyses of the highly sensitive square-wave polarograph and of the single-sweep oscillographic and a.c.-pulse instruments. H. M. Davies, J. Seaborn and H. I. Shalyosky present papers on the application of the differential cathode-ray instrument to 'subtractive' and 'comparative' polarography. Indeed, it is impossible in a review such as this to mention more than a small number of the eminent scientific authors who have made lucid and detailed contributions on newer instrumentation.

The three volumes abound in examples of practical analytical applications. The determination of trace impurities in semi-conductor materials; the estimation of dissolved oxygen; the reduction of pertechnetate ions; the polarography of molten nitrates, of chelate complexed ions, of ergothioneine and of various biologically important compounds are but a few of the experimental applications which receive detailed treatment.

Z. P. Zagorski presents a valuable paper on reference electrodes; the uses of solid micro-electrodes are discussed by several authors, and T. R. Mueller, C. L. Olson and R. N. Adams review the employment of boron carbide and carbon electrodes in voltametry.

The ramifications of the science of polarography within the overlapping fields of chemistry, biology and medicine are now so extensive that only a comprehensive collection of erudite papers of this type by many expert investigators could hope to present a realistic picture of present advances in practice and in theory. In these three volumes is an attempt at this herculean task, and the aim of the compiler has been largely achieved.

D. T. LEWIS

MEDICINE OF THE THORAX

Brompton Hospital Reports

Vol. 28, 1959. (A Collection of Papers by Members of the Staffs of the Hospitals for Diseases of the Chest—Brompton Hospital and the London Chest Hospital—and of the Institute of Diseases of the Chest.) Pp. xii+258. (London: Lloyd-Luke (Medical Books), Ltd., 1960.) 15*s.* net.

THE contents of this volume, which is as attractively produced as all its predecessors have been, maintain the high standard we have learned to expect from these reports. It is sad that this one should have to begin with a memorial notice to Sir Robert Arthur Young, who served both the staff and patients at the Brompton Hospital for so long. Joining the staff in 1905, he was one of the first physicians to realize the possibilities of thoracic surgery, and the long history of his life's work is admirably summed up here by the publication of the late Sir Gordon Gordon-Walker's address given at the memorial service. To read this address is to realize that Young was not only a great medical man but also a great human character.

The rest of the volume contains 16 articles on a variety of aspects of thoracic disease. Drs. L. H. Capel and J. Smart discuss in two papers aspects of effort intolerance; Drs. K. M. Citron, A. W. Frankland and J. D. Sinclair report on inhalation tests of bronchial hypersensitivity in pollen asthma, Drs. J. Pepys, R. Augustin and A. B. Paterson on common antigenic components of mycobacterial extracts and Dr. H. A. Fleming on differential lung function in atrial septal defect. Dr. Lynne Reid describes the connective tissue septa in the adult human lung and Dr. D. Ranger discusses the dangers of streptomycin with particular reference to its vestibular toxic effects. Dr. Paul Wood contributes two articles on pulmonary hypertension with reference to the vasoconstrictive factor and to reversed central shunt.

Surgery is represented by two articles by Sir Clement Price Thomas on the surgical treatment of pulmonary tuberculosis and on conservative and extensive resection for carcinoma of the lung, while J. B. Johnston and P. H. Jones discuss the treatment of carcinoma by lobectomy and sleeve resection of the main bronchus and W. K. Pallister describes a new endobronchial tube for the left lung. The value of the surgical contributions is greatly enhanced by the two articles by Sir Russell C. Brock, one of which is his Bradshaw Lecture on the present position of cardiac surgery (with its tribute to the work of Sir Charles Ballance and the part he played in the death of the superstition that the heart is untouchable), and the other is on direct operations for the correction of Fallot's tetralogy. Dr. J. G. Scadding contributes an opening article on the principles of definition in medicine, with special reference to chronic bronchitis and emphysema.

The bibliographies of all these articles are valuable, but would be more so if the titles of the papers quoted were given. The volume concludes with a list of papers published by members of the staff of the Brompton Hospital between August 1958 and July 1959 and with author and subject indexes to volumes 1–28. GEOFFREY LAPAGE

ELEMENTS OF PHYSICAL CHEMISTRY

Elements of Physical Chemistry

By Samuel Glasstone and Prof. David Lewis. Pp. v+758. (London: Macmillan and Co., Ltd., 1960.) 60*s*. net.

STUDENTS and teachers of physical chemistry will need no introduction to the *Elements of Physical Chemistry* by Glasstone and Lewis, the second edition of the book first produced by Glasstone in 1945. The major change in the new edition is a re-ordering of the chapters, so that after an initial outline of the properties of gases the reader is led through the realms of thermodynamics. This has the advantage of enabling such phenomena as vaporization of liquids and solids to be treated thermodynamically when first met, rather than phenomenologically as in the earlier edition. The molecular nature of matter is introduced in the billiard-ball approximation, in the chapter on the gaseous state via the kinetic theory, the more intimate details of the structure of atoms and molecules, and the direct evidence for their existence being left until a later stage. From this point onwards the book follows much the same well-established lines as the earlier edition. However, a new chapter on nuclear chemistry has been included. New material has been incorporated in all the chapters, to bring the book into line with contemporary developments.

The increased attention given to two of the basic theories of physical chemistry, namely wave mechanics and statistical mechanics, is welcome, although I feel that the section on the Schrödinger equation could well have been extended to indicate the way in which eigen values fall naturally out of the assumptions of the theory.

The book is written in such a way as to be readily understood by the student commencing the subject; moreover, the references given at the ends of the chapters should stimulate him to further reading of the subject. These references have been extended to include well-known review journals, as well as those to the *Journal of Chemical Education*, which were the sole source of additional information in the first edition.

To understand physical chemistry completely the student needs to acquire the facility to manipulate the results of experimental observation as well as the basic concepts. Development along these lines is encouraged by a new selection of problems at the end of each chapter, designed to set the reader thinking, and to develop his ability to interpret experimental results within the framework of physico-chemical theory.

The book is very well and attractively produced, and the figures have been well chosen to bring home to the reader the points in question. There is no doubt, in my mind, that the part played in the teaching of physical chemistry by the first edition will be more than adequately filled by the second.

A. MACCOLL

BRITISH FOOD ANALYSIS

The Composition of Foods

By Dr. R. A. McCance and Dr. E. M. Widdowson: Privy Council. Medical Research Council Special Report Series, No. 297. (London: H. M. Stationery Office.) 1960. (Third revised edition of Special Report No. 235.) Pp. viii+252. 30*s*. net.

THIS book of tables, giving the composition of a comprehensive selection of foods commonly eaten in Britain, is the third edition of the Medical Research Council Special Report Series No. 235, originally published in 1939. In 1942 the first edition was revised, the revisions including a change in the method of calculating the calorie value of carbohydrate, and the addition of 'National' 85 per cent flour and bread to the foods analysed. The second edition, published in 1946, included some other foods introduced into the nation's diet as a result of the Second World War. Both editions are well known to nutrition workers. The tables were the result of analytical work carried out under the direction of Prof. McCance since 1925. They showed the important organic and mineral constituents, with the exception of vitamins, of most of the foods in common use in Great Britain. Foods were analysed not only in the raw state but also as prepared for the table. The composition of many cooked dishes was given.

The basis of the third and latest edition remains the same. However, some foods no longer generally consumed have been omitted, and some of the many new proprietary foods which have appeared on the market since the War have been added. More than 100 new foods, including cereal preparations, soft drinks, sweets and alcoholic beverages, figure in this edition. Changes in the composition of various proprietary foods have made it necessary to revise values previously given.

Important and useful additions are two new tables showing the vitamin and amino-acid content of foods. The vitamin values are derived from assays made by other workers, while the amino-acid figures are a combination of the authors' own results and those of other workers.

In its revised form this well-known work will no doubt continue to serve nutrition workers and dietitians, retaining an honoured place among various other food composition compilations, national and international. The bulk of the tables is based on analyses of food samples carried out by the authors themselves, the results being given, for the main constituents, to the first decimal place. While such figures are, of course, accurate with respect to the samples analysed, they are not necessarily fully representative of the foods in question. Users of the tables should not forget that the composition of fresh foods varies widely even when variety and growing and harvesting conditions are uniform. Change in water content, for example, will obviously alter the whole composition of the food. All food composition tables, however carefully prepared, can in effect serve only as a guide in calculating the values of foods and meals. Joyce Doughty

FREE RADICAL TRAPPING

Formation and Trapping of Free Radicals

Edited by Arnold M. Bass and H. P. Broida. Pp. xvi+522. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 16 dollars.

ATOMS and free radicals, which normally react rapidly with surrounding molecules, can be 'trapped' in inert solids; if, for example, solutions in organic glasses are photolysed or products from a gas discharge are condensed at low temperatures. Free radicals have long been postulated as reaction intermediates, but only recently have many attempts been made to produce directly detectable concentrations of them in solids. That development has been rapid is shown by the size of this book. In 1954, seven laboratories independently published preliminary reports, their interest being mainly in determination of structures of radicals; in 1956, because of the slight possibility of production of sufficient concentrations of free radicals for use as rocket fuels, an intensive research programme was started at the National Bureau of Standards in Washington (under the direction of Dr. H. P. Broida).

Now that this burst of activity is over, research will proceed more slowly; this book will provide the background for the necessary period of consolidation. It begins with an excellent historical chapter by F. O. Rice, and there follow several chapters on methods of preparation and detection of trapped radicals. This approach, instead of arrangement by substances studied, is not easy to justify in the present state of knowledge and leads to considerable inconvenience—for example, data on trapped nitrogen atoms are scattered through eight of the sixteen chapters. However, it prevents the book from being a collection of research papers, and forces both authors and readers to reconsider the experimental problems of the field; indeed a certain amount of data relevant to the feasibility of trapping radicals appears here for the first time (Chapter 4).

A wide range of related fields and possible applications of free-radical trapping are dealt with in the later chapters. Some of these ("Rocket Propulsion") are highly speculative, others ("Radicals in Biological Processes") contain a great deal of background information and relatively few results; a shortening of the book, if accompanied by a reduction in the price, would certainly have been a great advantage. However, the editors' claim that this is a truly interdisciplinary field must be accepted, and among the new research workers for whose reference this book is written the spectroscopist will be glad to find a chapter on X-ray studies, and the biologist an elementary explanation of the application of electron-spin resonance, all in one volume.

B. Brocklehurst

A FRENCH LEXICON OF PLANT FORMATIONS

Les Formations Végétales et Paysages Ruraux

Lexique et Guide Bibliographique. Par G. Plaisance. (Publié avec l'aide du C.N.R.S.). Pp. 418. (Paris: Gauthier-Villars, 1959.) 3,000 francs.

LANDSCAPE, vegetation types and plant formations, as caused by factors of climate and soil and modified by man to suit his varied needs of food, pasturage and building, are basic factors in the evolution of languages and the history of civilization. Their ancient popular terminology has so often been the basis of place names and family names, and as such is studied by linguists, historians and geographers. In recent times they have become the special field of the botanical sciences of ecology, plant geography and phytosociology, which have introduced a disconcerting multitude of new words and phrases for the purposes of definition and classification.

To gather together the words and terms of several principal languages and continents for landscape, vegetation types and plant formations, from those of ancient dialects to the jargon of contemporary ecologists, has been the task of the author of this remarkable and fascinating dictionary. In the main *Lexique* each word, after definition, is given synonyms, examples of derivatives and main bibliographical references. After this there are special indexes of place names and family names derived from such words (for example, Fresnay (*frênaie*); Lafayette (*hêtraie*)); a trilingual, columnar lexicon for French, German and English terms; independent English and German indexes; another index, of botanical names; a vocabulary of technical terms used in ecology, plant geography and phytosociology; and, finally, a very extensive bibliography. The work is illustrated with small photographs and a number of maps. The maps of France showing the distribution, for example, of the many different dialect words for *hêtraie*, beech-wood, originating in fagetum, or of the great variety of words for expressing *défrichement*, are of absorbing interest.

Naturally, the work is strongest on the French side, and students of other nationalities will soon find defects and omissions, or will criticize the arrangement. I looked in vain, for example, for English 'down' and 'dune', and not enough attention has been given to recent Spanish publications and to Spanish words: for example, *pasto*, *pastizal*, *prado* and *pradera*, which appear in so many papers of keen, contemporary, Spanish agronomists should have been distinguished.

Nevertheless, every English ecologist and forester—one is tempted to say, every educated person—should examine this book, if only for repeated casual enjoyment and instruction.

N. Y. Sandwith

Spot Tests in Organic Analysis

By Prof. Fritz Feigl. Sixth, enlarged and revised English edition. Translated by Prof. Ralph E. Oesper. Pp. xx+675. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 65*s*.

THIS book is a revised and extended edition of Prof. Feigl's celebrated work on the application of the spot-test identification techniques to organic compounds of many different types. Translated by Prof. R. E. Oesper, of the University of Cincinnati, Ohio, the text of this sixth edition is lucidly presented in a most readable manner, with large legible print on good paper.

The individual items in the sections dealing with tests for functional groups, tests for individual groups, practical applications, etc. have been almost doubled in number when compared with the fifth edition. Extensive detail is given in addition relating to the identification of polymeric materials, synthetic rubbers and fibres, vitamins, antibiotics, and various pharmaceutical preparations. Each chapter carries an extensive bibliography of references, and the volume ends with a tabular summary of the limits of identification attained with macro-drop (0·05 ml.) investigations involving the application of specific spot-tests to solutions of those elements, groups and compounds which have been given detailed treatment in the text.

The working laboratory methods for spot-test analyses are illustrated by clear, diagrammatic representations of the apparatus employed, and the actual test procedures for individual cases are described with much attention to experimental detail.

This volume will be as welcome to students and to professional analytical chemists as its predecessors have been, and is yet another attempt to keep abreast of the ever-growing research, development and interest in this absorbing field.

D. T. LEWIS

Ciba Foundation Symposium on Hæmopoiesis

Cell Production and its Regulation. Edited by G. E. W. Wolstenholme and Maeve O'Connor. Pp. xii+490. (London: J. and A. Churchill, Ltd., 1960.) 60*s*. net.

IN recent years important advances in experimental hæmatology have been made, particularly by the use of radioisotopes and cell-transfusion techniques. These advances are amply reflected in the sixteen papers presented at the Ciba Foundation symposium on hæmopoiesis. The stimulating discussions which followed each paper are also recorded.

The dynamics of hæmopoiesis is a conspicuous feature of this book. Attention is focused on the problem of the primitive stem-cell, the production and fate of lymphocytes, and the inter-relationship of lymphoid and myeloid tissues. Factors which regulate the production and release of blood cells are also discussed. The subjects considered include: (1) granulocytopoiesis in human and animal bone marrows studied by radioisotopes; (2) hæmopoiesis following cell transfusions into irradiated animals; (3) the construction, from experimental data, of models illustrating the kinetics of erythrocyte production, lymphocyte formation in the thymus, and plasma cell production in lymph nodes; (4) humoral factors in hæmopoiesis, including erythropoietin, a leucocytosis-inducing factor and batyl alcohol; methods are described for the assay of erythropoietin, including the use of transfusion-induced polycythæmia to study erythropoiesis; and the mobilization of vitamin B_{12} reserves is described following acute blood loss; (5) observations on the patho-physiology of human leukæmic cells, certain findings contrasting with conventional views of the leukæmic process.

The Ciba Foundation is to be congratulated on its latest publication. The papers and their clinical implications will be of considerable interest to clinical and research hæmatologists. The book contains much that is topical in hæmatological research, including observations upon human material.

P. F. HARRIS

Unity and Diversity in Biochemistry

An Introduction to Chemical Biology. By Marcel Florkin. Translated from the French by T. Wood. (International Series of Monographs on Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, Vol. 1.) Pp. xiii+397. (London and New York: Pergamon Press, 1960.) 70*s*. net.

THIS monograph is presented as a collection of essays in which Prof. Florkin first reviews "the biochemical facts common to living beings", and then discusses biochemical differences between species. The treatment of unity in metabolic processes (Parts 1–4) is concise, yet complete enough to be of value to the general reader who wants an introduction to the present state of biochemical knowledge. Part 1 is a lavishly illustrated catalogue of the more widely distributed constituents of plants and animals. This collection is perhaps too detailed for an introduction to chemical biology (the structures of thirty-one carotenoids are shown), and most readers would welcome more comments on the relationship between structure and biological function. Part 2 includes a thoughtful discussion of thermodynamic aspects of biochemistry and a classification of enzymic reactions. Chemists and biologists will probably find Parts 3 and 4 the most rewarding in this book. These describe many of the reaction sequences which provide materials and energy for growth and survival of plants and animals.

In restricting a discussion of biochemical diversity to fifty-seven pages (Part 5) the author has been able to illustrate his arguments with only a few of the possible examples; comparative aspects of oxygen transport are considered in great detail. There is a welcome attention to biochemical differences between tissues of a single organism. Biochemical aspects of taxonomy and evolution are discussed briefly. Part 6 deals with the interaction of plants and animals with each other and with their inorganic environment, and concludes with the suggestion that loss of phosphorus to the sea may make life on the continents "a mere episode between two eras of marine existence".

Not everyone will agree with the author's choice of examples and references, but no other book deals with this wide range of topics so concisely, and the reader without biochemical training should find this a useful introduction to chemical aspects of biology.

M. R. ATKINSON

THE HÆMOGLOBIN PARTICLES

CHEMICAL AND GENETIC ASPECTS OF THEIR STRUCTURE*

By DRS. G. BRAUNITZER, N. HILSCHMANN, V. RUDLOFF, K. HILSE,
B. LIEBOLD and R. MÜLLER
Max-Planck-Institut für Biochemie, Munich

THIS article presents a summary of work on the primary structure of human hæmoglobin A which was begun five years ago in the Max-Planck-Institut für Biochemie; the results have been published during the past two years in various publications, and we wish to summarize and discuss them here.

The hæmoglobin particle is composed of four peptide chains, two identical α- and β-chains[1]. Human hæmoglobin consists of the Val-Leu-type (the α-chain[1–3]) and of the Val-His-Leu-type (the β-chain[1,4]). Our investigations were conducted on the main component of normal adult human hæmoglobin. In order to elucidate its chemical fine-structure we have split the globin particle with trypsin after denaturation with urea[5]; as first shown by Moore, Hirs and Stein[6] with ribonuclease, this enzyme is the simplest method of splitting a protein into a discrete number of definite breakdown products. For the separation of a peptide mixture we have developed a new procedure employing an anion exchange resin ('Dowex 1*X*2')[7,8] and pyridine acetate as a volatile ninhydrin-negative buffer. The essential principle of the method is that the separation depends only on the charge and also that peptides of very long chain-length can be eluted from the column. Since this method does not require desalting, it saves a considerable amount of labour (Fig. 1). The purification of the peptides is achieved by high-voltage electrophoresis and paper chromatography. By use of these procedures all the breakdown products of globin, which come from the α- as well as from the β-chain[9–13], could be separated, isolated and characterized quantitatively, by means of the automatic apparatus of Spackman, Moore and Stein (Beckman Instruments, Fullerton, Calif.). By these means the exact amino-acid composition of all tryptic breakdown products, with the exception of the three cysteine peptides, could be determined. The cysteine peptides could be separated and purified only after oxidation with performic acid. The amino-acid composition of these breakdown products will still have to be established more precisely, since destruction during oxidation is not ruled out. The knowledge of the exact amino-acid composition of these peptides is of primary importance for work on the amino-acid sequence still to be done, but not for the questions to be discussed here.

In extension of these investigations we have also developed a new, systematic procedure which makes possible the determination of the relative position of the tryptic peptides within the chain[14,15]. This is achieved almost systematically by splitting tryptic peptides with pepsin and by splitting those peptic peptides containing lysine and arginine with trypsin. A schematic presentation of the method is illustrated in Fig. 2. In this way identical peptides can be obtained which originate from the same section of the peptide chain, from which the sequence of the tryptic peptides can be concluded without difficulty.

In this manner the twenty-seven tryptic peptides of human hæmoglobin—thirteen from the α- and fourteen from the β-chain—could be assigned unambiguously to their positions within the peptide chains, and the partial formula of the α- and β-chains of the main component of normal adult human hæmoglobin could be established. In order to obtain a deeper insight into the primary structure of the hæmoglobin particle, the amino-acid sequence of the tryptic peptides was investigated. For this purpose conventional chemical (dinitrophenol method[16], Edman degradation[17,18], hydrazinolysis[19–21]) and enzymatic methods were employed. All the sequence studies were subjected to continuous control by quantitative analysis. For these controls a simplified amino-acid analyser (apparatus from Bender and Hobein, Munich)[22] was used. Up to the present, we have been able to fix the positions of about two-thirds of the amino-acid residues.

Homologous Architecture of the Chains

Comparison of the tryptic peptides of the α- and β-chain already shows that several breakdown

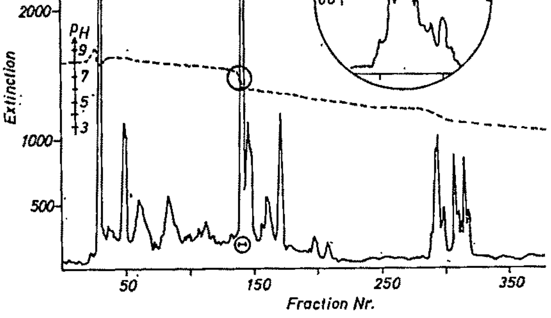

Fig. 1. Chromatography of peptic peptides of the α-chain on 'Dowex 1 *X* 2'. Experimental conditions: 1 per cent collidine, 1 per cent pyridine acetate *p*H 8·4. Gradient 1 : 0·1 per cent acetic acid. Gradient 2: 2 *N* acetic acid; 50 μM protein. Column, 150 cm. × 3 cm.; temperature, 35°; flow-rate, 80 ml. per hr.

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* Substance of a paper presented to the Hemoglobine-Structure Workshop on December 14, 1960, of Endicott House, Massachusetts Institute of Technology, Boston, Mass.

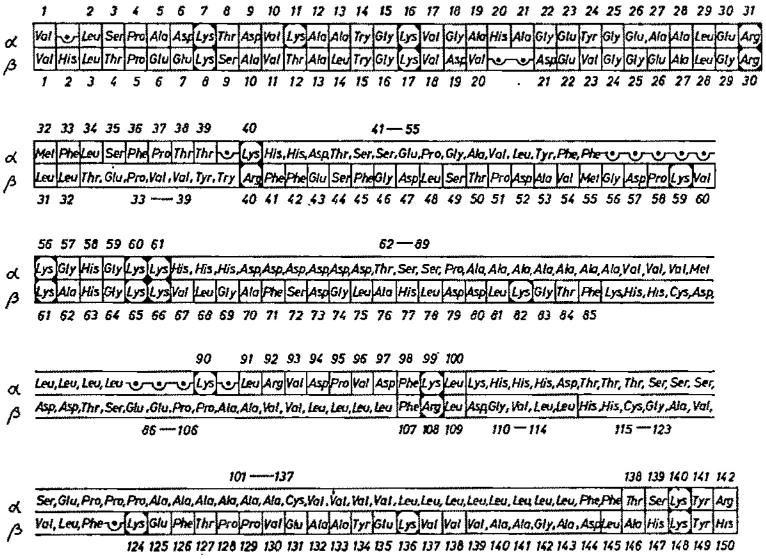
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
α Val Leu Ser Pro Ala Asp Lys Thr Asp Val Lys Ala Ala Try Gly Lys Val Gly Ala His Ala Gly Glu Tyr Gly Glu,Ala Ala Leu Glu Arg
β Val His Leu Thr Pro Glu Glu Lys Ser Ala Val Thr Ala Leu Try Gly Lys Val Asp Val Asp Glu Val Gly Gly Glu Ala Leu Gly Arg
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
32 33 34 35 36 37 38 39 40 41—55
α Met Phe Leu Ser Phe Pro Thr Thr Lys His,His,Asp,Thr,Ser,Ser,Glu,Pro,Gly,Ala,Val,Leu,Tyr,Phe,Phe
β Leu Leu Thr,Glu,Pro,Val,Val,Tyr,Try Arg Phe Phe Glu Ser Phe Gly Asp Leu Ser Thr Pro Asp Ala Val Met Gly Asp Pro Lys Val
31 32 33—39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
56 57 58 59 60 61 62—89
α Lys Gly His Gly Lys Lys His,His,His,Asp,Asp,Asp,Asp,Asp,Asp,Thr,Ser,Ser,Pro,Ala,Ala,Ala,Ala,Ala,Ala,Ala,Val,Val,Val,Met
β Lys Ala His Gly Lys Lys Val Leu Gly Ala Phe Ser Asp Gly Leu Ala His Leu Asp Asp Leu Lys Gly Thr Phe Lys,His,His,Cys,Asp,
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85
90 91 92 93 94 95 96 97 98 99 100
α Leu,Leu,Leu,Leu Lys Leu Arg Val Asp Pro Val Asp Phe Lys Leu Lys,His,His,His,Asp,Thr,Thr,Thr,Ser,Ser,Ser,
β Asp,Asp,Thr,Ser,Glu,Glu,Pro,Pro,Ala,Ala,Val,Val,Leu,Leu,Leu,Leu Phe Arg Leu Asp,Gly,Val,Leu,Leu His,His,Cys,Gly,Ala,Val,
86—106 107 108 109 110—114 115—123
101—137 138 139 140 141 142
α Ser,Glu,Pro,Pro,Pro,Ala,Ala,Ala,Ala,Ala,Ala,Cys,Val,Val,Val,Val,Leu,Leu,Leu,Leu,Leu,Leu,Leu,Leu,Phe,Phe Thr Ser Lys Tyr Arg
β Val,Leu,Phe Lys Glu Phe Thr Pro Pro Val Glu Ala Ala Tyr Glu Lys Val Val Val,Ala,Ala,Gly,Ala,Asp Leu Ala His Lys Tyr His
124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150

Fig. 3. The homologous structure of the α- and β-chains of the main compound of the normal adult human hæmoglobin. The homologous and identical sequences of the α- and β-chains of human adult hæmoglobin have been placed under one another. The sequence blanks resulting from such a comparison have been left free. The amino-acid residues the positions of which have been established are boxed in, thus $\boxed{\text{Ala}}$. Those residues the positions of which have not yet been fixed are represented in their sequence of elution from the amino-acid analyser, for example, Ala

products of both chains are identical or very similar; free lysine can be isolated from both, which is evidence of a lys-lys-sequence; moreover, the peptides Ala-His-Gly-Lys, Gly-His-Gly-Lys, Tyr-His, Tyr-Arg also show similarities. It became clear after determination of the position of the tryptic peptides within the chain and especially after establishing the amino-acid sequence within these peptides that the two chains are constructed in a homologous fashion[23]. They behave, with respect to each other, like proteins of different species.

In order to facilitate comparison, we have placed both chains together in Fig. 3. As can be seen, a large number of identical and homologous sequences occur in both chains. It is noteworthy that the basic amino-acids lysine and arginine occur at very similar positions in both chains: it is already apparent from sequence analysis that corresponding sections of the chain bear the same net charge. Both chains contain a basic centre, although the β-chain contains one additional basic residue:

(α) Lys-Gly-His-Gly-Lys-Lys (56–61)
(β) Lys-Val-Lys-Ala-His-Gly-Lys-Lys (61–66)

It is of further interest that the cysteine in both peptide chains—as one can tell from present investigations—is located in the last third of the chain.

The 'Blanks' in the Sequence

The individual homologous sequences of the α- and β-chain can only be made to match by omitting several amino-acids from an opposing position[23]. For example, the histidine in position 2 of the β-chain appears to have no partner in the α-chain. The homologous structure of the two chains can only be preserved by omission of this amino-acid: or, in other words, the α-chain has a 'blank' in the sequence in the equivalent position. The β-chain shows a similar behaviour in that it likewise possesses no partners for the amino-acids His-Ala (position 20–21) in the α-chain. There can be no doubt that the sequences (α) 56–61 Lys-Gly-His-Gly-Lys-Lys and (β) 61–66 Lys-Ala-His-Gly-Lys are homologous. It is, however, surprising that these sections in the chains are shifted five amino-acid residues apart. While the sequence of the preceding peptide in this section of the β-chain is already known, the equivalent peptide from the α-chain has not been determined analytically. It would be expected that a restriction for five amino-acid residues of the α-chain occurs in that part of the chain. As reported in earlier communications[12,13], and as is evident from Fig. 3, the constitution of the last third of both peptide chains is not yet known unequivocally. Although the exact number of amino-residues of the β-chain is not yet definite, because of uncertainties in the analysis of both cysteine peptides, it is already possible to say with certainty that the β-chain is larger than its homologous α-chain and contains approximately eight more amino-acid residues, whereas up to now only homologous chains with the same number of residues have been described.

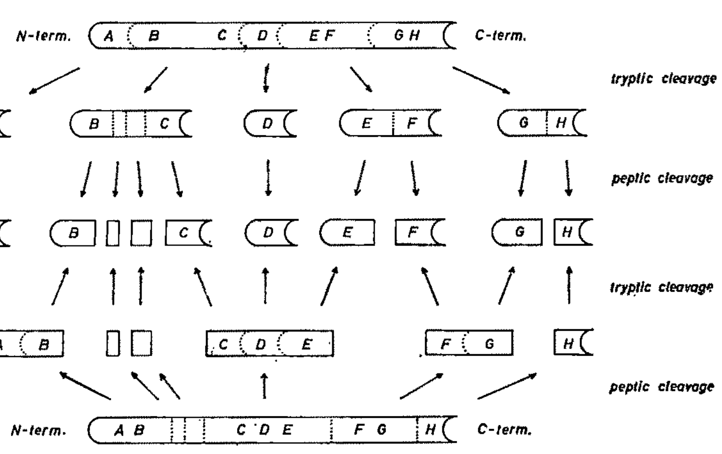

Fig. 2. Method for determining the position of the tryptic peptides within the chain

Theory

The structure of a protein reflects the structure of genetic material. A theory of protein structure is therefore equivalent to a theory of genetic substance. It must be assumed that originally a

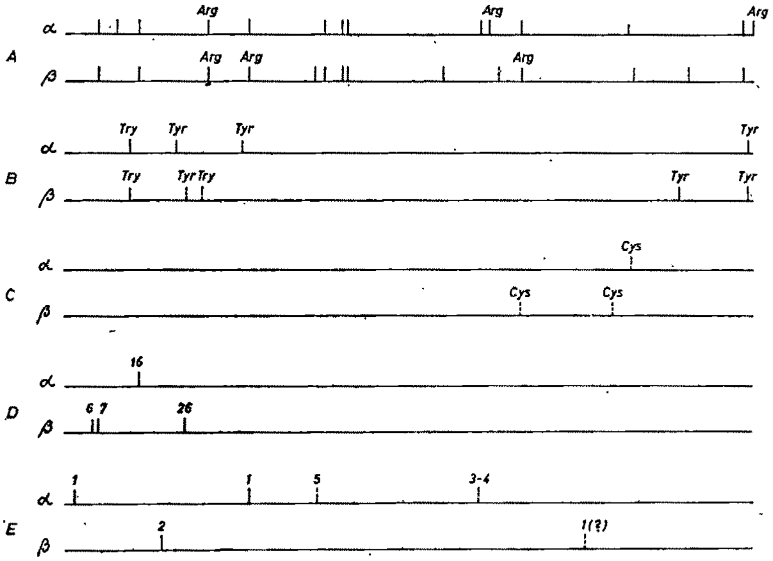

Fig. 4. Comparison of the peptide chains of the main component of normal adult human hæmoglobin. (*A*) Comparison of the position of lysine or arginine residues; (*B*) position of the amino-acids tyrosine and tryptophan; (*C*) most probable position of the cysteine residues; (*D*) position of amino-acids in which the specific genetically determined replacement of amino-acids occurs in pathological hæmoglobins (refs. 27–31); (*E*) position of blanks in the sequence; the broken lines mean that the exact position is not yet established; the numbers indicate the number of amino-acid residues missing in the chain and referred to as 'blanks'

simple hæmoglobin existed; it consisted of a single peptide chain which corresponded to an equivalent complementary piece of genetic material. During evolution and further differentiation, a duplication of the genetic material must have occurred, which, however, from that point developed and differentiated independently, until it corresponded to the double peptide chains found at the present time. These changes must have occurred a long time ago, since the differences between the chains are rather extensive. Besides these changes, the genetic material must have been subjected to a restriction; this is probably the simplest interpretation of the sequence blanks in the protein structure of hæmoglobin. In other words, the α- and β-chains behave like different species of peptide chains. Phylogenetically, however, the difference between the α- and β-chains is greater than the difference which has, for example, been found in the various kinds of insulin[25], where only an exchange of peptide chains of equal length has been established. In view of this finding, it seems to us that further essential insight into the structure of proteins has been achieved.

Sanger[25] was able to show—as indicated above—that proteins of various sorts differ by replacement of certain amino-acids. We believe that we have demonstrated experimentally, for the first time, a restriction at the molecular level. This finding can therefore only be explained if two complementary chains occur in hæmoglobin, whereby one in a certain sense serves as a standard for comparison and vice versa. The question of the linear structure of a molecule appears to us to be of the greatest importance for an understanding of evolution on a molecular basis. These results provide fresh stimulus for a comparison of proteins of various species, and also of the investigation of proteins with several peptide chains. It remains to be seen whether findings in other proteins will extend the results presented here.

A year ago, a three-dimensional Fourier analysis of horse hæmoglobin with a 5·5-Å. resolution was reported in *Nature*[26]. A resolution of 2·0 Å. appears now to be possible. The partial formula of human hæmoglobin presented here already contains all the essential outlines of its primary structure. It is only necessary to complete about 110 sequences, which, however, offer no further methodological difficulties.

In order to give a more complete and clearer view of the position in this field of work, we have arranged several reference points in the structure of hæmoglobin peptide chains opposite to each other in Fig. 4.

Further details of these results will be presented in *Hoppe-Seyler's Zeitschrift für physiologische Chemie.*

We wish to thank Prof. A. Butenandt, director of this Institute, for his generous support of this work. These investigations have been aided for the past several months by a grant from the Deutsche Forschungsgemeinschaft. We also wish to thank Miss B. Schrank, K. Haselhorst, R. Pagel and Mr. O. Ilg for assistance.

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POLLEN ANALYSIS IN UGANDA

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THE vegetations of the past thousands of years can be reconstructed accurately by isolating and identifying the pollens which are preserved with their diagnostic features intact in the anaerobic sediments of fen and lake. In Europe this work, known as pollen analysis, has established a succession of vegetation types which appeared since the retreat of the last ice sheet some 10,000 years ago, and active work delves into more remote eras. These vegetation types probably arose in response to the different climates which prevailed in the past; and although there are many factors which obscure a straight correlation between vegetation and climate it is entirely true to say that pollen analysis has provided the most accurate and consistent story of Europe's environmental past. The vast body of facts which has been gleaned may be appreciated very readily by browsing through Godwin's[1] recent book *The History of the British Flora*, which has the telling sub-title *A Factual Basis for Phytogeography.* All this has been accomplished by a few enthusiastic workers within the past thirty years. The question I now wish to consider is: Can pollen analysis give an equally informative record of the Quaternary history of East Africa?

This is the question which Hedberg[2] put into many minds when he indicated recently that pollen analysis might be successful on the lakes and fens of tropical East Africa. In Uganda it would certainly be very good to have new and independent evidence for the history of vegetation and climate in the Quaternary period, because it is certain that Uganda has suffered an over-emphasis of correlations based on climatic interpretations of important stratigraphic sites, such as those alongside the Kagera River. Wayland[3], who has travelled widely in Uganda, and was for many years the director of its Geological Survey, believes firmly that the Kageran stratigraphy supports an interpretation—perhaps not wholly—in terms of pluvial and inter-pluvial periods. Brooks[4] suggested that these might have been the tropical counterparts of the glacial and interglacial periods of the north temperate region. Wayland, however, stresses that the terms 'pluvial' and 'inter-pluvial' are unfortunate because in some minds they conjure up fantastic ideas of Noachian deluges separated by periods of extreme desiccation. Bishop[5] is an informed antagonist to the out-and-out climatic ideas, and it is instructive to compare his and Wayland's interpretation of the unquestionably important Kageran stratigraphy. Much of the difficulty and ambiguity in the evidence springs from the fact that beaches and terraces in East Africa cannot be straight away linked to climatic fluctuations, for changes in drainage and in lake-levels may have originated in tectonic movements, which have been extensive between the eastern and western rift valleys and around the Lake Victoria basin[6].

Part of the past year was spent in searching for suitable pollen-bearing deposits in Uganda, and, having had the good fortune to discover some excellent sites, I am sure that many of my colleagues will be interested in their whereabouts, and in the results from our first long pollen profile from this region.

Organic deposits, which have preserved pollen, are not common in Uganda because in the continuously warm climate the bacteria work very efficiently and all plant debris is rapidly mineralized. Thus, although there are vast swamps[7,8] of papyrus (*Cyperus papyrus*) and tall grass (*Miscanthidium violaceum*) all around the shores of the Lake and on the rivers, these are underlain usually by fine clays. These swamp clays, which are useless for pollen analysis, occur widely in the kingdom of Buganda, lying to the east and west of Kampala. The altitude of the region is about 4,000 ft.; the temperature ranges between 60° and 80° F.; the rainfall varies from 30 to more than 70 in. per annum, according to the locality; and the potential evaporation is a little more than 40 in. per annum. But at 6,000 ft. as near Kabale (Fig. 1) the temperature-range is 50–70° F., and the more sluggish bacterial industry allows peat to form and accumulate, and its depth is ultimately decided by drainage. The rainfall around Kabale may be about 40 in. a year and the annual potential evaporation about 30 in. In the Kashambya valley, near Kabale, Dr. Lind[7] found a maximum of a little more than 6 metres of peat, and in the Ishasha Valley—which is about the same altitude as the Kashambya—I have drilled through 14 metres in one place and reached the base, but through 18 metres in another place and not touched solid bottom. These, however, are possibly rapidly formed peats; but we find splendid compact peats and muds, which have formed probably slowly over many thousands of years, in the Rukiga Mountains, which are a short distance away, between Kabale and Kisoro.

Geologically speaking[9], the Rukiga Mountains consist of a 27-mile-wide belt of strike-ridges with intervening valleys—which are mature and very steep—descending often to a depth of 1,000 ft., and sometimes to 1,800 ft. This is the little Switzerland of Uganda, and it is much loved by all who know its hard-working, cheerful people, and its beautiful views of hilltops and island-studded lakes. As with most of Uganda, this countryside has been modified by changes of the Earth's crust, and here these movements have originated from the western rift valleys. But the lakes and fen-filled valleys, for the most part, owe their origin to drainage which has been impounded behind small volcanoes and lava flows. The now dormant and very lovely volcanoes, Muhavura, Mgahinga and Sabinio, are at the very south-west of the Rukiga Mountains, and they are part of the larger volcanic field known to geologists as the Bufumbira Bay. There are also small volcanic centres, such as Muko in the heart of the Rukiga Hills; the deposits around Muko are very interesting.

Muko is a tiny village from which a long, extremely narrow, and steep-walled valley runs into the north-west. This valley is neatly dammed by a cluster of small craters which rise to about 200 ft. behind the village. Drainage of the valley accumulated behind

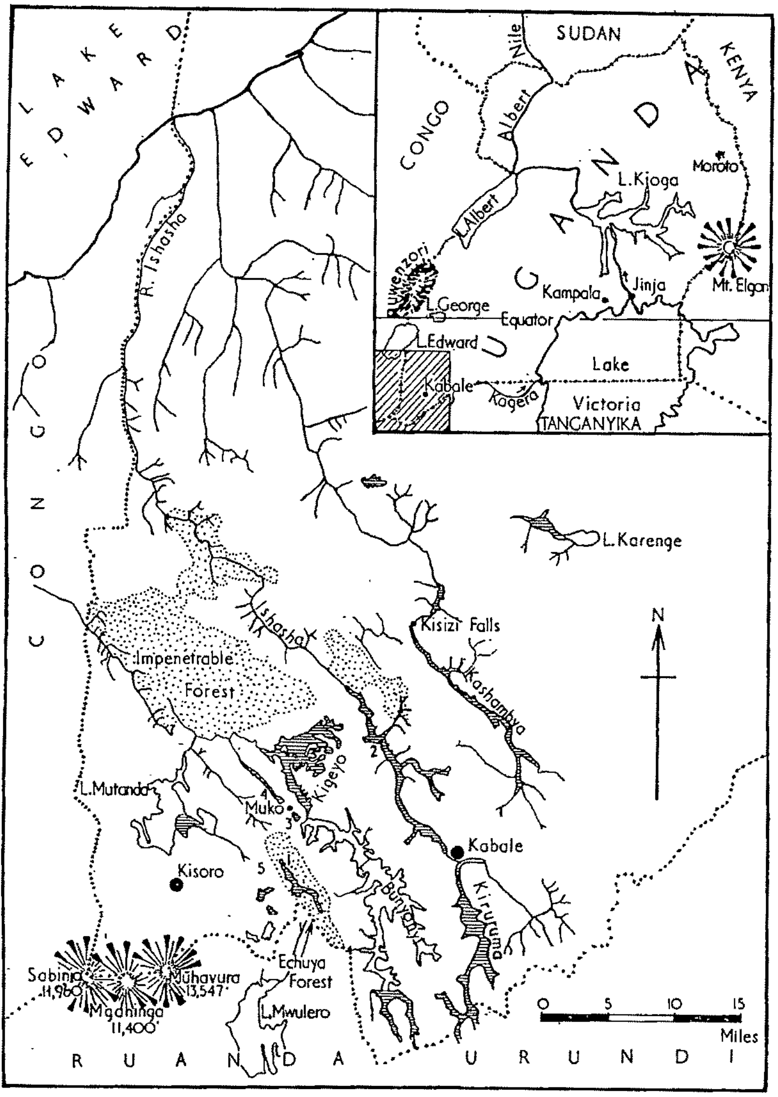

Fig. 1

this dam and a long lake formed, and this is now almost entirely filled in by lake muds and peats. In this valley, and just a short distance back from the volcanic bar, samples were collected through 20·5 metres of lake muds, and the base was not touched. The composition of the peat at that depth leads me to believe that there may be at least another 4–5 metres before the floor of the valley.

From Muko several sluggish lava streams made their way down the valley to the south-east so that finally they blocked the larger valley within which Lake Bunyonyi afterwards formed. Bunyonyi is an interesting lake with no indigenous fish, and this is remarkable because its overflow goes through Kigeyo fen and ultimately into Lake Mutanda, which has fish. But Bunyonyi waters are periodically deoxygenated. Now, part of the lava stream which halted at the north-west end of Lake Bunyonyi was diverted a little to one side by a rocky outcrop, and it impounded a smaller lake (site 3, Fig. 1) in a tributary valley. This lake has been completely infilled, and a drilling, believed to be near the centre of the deposit, gave 20 metres of lake muds, clay-muds and algal muds. The bottom, which was touched at this depth, was hard and presumably was a flooring of lava; the other dominant rock of the area is relatively soft Karagwe–Ankolean shale. Now, since the lake developed behind the lava flow and the lake muds rest on the lava, it is obvious that there are attractive opportunities here, and in the valley behind Muko (site 4), for correlating by pollen analysis and perhaps dating by radiocarbon the volcanic outbursts. Geologically, the lava flows should be relatively young because Lake Bunyonyi appears to have no high-level beaches, which means it has not yet had sufficient time to cut down the level of the outlet which flows over lava below Muko.

Other excellent deposits, and some of them similarly dammed behind lava flows, have been found on the Kisoro side of the Rukiga Mountains; one of these at a small village called Butongo (site 5) has been carefully sampled. Here a late stage of lake colonization has just occurred with a thick, floating mat of *Sphagnum* laced together with *Miscanthidium*. The drill penetrated very compact lake muds, lake clays and algal muds, this time to a depth of 20·5 metres. Near the base there was black ash in the mud, and volcanic pebbles.

The most elevated deposit we know of in the Rukiga Mountains is in Muchoya Valley (site 1); it is sometimes known as Elephant Valley. This valley is entirely enclosed by the Echuya forest reserve, which is of almost undiluted and dense bamboo (*Arundinaria alpina*). The valley has never been blocked by lava, but its drainage has been impeded by tilting, and for some time in the past there has been a long lake. The stratigraphy of the valley-filling was studied by drillings along a transect, and this proved a flat-bottomed valley, filled for the most part with lake muds. These are overlain by fen forest peat, and this in turn by a fen peat built up of tussocky sedges. The maximum depth of the deposit was 11 metres, and samples were collected at 2- and 5-cm. intervals, but with great difficulty in the lowest few metres because the muds were very firm and compact. It is from this valley that we now have the first long pollen profile from East Africa. I believe that this is an extremely important profile because of the position of this Muchoya deposit in the vegetational zones of the mountains. I worked on the theory, when choosing it, that a change of climate would have induced a movement of the altitudinally zoned vegetation of the Rukiga Mountains. Thus, there might be a record in the wind-borne pollen of a replacement of the bamboo by the succeeding zone of *Hagenia*, or by the underlying zone of Montane rain forest. Probably many readers are unfamiliar with these zones[10] of the East African mountains. There are three main belts: (1) Montane forest belt, 5,576–7,544 ft., 9,840–10,824 ft.; (2) Ericaceous belt, 8,504–11,152 ft., 11,480–13,448 ft.; (3) Alpine belt, 13,448 ft. upward. But the Rukiga Mountains with their peaks between 8,000 and 9,000 ft. are not high enough to harbour, to-day, the ericaceous and alpine belts, and indeed do not have even all the montane forest belt. This last-mentioned may have three zones, which reading from the base upward are: (1) montane rain forest zone; (2) bamboo zone; (3) *Hagenia* zone. The uppermost of these, the *Hagenia* zone, is absent to-day from the Rukiga range, and the bamboo consequently extends to the hilltops. The Impenetrable Forest, on slightly lower hills to the north-west of Muko, has only a few small areas of bamboo, and is dominated by rain-forest tree genera, including *Parinari* (Rosaceae), *Piptadenia* (Mimosaceae) and *Chrysophyllum* (Sapotaceae).

The entire pollen profile from Muchoya fen includes many genera of wind-borne pollen, and it would be confusing to reproduce it here where there is no space for an adequate introduction to the ecology of the plants involved. For simplicity, therefore, I have shown only two curves, *Hagenia* and *Stoebe*. *Hagenia*[11] is a rosaceous tree up to 50 ft. in height, with imparipinnate leaves and handsome panicles of flowers; the female reddish, the male white. *Stoebe*[12] belongs to the Compositae, and is a shrub up to 30 ft. in height, deceptively ericoid in appearance. According to Hedberg[12] it is "Co-dominant with *Philippia* spp. and *Erica* in forest or scrub on dry localities, sometimes scattered in grassland, from the (upper part of the) Montane forest belt, throughout the Ericaceous belt, and occasionally reaching into the lower part of the Alpine belt".

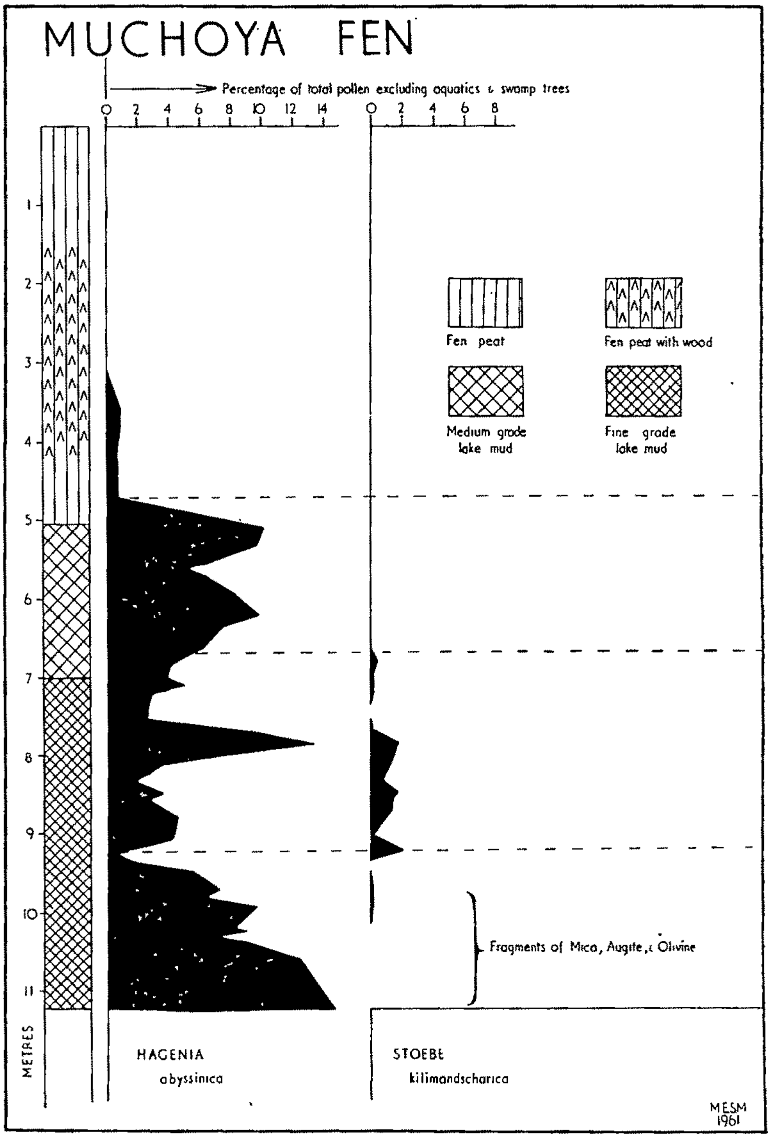

Fig. 2

Turning our attention now to the pollen profile (Fig. 2), we observe that, as the deposit began to accumulate, the surrounding hillsides were filled in with large amounts of *Hagenia*. Now this is very significant, because, as I have pointed out, *Hagenia* does not occur to-day on the Rukiga Mountains, except as occasional trees along the roadside and in clearings in the bamboo. From being initially high, the *Hagenia* percentages fall to a minimum close to 9 metres, and this could be regarded as completing the first zone of the profile. *Stoebe* was sparingly present in this first zone, but it becomes abruptly more prominent at the *Hagenia* minimum and its higher values persist to mark out, perhaps, a second zone in which, apart from a peculiar peak at 8 metres, *Hagenia* values are fairly low. At present we cannot explain these events because we know too little of the ecology of the plants. It is possible that this second period was one of greater cold, because *Stoebe* is more common in the ericaceous belt than in the montane forest belt. I believe, too, it is worth noting that *Stoebe* is not to-day known from the Rukiga or Ruwenzori Mountains, but it is recorded from western Congo, from Mts. Moroto and Elgon in Uganda, and from mountains in Kenya.

As *Stoebe* dwindles, the *Hagenia* recovers and reaches higher and sustained values which may mark out the third zone of the profile. This zone ends with the abrupt decrease of *Hagenia*, and I assume it was replaced entirely by bamboo, which has persisted to the present. Bamboo may, of course, have been intermingled with the *Hagenia* in the first three zones, but we have no evidence of this; and, in fact, its presence will not necessarily be recorded in the pollen. It is a strange fact that the bamboo on the Rukiga Mountains has never been found in flower, and the Africans of the area have no knowledge of its flowering at any time in the past. I think they are likely to have observed such an event because the plants die after flowering. I have heard it said that in the Far East large tracts of bamboo forest flower at long time-intervals, and the flowering is regarded as a bad omen. The bamboo fruits are much desired by rats which invade the area and afterwards set about ravaging stores of grain, so that ultimately famine may result.

Within limits, bamboo can be inferred in the upper part of the Muchoya profile from other observations. The bamboo is always accompanied by some plants which may exist also in the Montane rain forest. Thus one finds *Afrocrania* (Cornaceae), *Dombeya* (Sterculiaceae), *Lachnopylis* (Loganiaceae), *Mimulopsis* (Acanthaceae) and *Ilex* (Aquifoliaceae)[11]. Where we find pollen of these plants, but not of rain forest genera such as *Parinari* (Rosaceae), *Piptadenia* (Mimosaceae) and *Chrysophyllum* (Sapotaceae), then we may tentatively infer the presence of bamboo forest, though perhaps some of the first-mentioned genera could occur also in *Hagenia* scrub. I must add that bamboo does flower in some parts of Africa, and in my type-collection there is pollen taken from plants on Mt. Elgon and from the Aberdares in Kenya. The pollen is very large, and this, with the distinct pore morphology, distinguishes it from many pollens of the wild grasses.

With only this pollen profile for evidence, and little knowledge of the ecology of *Hagenia*, *Stoebe* and *Arundinaria*, one tends to fall back on climatic change as a cause for the former dominance of *Hagenia* and its replacement by bamboo. But we must be careful not to leap to a climatic interpretation because we are unable to discuss other perhaps potent ecological factors. We should also learn a lesson from the evolution of thought about European pollen profiles. These, at first, were explained almost wholly in terms of climate, but the most recent discussion[13] seems to find little room for climate and brings forward prominently succession, competition, and human interference. Therefore, it is right that we should emphasize the possible importance of these other causes for the 'shape' of the Muchoya profile.

Human interference perhaps may be ignored, because it is believed that the movement of people into the upper, cool parts of the Rukiga Mountains has been recent. But a reasonable explanation, on the other hand, in terms of ecological succession, could be constructed. Consider that *Hagenia* might arise in the place of bamboo if this vanished because of fire, or as a natural consequence of its flowering. I raise the possibility of fire because of the fragments of mica, augite and olivine in the lower several metres. These fragments must have come from a volcanic eruption, and, of course, if much ash had fallen on the landscape then fires might indeed have swept the bamboo. Afterwards it might not have recovered, for, unless the fire had immediately followed a rare flowering, there would have been no bamboo fruits to have given a new generation. But it seems improbable that there was a fire, and there is no layer of ash in the stratigraphy; and indeed the fragments of rock I mention are very minute and few. Since at present these considerations do not lead us to anything promising, we are possibly justified in assuming that the replacement of *Hagenia* by pure bamboo was caused by a change of climate—perhaps an amelioration—some hundreds or thousands of years ago. Without radiocarbon assay there is no possibility of dating any part of the profile. Interpretation of the profile will be very much easier when we have further profiles from the Rukiga Mountains. I have now commenced work on the 20·5-metres deep profile from Butongo (site 5). This site is not now in the bamboo forest, but is in the cultivated zone only 600 ft. below the present lower limit of the bamboo.

I have said nothing of the deposits actually hidden beneath lakes such as Victoria and Bunyonyi. These deposits cannot be sampled with the Hiller drill which was used for the other places I have mentioned. A special device is necessary to obtain deep-water samples, and Lake Bunyonyi is about 130 ft. deep. We hope to use an ingenious device created by Dr. F. J. H. Mackereth[14] at the Freshwater Biological Association's Laboratories at Windermere, England. It is powered by compressed air and obtains cores 6 metres in length, and it can work down to a depth of 250 metres of water. Because the diameter of the core is 1½ in. it should be possible to study pollen, plankton and chemistry. This should prove especially interesting in Lake Bunyonyi which, as I said earlier, is periodically deoxygenated and is without indigenous fish.

I hope this short account has indicated the richness and fascination of the field open to the pollen analyst in Uganda. I wish to thank the many friends from whose foresight and co-operation I have benefited. I am intensely grateful to Dr. Edna Lind, head of the Botany Department at Makerere College. Dr. Lind perceived the possibilities for pollen analysis in Uganda and arranged for me to be sponsored by the Nuffield Foundation and by Makerere College.

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OBITUARIES

Prof. T. R. Elliott, C.B.E., F.R.S.

T. R. ELLIOTT was born in 1877, in County Durham, and went to Cambridge in 1896, where he took the Natural Sciences Tripos and later was elected to a number of already famous studentships. It was while he held these that Elliott carried out a series of researches in physiology which, in the words of Sir Henry Dale, "were of well-nigh incomparable brilliance and authority, for a worker so young in years and experience".

His interests lay in the effects of the autonomic nervous system, and his attention was caught early by the resemblance between these effects and those of adrenaline. These researches led him, far in advance of his time, to propose the concept of the chemical transmission of nerve impulses, thereby anticipating by nearly a quarter of a century the flood of productive work in this field. His election to the Royal Society in 1913 was a well-merited recognition of his brilliance.

In 1906, Elliott resumed his medical studies and, four short years later, he was appointed physician to University College Hospital, London. There he became closely associated with Thomas Lewis. This was the turning-point. The traditional structure of education in medicine had long been giving cause for concern, and the appointment of a Royal Commission under Lord Haldane to report on university education in London was the occasion for its searching analysis. Among the proposals made was that recommending the creation of whole-time professorial posts in clinical subjects. But the First World War came and delayed its implementation, and Elliott was in France by September 1914. Then followed four years of intensive work in which Elliott became the projection into the battlefield of the medical knowledge and skills which Walter Fletcher, of the old Medical Research Committee, was brilliantly mobilizing at home. As Elliott himself admitted, it was here that he discovered his power for combining science and administration.

The War ended; attention returned to the Haldane Report and Elliott became, inevitably, one of the first whole-time professors of medicine in London. It was expected that this would herald a spate of researches in the clinical field comparable to those that distinguished his early days in physiology. That this did not happen was largely Elliott's conscious decision. He recognized that, if clinical research was to draw level with the experimental biological sciences, then his generation had to sacrifice their personal advancement to developing and organizing the new scientific medicine; and the state of medical progress in Britain to-day is the present memorial to him and his fellows. The advancement of knowledge was his criterion. From the earliest days he was associated with the Medical Research Committee, and he was the only man who has ever served three full terms as a scientific member of its successor, the Medical Research Council. As an indication of the respect in which his judgment was held, it may be mentioned that, young as he was and not a member of the Committee, his advice was asked on the appointment of the first secretary, and it was he who suggested Walter Morley Fletcher.

But in the notices that have appeared since Elliott's death there has been one note common to all—respect for him as a man. Many men have been intelligent, upright and courageous, but in addition Elliott possessed an almost disconcerting ability to detect bias and an indifference to credit that, but for his tolerance, would have made him forbidding. It is no wonder that he remained the trusted counsellor of men with great responsibilities, or that, in need, his was the aid that was sought.

H. P. Himsworth

Rev. J. E. Hull

With the death of the Rev. J. E. Hull in October 1960 at the advanced age of ninety-seven we have lost the last of the Rev. O. Pickard-Cambridge's pupils in the study of spiders.

After leaving St. Bede's College, Hull took a degree in mathematics in the University of Durham before returning to St. Bede's as vice-principal in 1890. Later, his work as a clergyman in various Northumberland parishes confined his natural history work mainly to that county.

He shared with Dr. A. R. Jackson and W. Falconer the chief credit for making known the spider fauna of northern England. He described two British spiders new to science, and six of his proposed new genera have survived. He also did extensive work on mites and edited a northern quarterly journal, *The Vasculum*. His last paper was published when he was ninety-two.

W. S. Bristowe

Dr. M. F. E. Nicolai

Dr. M. F. Emilie Nicolai, algologist and biophysicist, director of the Hydrobiological Instituto at Hilversum, Holland, died suddenly at her home in Holland on March 13. There can be few people whose passing has brought sorrow to such a wide circle of devoted friends.

Dr. Nicolai was born at The Hague on June 27, 1900 where she received her early education. In 1918 she entered the University of Leyden as a student in biology and in the subsequent years she studied botany, zoology, mineralogy, geology, chemistry and physics. Her doctor's degree was awarded in 1929 on a thesis "On Changes in Permeability in the Root Cells of *Sinapis alba*". She remained associated with Leyden until 1942 first as assistant, and later head assistant, to Prof. J. M. Janse and then, from 1931 onwards, to Prof. L. G. M. Baas Becking. During this time her published work lay in the fields of physiology and ecology, though one of her papers (on chlorophyll multifilms, with Dr. C. Weurman) already indicated her interest in the application of physical principles and methods in biology. Her first serious work in the fields both of algology and in biophysics came during this period when, in 1937, she visited the Eidgenossische Technische Hochschule, Zurich, to work under Prof. Dr. A. Frey-Wyssling on the cell wall structure of *Chaetomorpha*. It was no mere coincidence that at this same time my own interests had turned toward the related genus *Cladophora*, causing us afterwards to work closely together for so many years. In 1942 Dr. Nicolai transferred to the Fibre Research Institute, Delft, under Dr. J. R. H. van Nouhouys, and in 1946 became head of the Biological Department. Though, so far as I know, she never worked with him, she was deeply impressed by Prof. G. van Iterson, jun., and it was largely through his influence that in 1947 she came to England first as my research assistant and then as lecturer at Leeds. This was the beginning of a fruitful collaboration on wall structure in the algae lasting almost ten years, opening up a new field and laying down a foundation of work for many years still to come. It was during this period that she made her name as an algologist. Our collaboration terminated when her loyalty to her country led her in 1957 to accept the position of director of the newly founded Hydrobiological Institute in Holland, though she frequently returned to Leeds.

Dr. Nicolai was a botanist of wide experience and a first-class investigator, and for this alone her death would be a serious loss. But she was much else beside. Warm-hearted and generous and with friendliness overflowing to all, 'Nick's' intuitive understanding of, and sympathy with, the problems of her associates never allowed her to speak ill of anyone. Though small in stature, she was always dignified. To many of us her calm unruffled manner was a source of constant strength, and we are all the better for having known her. Few women scientists can have been so well beloved as was 'Nick' by people in so many walks of life. Wherever she went, in Europe or farther afield, in shops or in the rooms of learned societies, she was soon surrounded by her friends.

R. D. Preston

NEWS and VIEWS

Royal Society : Foreign Members

The following have been elected foreign members of the Royal Society: Prof. Olaf Holtedahl, professor emeritus of geology in the University of Oslo and member of the Norwegian Academy of Sciences, Oslo, distinguished for his outstanding contributions to geology, particularly of Norway and the arctic regions; Prof. Solomon Lefschetz, Henry B. Fine professor emeritus of mathematics in Princeton University, Princeton, distinguished for his work in the fields of algebraic geometry and topology; Prof. Elmer Verner McCollum, professor emeritus of biochemistry in Johns Hopkins University, Baltimore, distinguished for his services to the science of nutrition; Academician Aleksandr Nikolaevich Nesmeyanov, president of the Academy of Sciences of the U.S.S.R., Moscow, distinguished for his contributions to organic chemistry, particularly on organo-metallic compounds.

Civil Engineering at University College, London : Prof. A. H. Chilver

Dr. A. H. Chilver, who has been appointed to the Chadwick chair of civil engineering in University College, London, in succession to Emeritus Prof. H. J. Collins, was born in 1926. He was educated at Southend-on-Sea High School for Boys and at the University of Bristol, graduating in 1947. He was awarded the Albert Fry Prize in 1947. After leaving Bristol, Dr. Chilver worked for a year with London Midland Region of British Railways on structural steelwork design. He then returned to the Civil Engineering Department, University of Bristol, in 1948, as a research student. He was appointed assistant lecturer in civil engineering in the University in 1950 and eventually lecturer in 1952. He left Bristol in October 1954 on his appointment as a University demonstrator in the Engineering Department, Cambridge. He was promoted to a University lectureship in 1956 and was elected into a fellowship at Corpus Christi College in January 1958. Dr. Chilver's main fields of research are structural stability and related problems; stability of thin-walled metal structures, stability of complete frames, buckling of shells; structural safety; loads on engineering structures and strength of structural materials. His publications consist of various papers on structural stability published in engineering journals, an elementary text-book on theory of structures written in collaboration with R. J. Ashby and another on strength of materials written in collaboration with J. Case.

Chair of Mechanics at Cambridge : Prof. D. C. Johnson

Prof. D. C. Johnson, professor of mechanical engineering in the University of Leeds, has been appointed to a chair of mechanics in the University of Cambridge. Prof. Johnson was born in 1915. He was educated at Mill Hill School and was a Minor Scholar at King's College, Cambridge, during 1934–37. He was placed in the first class in the Mechanical Sciences Tripos in 1937, gaining distinction in heat and heat engines. After leaving Cambridge, Johnson worked during 1937–47 for Rolls-Royce, Ltd., Derby. He returned to Cambridge in 1947 as a University demonstrator and was appointed a University lecturer in 1948. He was appointed secretary to the Faculty Board of Engineering in the University and elected into a fellowship at Trinity Hall in 1954, resigning in 1955 to take up the chair of mechanical engineering at Leeds University. Prof. Johnson's main field of research is vibrations, on which subject he has lectured widely and contributed several papers. His latest publication, "The Mechanics of Vibration", was written in collaboration with Prof. R. E. D. Bishop and published by the Cambridge University Press in 1960.

Nature Conservancy Appointments

The Nature Conservancy has appointed Prof. J. B. Cragg as director from June 1, 1961, of the Merlewood Research Station, Grange-over-Sands, and Dr. K. Mellanby as director from October 1, 1961, of the new Monks' Wood Experimental Station, to be built on a recently acquired site near Huntingdon.

Prof. J. B. Cragg

Prof. Cragg has had some twenty-five years experience of university work. In addition to his academic duties he has served on a range of major official committees and has visited Israel, West Africa and the Antarctic. He was appointed reader in zoology in the University of Durham (Durham Colleges) in 1946 and then professor in 1950, during which period he has written extensively on problems of conservation and ecology. Prof. Cragg is president of the British Ecological Society and chairman of the Agricultural Research Council's Pest Infestation Research Board. He has also played an active part in the affairs of the Institute of Biology as member of Council, editor and vice-president. The main research at Merlewood, which the Nature Conservancy set up in 1952, concerns the study of woodland ecology, with an emphasis on nutrient cycles. Moor House Field Station, on the Westmorland Pennines near Alston, Cumberland, will also be directed by Prof. Cragg.

Dr. K. Mellanby, C.B.E.

Dr. Mellanby is one of the few senior entomologists with experience in both medical and agricultural entomology. His main interest has been concerned with the reactions of insects to temperature and humidity, a subject on which he has written many papers. He has carried out much research work in Britain and other countries (mainly tropical countries). In 1955, he became head of the Department of Entomology of Rothamsted Experimental Station.

He is president of the Association for the Study of Animal Behaviour and vice-president of the Royal Entomological Society. The establishment of the Nature Conservancy's new Experimental Station at Monks' Wood (adjoining the National Nature Reserve) will provide Dr. Mellanby with a challenge and an opportunity for which his broad experience is especially relevant. When building is completed in 1962, the Station will house three teams studying the effects of toxic chemicals on wild life, ecological management of native woodlands and scientific problems of conservation. The Station will also form a base for field studies of the diploma course on conservation of University College, London, and for other university and related activities. The Station is already functioning in embryo in temporary premises, and it is hoped that it will be fully operative by 1963.

The James Craig Watson Medal : Prof. O. H. L. Heckmann

The James Craig Watson Medal of the U.S. National Academy of Sciences has been awarded to Prof. Otto Hermann Leopold Heckmann in recognition of his noteworthy contributions to astronomy. The Medal, established in 1874 by the bequest of Dr. Watson to recognize astronomical discoveries or work "worthy of special reward", was presented to Dr. Heckmann at the annual meeting of the Academy on April 24. As professor of astronomy in the University of Hamburg and director of the Hamburg Observatory at Bergedorf, West Germany's leading observatory, Dr. Heckmann is widely known for his work in astrometrics. Under the direction of his predecessor, R. Schorr, the Hamburg Observatory undertook about 1930 the enormous task of photographing and measuring the positions of the stars of the northern hemisphere. This work, known as the *AGK*2, was published in fifteen volumes. Dr. Heckmann initiated a repetition of it, enlisting the cooperation of a group of observatories in Canada, England, France, West Germany, East Germany, the U.S.S.R., and the U.S. Naval Observatory in Washington, to join in a concerted effort to derive, by new observations, the proper motions of 180,000 stars. Completion of the new catalogue, *AGK*3, is expected in about three years. Dr. Heckmann is internationally regarded as an expert in the field of relativity and cosmology. His work in this field includes not only contributions to the theory of cosmology but also critical assessments of the observational and experimental basis of cosmological theories.

The CIBA Fellowship Trust : Awards

The following were recently awarded CIBA fellowships: Mr. R. F. C. Claridge (University of Auckland and University of Cambridge), for post-doctoral work at the University of Cologne (radiochemistry); Dr. J. S. Littler (University of Oxford), for post-doctoral work at the Federal Technical Institute, Zurich (chemistry); Mr. H. Stern (University of Oxford), for post-doctoral study at the University of Brussels (theoretical physics); Mr. F. I. B. Williams (McGill University and University of Oxford), for post-doctoral work at the University of Liège (solid-state physics). Fellowships previously awarded to the following were renewed for a further year: Dr. V. P. Arya, at present working at the University of Stockholm (natural-products chemistry); Dr. W. D. Hamilton, at present working at the University of Uppsala (nuclear physics); Dr. R. Grinter, at present working at the Federal Technical Institute, Zurich (physical organic chemistry); Dr. B. L. Mordike, at present working at the University of Göttingen (physical metallurgy).

Scientific Development and the Budget

In his Budget speech on April 17, the Chancellor of the Exchequer, Mr. Selwyn Lloyd, expressed the view that productivity in Britain is being held down by shortages of skilled labour and that he is not satisfied that both sides of industry are invariably tackling this problem with sufficient energy. There are too few scientists and technologists. There are features in the tax system which make for inadequate rewards for initiative and enterprise. Some industries are insufficiently accustomed to competition and insufficiently aware of the need for technical improvement if they are to hold their own with their overseas competitors. Labour relations, standards of management and techniques of selling are sometimes below what is needed to-day, and certain industries are still, in his view, handicapped by restrictive practices which are completely out of date.

These comments were welcomed by Mr. H. Wilson, who wished to see them matched by action in scientific education and also measures to encourage scientific research in industry. He attributed the loss of young scientists and technologists to America to the poor prospects of promotion and inadequate salaries offered by many industries in Britain, and wished to see taxation on the middle ranges of scientists and executives reduced. The over-riding priority is to encourage science and technology. The total expenditure on scientific research, Mr. Wilson remarked, is £478 million a year, of which £370 million comes from public funds, while 52 per cent of the national expenditure on research and development goes on defence. Calling for a crash programme to train more scientists and engineers and a new policy for deploying them, Mr. Wilson urged even more a new and determined drive to apply science in industry. He suggested a wider use of the National Research Development Corporation and an expansion by 20 per cent of the 15,000 scientists at present employed on civil research and development.

The President of the Board of Trade, Mr. R. Maudling, agreed that we need much more research and development in British industry and also a rapidly expanding programme, as we now have, of scientific and technical education. Defence requirements, however, still limit the transfer of scientists and technologists from defence research to civil research. Mr. H. Nicholls thought that the Chancellor's surtax proposals would play some part in keeping in Britain the 2,000 scientists now leaving the country every year to work in competitor countries; the net loss, he pointed out, is about £1,000. Mr. D. Ginsberg, however, complained that the Budget did nothing to assist scientific research.

The Colonial Development Corporation

In a statement in the House of Commons on April 27, the Secretary of State for the Colonies, Mr. Iain Macleod, said that to ease the financial position of the Colonial Development Corporation, the Government had decided to release the Corporation from the obligation to meet the liability of accumulated unpaid interest on long-term Exchequer advances, now amounting to about £11 million, when they become due; the sum is to be placed in a special account in a way similar to that proposed by the Sinclair Committee, where interest will not be payable. The Corporation's debt of about £9 million, arising from projects abandoned by the Corporation, would also be placed in this account. Towards these two liabilities the Corporation would pay to the Exchequer 60 per cent of its net annual profits in excess of £250,000. The Government proposed to review these arrangements in three years time in the light of experience gained.

In future loan transactions, the period over which repayments to the Exchequer would be made would conform generally to that of the capital repayments which the Corporation would be receiving from third parties. For investments in shares, the Corporation would generally draw long-term advances for a 40-year period, and for this type of investment only the Corporation would not be required to service the Exchequer advances during the first seven years. Mr. Macleod said he was satisfied that these arrangements would be of real assistance to the Corporation and that they largely followed the recommendations of the Sinclair Committee. The Government had been unable to accept the recommendation that part of the advances should be converted to an equity share-holding in the Corporation, as this would have amounted to a subsidy over which there would not be full Government or Parliamentary control. In reply to questions, Mr. Macleod said that while the Corporation could bring to fruition projects it may have started in newly independent countries, its main effort should be made in starting new projects in the Colonies where the need was very great.

A Radio Telescope for the Department of Scientific and Industrial Research

A FULLY steerable radio telescope is to be built at a site near Crowthorne, Berkshire, by the Ministry of Works for the Radio Research Station of the Department of Scientific and Industrial Research. It is expected to be completed and in operation towards the end of 1963 at an estimated overall cost of £250,000, including buildings, equipment and site services. The instrument, which will have a parabolic aerial about 80 ft. in diameter, will have high accuracy and a faster tracking speed than the 250-ft. radio telescope at Jodrell Bank, an essential requirement for following Earth satellites and determining orbital data. It will be used to receive weak signals from distant space vehicles transmitting at ultra-high frequencies. In the field of radio astronomy, an application of the telescope will be a study of radio noise from the Sun, including sudden increases in radio emission which are related to disruption in short-wave communications. Also of interest is the radio noise from planets. The telescope will be valuable in extending investigations of the properties of the upper and lower atmospheres which are important in both terrestrial and space communications. This feature will assume even greater significance in the next few years, when satellites for communications, as well as for research purposes, are likely to come into use. In considering this project, the Council for Scientific and Industrial Research was advised by the following sub-committee: Sir Bernard Lovell (University of Manchester), Prof. Martin Ryle (University of Cambridge) and Dr. J. S. Hey (Royal Radar Establishment, Malvern).

Committee on Hydrological Research

IN reply to a question in the House of Commons on April 18 asking for the creation of an organization to conduct basic research into the conservation and utilization of water supplies in Britain, Mr. D. Freeth, the Parliamentary Secretary for Science, said that the Department of Scientific and Industrial Research has set up a Committee on Hydrological Research, under the chairmanship of Dr. B. K. Blount: "to survey and assess current research relevant to water conservation in the United Kingdom; to identify needs for further research; to allocate priorities and to determine where research should be done and to co-ordinate the work as necessary". The Committee has already held its first meeting and the Ministries of Agriculture and Local Government, as well as other bodies, are co-operating fully.

The membership of the Committee is as follows: Dr. B. K. Blount (*chairman*), Department of Scientific and Industrial Research; Dr. E. C. Childs (Agricultural Research Council); Dr. H. L. Penman (Agricultural Research Council); Mr. E. A. C. Johnson (Ministry of Agriculture, Fisheries and Food); Mr. J. K. Wilson (Department of Agriculture for Scotland); J. E. Munro (Department of Health for Scotland); Mr. R. F. Wood (Forestry Commission Research Station); Mr. C. H. Spens (Ministry of Housing and Local Government); Mr. A. C. Boulton (Surface Water Survey, Ministry of Housing and Local Government); Mr. P. J. Meede (Meteorological Office); Dr. E. B. Worthington (Nature Conservancy); Prof. M. V. Laurie (Department of Forestry, University of Oxford); Mr. P. O. Wolf (Department of Civil Engineering, Imperial College, University of London); Mr. N. A. F. Pountree (Institution of Water Engineers); Mr. M. Nixon (River Boards Association); Dr. R. C. Allen (Water Research Association); Dr. S. Buchan (Geological Survey and Museum); Mr. F. H. Allen (Hydraulics Research Station); Dr. B. A. Southgate (Water Pollution Research Laboratory).

Malaria Eradication Campaign

IN replying on an adjournment debate on the United Kingdom's contribution to the malaria eradication campaign on April 26, the Parliamentary Secretary to the Ministry of Health, Miss E. Pitt, said that the United Kingdom contributes already in four ways: through the regular budget of the World Health Organization; through the United Nations International Children's Emergency Fund; through the United Nations Technical Assistance Fund; and through the grants under the Colonial Development and Welfare Acts, the total expenditure under which for the purpose during the past twelve years was £760,000. In 1961 the contribution from the Malaria Eradication Special Account was about one-third of all that expended by the World Health Organization on malaria eradication, and amounted to 5·7 million dollars. The regular budget contributed 714,000 dollars, and the extended programme of technical assistance 639,000 dollars. The Special Malaria Fund of the Pan-American Health Organization contributed 3 million dollars and the United Nations International Children's Emergency Fund 8 million dollars, making a total of nearly 19 million dollars.

The Malaria Eradication Special Account was established in 1955 on the initiative of about 25 countries, but at the start the major Powers were opposed to the financing of such a scheme by soliciting voluntary contributions from Governments, and it was not until 1957 that the United States changed its policy and contributed 5 million dollars to the Account. The British Government maintained the view that such an eradication campaign should be financed through the regular budget of the World Health Organization (to which the United Kingdom contributes 7 per cent), and at the General Assembly of the Organization in New Delhi in February the United Kingdom supported a scheme which would put any shortage in voluntary contributions immediately on the regular budget. The administrative and operational costs of more than 600,000 dollars had already fallen on the regular budget in 1961; an additional 2 million dollars would fall on it for 1962, double this for 1963; and the whole 6 million dollars in 1964, an increase of more than 20 per cent in the 1962 budget of 23·6 million dollars. This would involve further contributions from the United Kingdom of 140,000 dollars in 1962, 280,000 dollars in 1963 and 420,000 dollars in 1964. Under the scheme adopted, which gives credits to the member countries carrying out malaria eradication campaigns and have low *per capita* incomes, a group of about 30 countries with relatively large *per capita* incomes will share the burden which in the past was borne by a few volunteers but mainly by the United States, which contributed about 90 per cent. Miss Pitt said that in the future the Director-General of the World Health Organization would be able to plan more effectively, since he could rely on the resources of his regular budget, and she also emphasized the substantial contribution from Britain on the technical side in research and training facilities.

Public Health Nursing

THE nursing profession throughout the world is faced with a severe shortage of recruits to its ranks. In the developed countries an increased burden is being placed on its shoulders by the growing complexity of medical services, treatment and equipment; the demand for nurses is rising at a time when the supply is falling off as a result of the availability elsewhere of better-paid jobs in easier working conditions. In the less-developed countries the demand for nurses is enormous; but the supply is restricted by unwillingness to allow women to become nurses, by the low status of nursing, and, above all, by the absence of the money to pay them. This crisis in the nursing profession has led to a re-appraisal of the functions of nurses and to various attempts to use auxiliary workers for the less-essential tasks of nursing, thus freeing nurses for the more-essential. A volume in the series of *Public Health Papers* by the World Health Organization contains a collection of papers by nursing and other experts from all parts of the world, who discuss the training and functions of public health nurses, the basic curriculum and the changes that the new situation is bringing about and should bring about in it, the importance of team work, the value of knowledge of administration, the role of the auxiliary worker, and the fresh approach now required of public health nurses (No. 4 : *Aspects of Public Health Nursing*. Pp. 185. 5 Swiss francs; 8*s*. 6*d*.; 1.75 dollars; by various authors. (Geneva: World Health Organization; London: H.M.S.O.) 1961). There are several contributions describing how countries—such as Brazil, France and the U.S.S.R.—have dealt with the problem of the auxiliary worker. A final paper discusses the difficulties met by international nurses working in other countries with strange cultures, different standards and attitudes.

Rural Research in C.S.I.R.O.

AMONG the many periodicals that nowadays come to our libraries, those of the Commonwealth Scientific and Industrial Research Organization of Australia have for long maintained a high value, and *Rural Research in C.S.I.R.O.* has for some years now kept agriculturalists well informed about the progress of agricultural research in Australia. The issue for December 1960 (No. 34, obtainable from the Agricultural Liaison Section of the Commonwealth Scientific and Industrial Research Organization, 314 Albert St., East Melbourne, C.2, Victoria) discusses the experimental work being carried out in Queensland on the rearing of cattle in a tropical environment, and the characteristics of the coats of the cattle in relation to this. Other articles deal with: research on the wool of sheep; sheep fluorosis; improved cobalt treatment of sheep; the use of the electron microscope in agricultural research (illustrated by striking photographs); soils and sulphur deficiency in soils; and new techniques which can extend the storage life of apples.

Bering Strait Dam

P. M. BORISOV has outlined a project, in the October (1959) issue of *Literaturnaya Gazeta*, of a 90 km. long dam across the Bering Strait equipped with powerful pumps pumping cold Arctic Ocean water into the Pacific Ocean at the rate of 500 km.^{3} in 24 hours. Such a project, according to Borisov, would increase the flow of warm Atlantic Ocean water into the Arctic Ocean and change the climate of the Arctic regions. This project is criticized by D. A. Drogaitzev (*Priroda*, 6, 35; 1960), who, on the basis of detailed calculations, argues that such a project would displace the locus of Atlantic Ocean cyclones to the region of the Barentz Sea. Such a displacement would certainly change the climate of Northern Europe and Western Siberia, but this change will produce colder winters and hotter summers and will lead to the displacement of the desert belt from the region of North Africa and Central Asia to the north of Europe. At the same time the northern Pacific region will also undergo a climatic deterioration.

The Manchester Museum

IN the annual report of the Manchester Museum for 1959–60, special mention is naturally made of three additions to the exhibition space. The special Japanese gallery to house the Lloyd collections has been completed, a coin room has been provided, and certain re-allocations with the University have made possible botanical offices and laboratories and the re-organization of the great herbarium. Two outstanding purchases were made during the year—two Celtic gold bracelets dug up in 1829 at Malpas in Cheshire and an Iron Age gold necklace found at Burnley in 1801. By the aid of various funds and a subscription list it was possible to purchase these three objects. A bequest of great importance was the Leslie Armstrong collection excavated from Pin Hole Cave, Derbyshire. It comprises about 14,500 bones, bone flakes, antlers and teeth. The report closes with an impressive list of publications by the staff.

The Birmingham Museum of Science and Industry

BIRMINGHAM CITY COUNCIL has approved the rebuilding of the Museum of Science and Industry.

Shortage of space for display and storage has prompted the Council to rebuild on the present site. Some of the very old buildings are structurally unsound, and would be most uneconomical to maintain in good repair. They will be demolished and replaced by a new building which will incorporate any suitable existing buildings. The scheme for rebuilding includes a new Locomotive Hall, an administrative block and an Engineering Hall. The scheme will be carried out in three phases over a period of several years. The total cost including professional fees will be in the region of £655,000.

Meteorites in the U.S.S.R.

IN the first quarter of the last century, certain curiously shaped hollows were discovered on the land surface of the Island of Sarema in Estonia, but it was only in 1927 that I. A. Reinwald produced the correct interpretation of their origin. As reported by E. L. Krynov (*Priroda*, 7, 55; 1960) these hollows represent meteorite craters. They were studied in great detail by Reinwald and after his death in 1945 by A. O. Aaloe, who carried out a systematic excavation of these craters and collected a large amount of fragments and dust belonging to iron meteorites. The disposition of the seven craters of the Kaalijarvi region suggests that, as in the case of the craters of Henbury in Australia, the Estonian craters are situated within an elliptical area of meteorite dispersion produced by a fall of meteorites which moved along a line stretching from south-south-west to north-north-east.

Reports of two meteorite falls in the U.S.S.R. have been forthcoming during the past year. According to M. A. Kashkai *et al.* (*Priroda*, 9, 109; 1960) an iron meteorite fell in Yardymly region, S.W. of Baku, Azerbaijan, at 8.5 a.m. November 24, 1959. This meteorite apparently broke into fragments before its fall as five distinct falls were registered. The collected fragments vary in weight from 360 gm. to 127 kgm. Calculations show that before fragmentation the meteorite was moving from the S.W. to the N.E. V. A. Yarmoluyk reports (*Priroda*, 11, 100; 1960) that a number of eyewitnesses observed a flight of a bolid accompanied by a flash and sound effect at 17h. 30m. local time on December 22, 1959, near the settlement Nurki, Dzhudzhur Range, fringing the Okhotsk Sea coast. It is presumed that these effects were caused by a meteorite fall, but no fragments of such have as yet been found in this region.

Artificial Melting of the Arctic Ice

A CONSIDERABLE handicap to navigation in the Arctic Ocean is presented by the delay of melting of ice in bays and river estuaries as compared with that in the open ocean. As described by I. S. Peschansky (*Priroda*, 7, 49; 1960), a most successful acceleration of the process of melting of ice has been achieved by dusting the surface of the ice with various dark-coloured materials along certain definite navigation lines. This allows a greater play of solar energy and the formation of 'radiation' channels a month and a half before the normal melting period of the ice.

Flora of the Rugby District

A GOOD example of the way an educational institution and the neighbouring community can work together is provided by a publication describing the flora of Rugby and its environs. It has been prepared by a former scholar, D. E. Allen, with records supplied by boys of the school and members of the town. To the original *Flora*, which was first published in 1957, has now been added a supplement providing details of recent discoveries (Rugby School Natural History Society. A First Supplement to *The Flora of the Rugby District*. Pp. 4. By D. E. Allen. (Rugby: George Over, Ltd.) 1*s*.).

The Royal Irish Academy

AT a meeting of the Royal Irish Academy in Dublin on March 16, Prof. John L. Synge, director of the School of Theoretical Physics in the Dublin Institute for Advanced Studies, was elected president. Prof. J. Doyle, Prof. J. J. Tierney, Prof. B. Ó. Cuív and Prof. F. E. Hackett were re-elected as secretary, secretary for polite literature and antiquities, secretary for Irish studies, and treasurer respectively. On the retirement of Dr. A. Farrington, who had been assistant secretary since 1928, Mr. Liam de Paor was elected executive secretary. The following were elected honorary members: in the Section of Science, Sir Harold Jeffreys, Cambridge, Edgar Lederer, Gif-sur-Yvette, and Lamoraal Ulbo de Sitter, Leyden; in the Section of Polite Literature and Antiquities, Roman Jakobson, Harvard, and Jerzy Kuryłowicz, Cracow. Mr. J. C. Beckett, Dr. J. G. Belton, Dr. J. W. Harman, Mr. J. P. Haughton, Dr. N. A. Porter, Dr. Yasushi Takahashi and Mr. F. G. A. Winder were elected members of the Academy.

The Royal Institution

AT the annual meeting of the Royal Institution, held on May 1, the following officers were elected for the year 1961–62: *President*, Lord Brabazon of Tara; *Treasurer*, Mr. W. E. Schall; *Secretary*, Brigadier H. E. Hopthrow; *Managers*, Mr. F. G. Brown, Sir Harold Roxbee Cox, Mr. E. R. Davies, Prof. Alexander Haddow, Mrs. H. K. Hawkes, Dr. R. Holroyd, Miss E. Henrietta Jebens, Mr. L. B. W. Jolley, Prof. L. A. Jordan, Sir Ben Lockspeiser, Dame Kathleen Lonsdale, Dr. H. Lowery, Mr. W. R. Stevens, Wing-Commander H. F. Tiarks and Prof. W. D. Wright; *Visitors*, Mr. A. D. Baynes-Cope, Mr. A. D. R. Caroe, Mr. Norman Clarke, Miss F. M. Eastwood, Mr. A. H. Ewen, Dr. D. H. Follett, Mr. G. J. Gollin, Mr. N. P. W. Moore, Dr. A. A. Moss, Mr. J. D. Peattie, Mr. Frank Poperwell, Mr. F. Y. Poynton, Dr. H. R. Rishworth, Miss D. Sidebottom and Mr. H. Stanesby.

The Geological Society of London

THE following were elected officers and council of the Geological Society of London at the annual general meeting held at Burlington House, Piccadilly, on April 26: *President*, Prof. S. E. Hollingworth, Yates Goldsmid professor of geology in the University of London; *Vice-presidents*, Prof. T. Neville George, professor of geology in the University of Glasgow; Dr. D. R. Grantham; Sir William Pugh, emeritus professor of geology in the University of Manchester, and lately director of H.M. Geological Survey; Dr. C. J. Stubblefield, director of H.M. Geological Survey; *Secretaries*, Mr. J. M. Edmonds, curator of Geological Collections, University Museum, Oxford; Dr. P. A. Sabine, principal geologist, H.M. Geological Survey. *Foreign secretary*, Prof. O. T. Jones, emeritus professor of geology in the University of Cambridge. *Treasurer*, Mr. P. Evans. *Ordinary members of* *council*, Prof. J. G. C. Anderson (Cardiff), Dr. H. W. Ball (British Museum, Natural History), Prof. K. C. Dunham (Durham), Dr. R. G. S. Hudson (Dublin), Prof. B. C. King (Bedford College, London), Dr. Dorothy Rayner (Leeds), Prof. R. M. Shackleton (Liverpool), Prof. J. Sutton (Imperial College of Science and Technology, London), Prof. P. C. Sylvester-Bradley (Leicester). The following were elected members of the Society: Mr. M. Black, lecturer in geology, University of Cambridge; Mr. P. T. Cox, managing director, B. P. Exploration Co., Ltd.; Mr. D. Curry; Dr. K. A. Davies, deputy director, Overseas Geological Survey; Dr. Vernon Wilson, district geologist, H.M. Geological Survey.

The Franco-British Pharmaceutical Commission

The Franco-British Pharmaceutical Commission was appointed in January 1950 by the Councils of the Pharmaceutical Society of Great Britain and of the Ordre National des Pharmaciens de France to promote co-operation in the field of pharmacy. The formation of the Commission was inspired by the Cultural Convention held by the Governments of France and Great Britain in 1948 at which the hope was raised that " . . . in each country the fullest possible knowledge and understanding of the intellectual, artistic, scientific, technical and educational activities and of the history and ways of life of the other" should be promoted. In the spring of each year since 1951 a team of French lecturers has visited towns in Great Britain, and a British team has gone to France. More recently, British experts have visited France (1960 Lille; 1961 Lyons) to study aspects of the practice of pharmacy there. Three British pharmacists have done research work at the University of Paris, and one is going to Bordeaux during 1961/62. Through the two Societies, the Commission is able to obtain information on most pharmaceutical subjects and to facilitate the exchange of scientific material, for example, specimens of drugs or medicines, between interested parties in the two countries. Week-end congresses, well attended by both French and British pharmacists from all parts of the two countries, have been held in the spring, alternately in France and Great Britain. The ninth Congress, which will be attended by about 100 French and 100 British, is to be held in Jersey during May 12–15, 1961. The secretary of the British Section of the Commission is Sir Hugh Linstead. Further information can be obtained from the Pharmaceutical Society of Great Britain, 17 Bloomsbury Square, London, W.C.1.

Announcements

Prof. John Stanley Griffith of the Department of Chemistry, University of Pennsylvania, has been awarded the Faraday Society's Marlow Medal, for his work on ions of the transition metals.

Dr. Max F. Perutz, director of the Medical Research Council Unit for Molecular Biology, Cambridge, delivered the 1961 Chaim Weizmann Memorial Lecture at the Weizmann Institute of Science, Rehovoth, on April 18, when he was made an Honorary Fellow of the Institute for "his major contribution to the science of X-ray Crystallography applied to macromolecules and for his distinguished part in the elucidation of the hæmoglobin molecule".

The Lawes Agricultural Trust Committee has appointed Dr. C. G. Johnson as head of the Entomology Department, Rothamsted Experimental Station, from October 1, in succession to Dr. K. Mellanby, who will become director of the Monks' Wood Experimental Station of the Nature Conservancy (see p. 488).

The Right Hon. Lord Clitheroe has been appointed a vice-chairman of Tube Investments, Ltd. Sir Ben Lockspeiser, having reached retirement age, and Sir Francis de Guingand have resigned from the Board. Sir Francis is returning to the Union of South Africa, where he will continue to be in charge of Tube Investments interests in Southern Africa. Prof. J. M. Kay, professor of nuclear power at the Imperial College of Science and Technology, University of London, has been appointed to the Board of Tube Investments, Ltd., as director of research and development from July 1.

The Academy of Sciences of the U.S.S.R. has issued two publications in Russia relating to the International Geophysical Year—(1) *Information Bulletin I.G.Y.*, No. 8, 1960. This contains a number of articles and reports on a variety of subjects such as geomagnetism, the ionosphere, glaciology and oceanography. (2) *Bibliography of publications relating to the I.G.Y.* (in Russian). This comprises some 800 items published in 1959, which are ordered and classified according to subjects.

The third international symposium on "Phytopharmacy and Phytiatry" is being held at the State Agricultural University, Ghent, on May 9. Further information can be obtained from the State Agricultural University, Coupure Links, 235, Ghent.

The National Physical Laboratory, Teddington, Middlesex, is organizing two open days during May 10–11. To be included in the programme are two lectures: "The N.P.L. and Industrial Measurement Standards", by Dr. H. Barrell; "Research in the Basic Physics Division", by Dr. J. A. Pople. Further information can be obtained from the Director, National Physical Laboratory, Teddington, Middlesex.

The German Society for Polar Exploration is holding a meeting at Münster during May 11–14. The theme of the meeting will be "New Results in Polar Exploration", and it will include a symposium on "Scientific Problems of Exploring Greenland". Further information can be obtained from the Deutsche Gesellschaft für Polarforschung, E.V., Münster, West Germany.

The International Atomic Energy Agency is organizing an international conference on "Nuclear Electronics", to be held in Belgrade during May 15–20. An exhibition is being organized in conjunction with the conference, and will be open during May 13–21. Further information can be obtained from the International Atomic Energy Agency, Vienna I, Austria.

A meeting of the Biochemical Society is being held in University College and Trinity College, Dublin, during May 25–26. A meeting of the Association of Clinical Biochemists will follow at University College on May 27, and included in the programme is a symposium on "Acid-base Balance". Further information can be obtained from Dr. W. J. Whelan, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1.

THE NATIONAL SCIENCE FOUNDATION, WASHINGTON

ANNUAL REPORT

THE tenth annual report of the National Science Foundation, covering the year ended June 30, 1960*, follows the pattern of the previous two reports. Besides the Director's statement and the chapter reviewing the Foundation's programmes for promoting basic research and education in the sciences (see p. 473) nearly half the report reviews the work of the Foundation during the year. The remaining half of the report consists of appendixes listing the members of the National Science Board, the Foundation's staff, committees and advisory panels, the grants made for basic research and other purposes, fellowship awards, publications, and the financial report.

Grants for research totalled 78 million dollars and the report summarizes briefly the programmes supported through the Foundation's Divisions of Biological and Medical Sciences, and of Mathematical, Physical and Engineering Sciences, its Office of Social Sciences, which for the first time operated for the full year; and, through the Office of Special International Programmes, in the Antarctic. Significant research developments in the year which are noted are the total synthesis of chlorophyll, the study of factors regulating behaviour in man, the discovery of the first synthetic antigen, the isolation and identification of the acetylcholine receptor protein from the electric tissue of the electric eel, the study of gravity response dependent on hereditary factors and the discovery that sub-units of a gene are arranged linearly within a gene. Progress has been made in determining the mechanism of action of vitamin B_{12}, the feasibility of studying bargaining behaviour in bilateral monopoly situations under controlled laboratory conditions has been demonstrated, and an archæological study of eastern Arizona has discovered evidence of a primitive people without pottery and probably without agriculture living in the midst of other tribes with far more advanced culture. A sensitive infra-red analyser has been applied to the measurement of atmospheric carbon dioxide, various isotope techniques are yielding important results in the study of undisturbed cores of sediment from the ocean bottom and a photoelectric image intensifier is giving significant help to astronomers.

Of the 1995 research grants made during the year, 891, totalling nearly 25 million dollars, were for biological and medical sciences; 916, totalling 31·5 million dollars, were for mathematical, physical and engineering sciences; 109, amounting to more than 2 million dollars, for social sciences, and 79, amounting to more than 3·5 million dollars, for Antarctic research.

During the year, in its seven fellowship programmes, the Foundation made 4,010 awards totalling 13·5 million dollars, of which 1,200 were pre-doctoral, 1,190 co-operative graduate fellowships, 580 summer fellowships for graduate teaching assistants, 180 post-doctoral fellowships, 75 senior post-doctoral fellowships, 285 science faculty fellowships for teachers, and 500 summer fellowships for secondary school teachers of science and mathematics. The Foundation also supported 649 institute programmes during the year. Of these, 412 were summer institutes attended by 22,000 teachers; 33 were academic-year institutes for 1,500 teachers and 209 were in-service institutes, attended by some 9,000 teachers on a part-time basis. Special projects in science education included programmes for secondary schools to foster interest in, and understanding of, science, mathematics and engineering, travelling science libraries, grants for visits of outstanding scientists to secondary schools, as well as special science demonstration lectures for secondary schools and a science clubs programme. Other programmes were concerned with undergraduate science education and supplementary training for teachers of science and the improvement of the content of courses.

The Scientific Man-power Programme of the Foundation makes available information on the resources of scientific technical personnel in the United States. A comprehensive report on the status of the scientific and technical man-power register, the Foundation's studies in this field and its projected plans for the future, was issued in January 1960. The Foundation's responsibilities in the dissemination of scientific information have also been extended, and besides endeavouring to improve the existing information services, the Foundation, through its Office of Scientific Information, is promoting a national research programme for developing techniques for handling information. As part of this effort it is vigorously fostering the co-ordination of effort in this field, both within the Federal Government and among private and professional organizations, while on the international level the Foundation has co-operated with the International Federation for Documentation and the Abstracting Board of the International Council of Scientific Unions; and has, with the European Productivity Agency, studied means for establishing a European Translations Centre. Projects in this field assisted during 1960 include a large-scale test programme at Western Reserve University for evaluating procedures developed for the automatic processing and searching of metallurgical literature; a programme on mechanization of processing and searching chemical literature, including research on the semantics of chemical literature; and the investigation of large files of information with a multi-level structure and self-organizing capability at the Electrade Corporation.

The Foundation, mostly through scientific societies, supports the translation, in whole or in part, of 45 Russian periodicals, and, in November 1959, sponsored a conference of editors and officers of 28 professional societies and academic institutions translating such periodicals to discuss means for improving the dissemination of translated periodicals among scientists in the United States. A series of national surveys on media of scientific communication was also launched, and some experiments with new publication techniques and methods were supported. The Foundation is now authorized to support international activities which may improve education in science in the United States.

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* National Science Foundation, Washington. Tenth Annual Report for the fiscal year ended June 30, 1960. Pp. xiii + 310. (Washington, D.C.: Government Printing Office, 1961.) 1 dollar.

THE INTERNATIONAL ACADEMY OF ASTRONAUTICS

THE International Academy of Astronautics, which now has a total membership of 76, coming from eighteen countries, was created by the International Astronautical Federation at Stockholm in August 1960, for the purpose of promoting the advancement of astronautical sciences. The Academy is working under a grant from the Daniel and Florence Guggenheim Foundation, and the French Government has provided offices for the Secretariat of the Academy in Paris.

The director of the academy is Dr. Th. von Kármán (United States) and the deputy directors are Prof. J. Pérès, dean of the Faculty of Sciences of the University of Paris, and Dr. F. J. Malina (United States). The initial membership of the Academy included 45 members, mostly leaders in space flight and in sciences related to astronautics. Prof. Niels Bohr has been elected the first honorary member of the International Academy of Astronautics.

The Academy has recently elected the following new members:

Basic Sciences Section: Prof. J. Bartels, University of Göttingen; Prof. D. R. Bates, Department of Applied Mathematics, Queen's University, Belfast; Prof. L. Biermann, director, Max Planck Institute for Astrophysics; Prof. S. Chapman, High Altitude Observatory, Boulder; Dr. H. Friedman, Atmosphere and Astrophysics Division, United States Naval Research Laboratory, Washington; Prof. L. Goldberg, Harvard College Observatory, Cambridge, Mass.; Dr. H. E. Newell, deputy director, Office of Space Flight Programs, National Aeronautics and Space Administration (United States); Prof. F. L. Whipple, Harvard College Observatory, Cambridge, Mass.

Engineering Sciences Section: Dr. A. Busemann, Langley Research Center, National Aeronautics and Space Administration (United States); Prof. E. Carafoli, director, Institute of Applied Mechanics, Bucharest; Prof. L. Crocco, director, Jet Propulsion Center, Princeton University; K. A. Ehricke, Convair Astronautics Division (United States); Prof. A. Ferri, Polytechnic Institute of Brooklyn; Prof. A. R. Kantrowitz, director, Evco Everett Research Laboratory (United States); Mr. M. J. Lighthill, director, Royal Aircraft Establishment, Farnborough; Prof. M. Roy, director, Office National d'Etudes et de Recherches Aéronautiques (France); Dr. H. S. Seifert, Stanford University; Dr. E. Stuhlinger, director, Research Projects Division, National Aeronautics and Space Administration, George C. Marshall Space Flight Center (United States); Dr. Martin Summerfield, Princeton University.

Life Sciences Section: Dr. R. Grandpierre, director, Aviation Medicine Research Center (France); Prof. U. S. von Euler, Department of Physiology, Karolinska Institutet; Dr. A. Graybiel, director of research, U.S. Naval School of Aviation Medicine (United States); Prof. T. Lomonaco, director, Research Centre for Aviation Medicine (Italy); Prof. R. Margaria, former director, Research Centre for Aviation Medicine (Italy); Dr. H. J. Schaefer, Research Department, United States Naval School of Aviation Medicine; Prof. G. Schubert, Physiological Institute, University of Vienna; Colonel J. P. Stapp, Aerospace Medical Center, Brooks A.F. Base (United States); Air Commodore W. K. Stewart, Royal Air Force Institute of Aviation Medicine, Farnborough; Dr. P. M. Van Wulften Palthe, National Aeromedical Centre (Netherlands); Sir Harold E. Whittingham, Chief Medical Officer, British Overseas Airways Corporation.

The next meeting of the Academy will be held in October 1961, in Washington, in conjunction with the annual congress of the International Astronautical Federation. During the meeting, the first Daniel and Florence Guggenheim International Astronautics Award will be presented. This international prize of £1,000 is to be given annually to an individual who has made outstanding contributions to astronautics during the five preceding years. The Academy will also review the work of the following committees: Publications, Awards and Fellowships, Lunar International Laboratory (LIL), and the Joint Commission of the Academy and the International Institute of Space Law on Technical Aspects of Space Law.

APPLIED BIOLOGY IN TECHNICAL COLLEGES

DEVELOPMENTS in the courses leading to the Diploma in Technology in Applied Biology at Bristol College of Science and Technology and at Brunel College of Technology, Acton, are described in the *Journal of the Institute of Biology* (8, No. 1; Jan. 1961).

The Diploma in Technology course in Applied Biology at Bristol began in October 1957 with eleven students, all college based. A previous survey had shown that opportunities for botanists and zoologists were very limited, but that there were increasing opportunities for trained biologists in the field of microbiology, pharmacology and biochemistry. The course was, therefore, arranged as a sandwich course for college-based students, designed to give a broad training in the early years with opportunity for specialization in the final year. As approved eventually by the National Council for Technological Awards, the course requires the study of botany, zoology, microbiology, physiology and chemistry in the first three academic periods in college, followed by biochemistry with a choice of either plant pathology, plant physiology, pharmacology, microbiology or entomology as a specialization in the final year, as well as a project occupying on average one day per week. General studies and tutorial work are an essential feature of the course.

The annual intake to the course is limited to between fifteen and twenty students. No student is admitted without interview, and, where it is felt that an insufficient number of suitable candidates present themselves, no attempt is made to fill the course; academic qualifications required for entry are either two Advanced Level passes at the General Certificate of Education, or a good endorsed certificate in biology, but at least equal emphasis is placed on personality. Applications for admission to the course are now coming forward at the rate of fifty to sixty each year; of these a high percentage is made up of students who have been offered university places, but have preferred a course leading to a Diploma in Technology. At the same time, some students withdraw their applications on being offered university places, although this happened only in one case during 1960. There has been some wastage on the course arising from a variety of causes.

Co-operation with industry has been very close, and, during the three years for which the course has now been running, staff have been cordially received during their industrial visits. Some thirty-five firms offer industrial training at various levels and in various fields, and a number of other firms are anxious to co-operate as student numbers grow. Before a student is assigned to a particular firm, his or her interests are discussed with the head of department and the senior lecturer in biology, and the advice of various lecturers sought; an approach is then made to the firm able to offer the most appropriate kind of training and a suitable programme is drawn up. The student is then called for interview by the selected firm and a final decision is made as to the general suitability of arrangements. A member of staff from the firm is chosen to supervise the industrial training and to maintain contact with the College.

A tutor visits the student on two or three occasions, and mid-way through these industrial periods students are brought together for a residential week-end at some selected college outside Bristol, along with students from other Diploma in Technology courses. This provides an opportunity to meet the College staff and discuss matters of general interest.

The break of six months in academic work presents problems of continuity. Difficulties are to a considerable extent overcome by close consultation between industry and the College. Students leave college with some academic assignments involving guided reading and the writing of essays. Every care is taken to ensure that this does not create too much of a burden by holding a staff meeting at which the amount of work to be given to students is finally determined. Details of the assignments are given to the industrial tutors so that they are fully aware of the work to be done by a student during the industrial period; in some cases, time to do this work is allowed for in the general programme. Over and above this, a student is required to write an account of the industrial training and of its relevance to the particular industry in which he finds himself.

This course, despite some initial difficulties, is proving to be of considerable value to students, and, while there is still room for experimentation, the general pattern is sound. All the present fourth-year students are assured of appointments on completing their course, and an increasing number of firms are willing and anxious to co-operate.

The course for the Diploma in Technology in Applied Biology also began at the Brunel College of Technology in October 1957; and, although the subjects studied differ in detail from those at Bristol, the general arrangements follow the same pattern

TREE PLANTING IN THE NATIONAL PARKS

A PUBLICATION has recently been issued jointly by the Standing Committee on National Parks of the Councils for the Preservation of Rural England and Wales and the Joint Action Group for the Protection of Dartmoor and Exmoor, entitled *The Case for Control of Afforestation of Open Land in National Parks**.

The problem of land use in our heavily populated island becomes yearly more and more complex. The public memory is short, and even those who are capable of pre-vision and of determining what is desirable in the long run cannot convince Governments of the appropriate action. This is especially true of tree-planting in its various aspects. Apart from a fraction of the land-owning class of earlier centuries it is unfortunately true that those in control have always been uninterested in, even antipathetic to, forestry as a commercial undertaking at home—so long as there existed readily available supplies of timber abroad. But for the lessons of two World Wars it is very doubtful if a State forest service would ever have been set up in Great Britain with the task of creating new forests and conserving existing woodlands. Since its establishment in 1919, the Forestry Commission has made good progress with its main task and its activities are widespread over most of Britain. It has clashed here and there with various land users, but, on the whole, its work is appreciated.

It would be wrong to imagine, however, that the Government has some overall plan for land use and that the various authorities who have an interest in land use are centrally controlled. It is not even the case that all the statutory authorities concerned in any way with tree conservation, tree-planting, afforestation and forestry are centrally controlled. Besides the Forestry Commission, in Britain, the following public bodies are interested in trees in one way or another—the local planning authorities under the Town and Country Planning Act of 1947, the Nature Conservancy, and the National Parks Commission. In addition, there is now the Timber Growers Association of private individuals engaged in commercial tree-planting enterprises. In some respects their interests clash, especially when the problem of conservation of amenities arises.

It is not a good thing that the various bodies interested in woodlands for various reasons should quarrel among themselves; they should be in the closest association, so that the woodland heritage can be preserved and extended, wherever possible, to fulfil to the best advantage all the recognized functions of protection, amenity, recreation and production. Hitherto, the tendency has been for

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* Standing Committee on National Parks and Joint Action Group *The Case for Control of Afforestation of Open Land in National Parks*, presented by the Standing Committee on National Parks of the Councils for the Preservation of Rural England and Wales; and the Joint Action Group for the Protection of Dartmoor and Exmoor. Pp. 24. (London: Standing Committee on National Parks, 4 Hobart Place, S.W.1, 1961.)

State action to concern itself too much with the commercial aspect of tree-planting. Afforestation has been almost entirely directed towards the establishment of short term 'crops' of trees and not to permanent woodlands adapted to satisfy demands for amenity, recreation and protection. In harmony with this tendency special fiscal concessions are given to private woodland owners who engage in commercial forestry. Taxation, in relation to tree-planting in Britain, is not easy to understand, more especially as the tree grower is also entitled to certain grants in aid. The Government takes with one hand and gives with the other, and it is not very clear why. A case can be made for State assistance to recreate forests, and a case can be made for a simple single basis of taxation of commercial forests, namely, on the profits of the enterprise instead of on the timber capital; but it is extremely difficult to segregate the commercial forest from the amenity woodland and to say how the State should act in relation to the latter. The ideal woodland is that which fulfils the triple functions of protection, amenity and production, and many landowners could teach the Forestry Commission a lesson to that effect.

The fiscal concessions allowed to tree-growers have led to a striking and relatively recent development in forestry circles. Commercial afforestation enterprises have been set up and have become especially active in southern England and Wales. The task which they have set themselves would not be financially attractive—it would not be economic—without the subsidies and fiscal concessions at present allowed. How successful these afforestation enterprises will be eventually, that is in the establishment of healthy permanent woodland, remains to be seen. Like the greater part of the Forestry Commission plantations they are, taking the long view, still experimental. If they pay the entrepreneurs it is solely because of the State assistance and taxation reliefs.

The position in respect of subsidies for tree-planting and of taxation relief to woodland owners certainly requires to be reconsidered; but whether it is desirable to give the Standing Committee on National Parks over-riding control on afforestation in such a way as to conflict with the duties of the Forestry Commission, or in any other way, is very doubtful.

The case for establishing such a control is put in a memorandum distributed by the Standing Committee on National Parks of the Council for the Preservation of Rural England and Wales and the Joint Action Group for the Protection of Dartmoor and Exmoor. They view with alarm the incursion of commercial afforestation into the Dartmoor and Exmoor National Parks in particular. They have a case. The solution does not lie, however, in resisting such encroachments but in trying to ensure that the State assistance on which the intruders depend is devoted to the creation of a type of forest which increases the amenities of the Parks while at the same time being productive.

There is no reason why the creation of these woodlands should result in the "squandering of any noble heritage of beauty" except in so far as the methods adopted are inappropriate. It is unfortunate that until recently State forest policy had subordinated too much the important protective and amenity functions of woodlands to the purely commercial function of producing timber, because it has placed the Forestry Commission in a weak position when it comes to discussing the best use of tree-planting associated with national parks.

In respect of Britain as a whole there are many districts where an afforestation policy of this kind would be welcomed, and which might benefit greatly from what is in effect the local expenditure of taxation revenues indirectly through the medium of private enterprise. Whether this is really a healthy development from the point of view of forestry is another matter. The production of figures in a book is a very different skill from that of creating and conserving a forest on the ground.

M. L. Anderson

PARENTS' VIEWS ON EDUCATION

During the past three years Political and Economic Planning has been carrying out an inquiry to determine how far the social services are meeting the needs of the people who use them.

Of the social services none can affect the life of the individual and the nation more radically than education, and, as a preliminary to a fuller report to be published later, Political and Economic Planning has produced a broadsheet recording the findings of the inquiry on the education service*. Since the object of the survey was to present the consumers' view of the social services, the broadsheet's approach to the education service must necessarily be a limited one. Parents' wishes cannot be equated with educational need. Parents may over-rate their children's abilities, or they may not understand what the school is trying to do. Nevertheless, it is part of the new plan for education that the parent should become more closely involved in the child's schooling. An understanding of parents' experience and opinions is, therefore, essential when considering the present achievements of the education service.

The main inquiry was carried out among families living in the Greater London area. It had been decided that the inquiry must be concentrated in one district and that the locality chosen should be a large urban area since this is the type of community in which most people live. It should also be large enough to contain a diverse population and to include several local authority areas with a variety of different arrangements for some of the social services. Greater London fulfilled all these requirements. To make sure, however, that the findings for London were not untypical of the rest of Britain, a second smaller inquiry was carried out in Northampton, a provincial town of quite different size. This second inquiry confirms that the London findings can be considered representative. A total of 734 families was successfully interviewed in the main inquiry. Since the social services to-day are not primarily concerned with hardship, or important only to limited sections of the population, it had been decided to study samples of all families with children under the age of 16.

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* *Planning*, Vol. 27, No. 448 (January 23, 1961): Parents' Views on Education. Pp. 1–32. (London: Political and Economic Planning, 1961.) 3*s*. 6*d*.

It is evident from the answers received during the survey that there is now considerable interest in education and appreciation of its possibilities. The mothers showed concern with the kind of school attended by their children, and they had often decided that there was some particular form of higher education or specific training that they would like their children to have. A high proportion of those with older children said that they wanted them to stay at school after they were fifteen. Although interest was greatest at the top of the occupational scale, it was also strong among the operative groups. It would appear that the attitude towards education at all levels of society is becoming more positive. A higher standard of living together with smaller families has lessened the necessity for working-class parents to turn the child into a wage-earner as soon as possible. Instead, there is a wider realization that educational qualifications may be a valuable investment and that very wide opportunities may be open to the talented child. The old grudging attitude towards the school as a place in which children were detained when they might usefully have been out at work appears to be dying.

Although the prevailing attitude towards the education service is not an unfavourable one, there is a substantial section of unsatisfied demand for particular schools. There was no intention during the inquiry to set up arbitrary standards in the field of education and to test whether or not these were reached in the families in the sample. Nevertheless, it was a general principle in the Education Act that parents should be able to get the kinds of schools and education for their children that they think the children need. Altogether 17 per cent of the mothers in the inquiry said that they had failed to get particular types of school that they had wanted. Six per cent were thinking of nursery schools and 7 per cent of secondary schools (mainly grammar and technical). There may be differing views on whether or not there is a real need for these services as distinct from a demand for them; the need for expansion and improvement in these two fields has already been accepted by the community and incorporated in the Education Act of 1944. While the proportions of present-day demand are small in comparison with the total sample, they are considerable when compared with the proportions who had used the types of school in question. (Five per cent of the families had used nursery schools, 12 per cent grammar schools and 6 per cent technical schools.)

The demand for grammar and technical schools and also, in some cases, the use of private schools, appears to be the result of parents' dissatisfaction with secondary modern schools. Some of their comments suggest that they regard the secondary modern school as offering inferior academic education and as not providing a specific enough training for a particular job. If the objects of the 1944 Education Act are fully realized and secondary modern schools come to be recognized as enjoying the equality of facilities and staffing with grammar schools, it seems possible that the demand for grammar schools may decrease. Similarly, if secondary modern schools play their part in the provision of technical education then parents are less likely to feel that their children are missing opportunities for occupational training. The present situation, in which certain secondary schools are obviously considered superior to others, is socially undesirable and harmful to the successful as well as the unsuccessful children.

It is important, however, that parents should be able to arrange for a grammar school place for those children who should benefit from it. One feature that emerges from the Political and Economic Planning survey is that at present the education service is being used most advantageously by families at the top of the social scale. The managerial and professional group and the supervisory and technical group have been more successful than others in obtaining grammar school places for their children. It is the managerial and and professional group that shows the strongest awareness of education.

The desire for further information is strong among the other groups, however, and the disparate use of the service cannot be entirely accounted for by less interest in education among these groups. When parents at the bottom of the occupational scale would prefer a grammar school place for their children they are, nevertheless, less likely to obtain it. It seems that there are a number of parents failing to get the education they would like for their children because the education itself is beyond their own experience.

The demands which parents make on the education service on behalf of their children may sometimes be unreasonable or because they are inadequately informed. On one hand, parents may identify their own social prestige too closely with their children's achievements or, on the other hand, they may be too little aware of the ends and means of education. It appears, for example, that the mothers questioned in the survey were concerned predominantly with the jobs their children would get and not with the enrichment of their personality. Nevertheless, it is important that parents should feel reasonably satisfied with their children's education.

TERRESTRIAL SPECTROSCOPY

By Prof. C. L. PEKERIS, Z. ALTERMAN and H. JAROSCH

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THE discovery of the Zeeman effect in 1896 came at a time when the basic concepts needed for its interpretation had been worked out by Lorentz[1]. Already in his first paper[2], Zeeman reported a verification of the polarization of the lines, an effect which Lorentz had advised him to look for. He gives a derivation, on the basis of the Lorentz theory, of the formula for the splitting of the original frequency σ_0 by a magnetic field H:

$$\sigma = \sigma_0 \pm \frac{eH}{2mc} \qquad (1)$$

This relation had been derived independently by Larmor. Larmor proved[3] that the effect of a magnetic field on the orbit of a charged particle is analogous to the effect of rotation on the frequency of a mechanical system. While the Zeeman effect provides ample verification of the electromagnetic part of Larmor's analogy, an experimental demonstration of the mechanical counterpart has been wanting. A striking case of the splitting of the natural frequency of a purely mechanical system by rotation presented itself recently when the records of the Great Chilean earthquake of May 22, 1960, were analysed. This earthquake excited the natural oscillations of the Earth. The gravest modes $n = 2$ (53·7 min.) and $n = 3$ (35·5 min.) appeared as doublets in the spectra of both the gravimetric records[4] and the strain-meter records[5].

The free oscillations of the Earth are governed by gravitational and elastic forces. Taking the distribution of density $\rho(r)$, and of the elastic constants $\lambda(r)$ and $\mu(r)$, as inferred primarily from seismic as well as from other geophysical data, we have determined the terrestrial spectrum for several proposed models of the Earth[6-9]. The periods deduced from spectral analysis of the strain-meter records (52 lines) agreed with the gravimetric values (49 lines) to within 1 per cent; and this was also the measure of agreement with the theoretical spectrum[9] for the Gutenberg model of the Earth, and to a lesser extent with the spectrum for the Bullen *B* model. Free modes ranging from spherical harmonic order $n = 2$ up to $n = 38$ were identified seismically, and up to $n = 41$ gravimetrically.

We have investigated the effect of rotation on the spectrum of the Earth by carrying out a first-order perturbation calculation in the parameter $\alpha = \omega/\sigma_0$, where ω denotes the angular rotation of the Earth. In the absence of rotation, the components of displacement u_0, v_0 and w_0 in a spherical system of co-ordinates (r, θ, φ) are given by:

$$u_0 = U_0(r)Y_{nm}, \quad v_0 = V_0(r)\frac{\partial}{\partial\theta}Y_{nm} \tag{2}$$

$$w_0 = \frac{V_0(r)}{\sin\theta}\frac{\partial}{\partial\varphi}Y_{nm}, \quad Y_{nm}(\theta, \varphi) = P_n^m(\cos\theta)\exp im\varphi \tag{3}$$

where a factor $\exp i\sigma t$ has been omitted. The characteristic frequencies $\sigma_0(n)$ are degenerate, and do not depend on the azimuthal number m. The introduction of a slow rotation $\omega(\ll\sigma_0)$ removes the degeneracy, each line $\sigma_0(n)$ being split into $(2n + 1)$ lines σ_n^m given by:

$$\sigma_n^m = \sigma_0(n) + m\tau(n)\omega \qquad -n \leqslant m \leqslant n \tag{4}$$

with

$$\tau(n) = \frac{\int_0^a \rho r^2(2U_0V_0 + V_0^2)dr}{\int_0^a \rho r^2[U_0^2 + n(n+1)V_0^2]dr} \tag{5}$$

in the case of spheroidal oscillations, and

$$\tau(n) = \frac{1}{n(n+1)} \tag{6}$$

in the case of torsional oscillations.

Table 1 gives the periods in the multiplets for the oscillations of spherical harmonic order $n = 2$ and 3 of the Bullen *B* model. These are based on the previously determined values[7] of $\sigma_0(n)$, and on equations (4) and (5). Also shown are theoretical intensities of the gravimetric and strain-meter lines for an observing station at Los Angeles ($\theta = 56°$). These were determined on the assumption of an explosive compressional point-source at the earthquake focus in Chile ($\theta_0 = 128°$).

Table 1. THEORETICAL AND OBSERVED MULTIPLETS FOR LOS ANGELES. $\theta = 56°$

n	m	T min.	Amplitude		Observed periods	
			Gravimetric	Strain	Gravimetric (ref. 4)	Strain (ref. 5)
2	−2	55·33	0·115	0·302	54·98	
2	−1	54·50	0·242	0·615		54·7
2	0	53·70	0·0015	0·0023		
2	1	52·92	0·242	0·615	52·80	53·1
2	2	52·15	0·115	0·302		
3	−3	35·99	0·396	0·401		
3	−2	35·83	1·257	1·46	35·87	35·9
3	−1	35·67	0·282	0·377		
3	0	35·50	0·624	0·873		
3	1	35·34	0·282	0·377		
3	2	35·18	1·257	1·46	35·24	35·2
3	3	35·03	0·396	0·401		

As for the periods, the feature of interest is the observed interval between the doublets, rather than the central frequency, since the latter depends on the particular Earth model assumed. We note the following:

(1) *Gravimetric.* In the case $n = 2$, the central line $m = 0$ should be very weak, and was actually not observed. The theoretical interval between the strongest $m = -1$ and $m = 1$ lines is 1·6 min., as against the observed interval of 2·2 min. Since, however, the $m = \pm 2$ lines are theoretically half as strong as the $m = \pm 1$ lines, the observed lines may represent the unresolved pairs $m = 1$ and 2. The average periods of the pairs, weighted according to their amplitudes, are 54·77 min. and 52·67 min., giving an interval of 2·1 min., which is close to the observed interval of 2·2 min.

In the case $n = 3$, the strongest lines should be the pair $m = \pm 2$, which are very close to the observed two lines. The $m = 0$ line should be half as strong, and should have been observed if the noise-level were low enough and the resolution adequate.

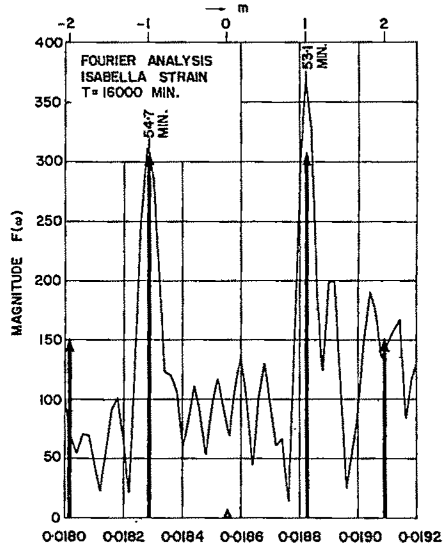

Fig. 1. $n = 2$. Arrows show positions and amplitudes of multiplet. ——, Spectral intensity of Chilean earthquake observed at Isabella by Benioff, Press and Smith (ref. 5)

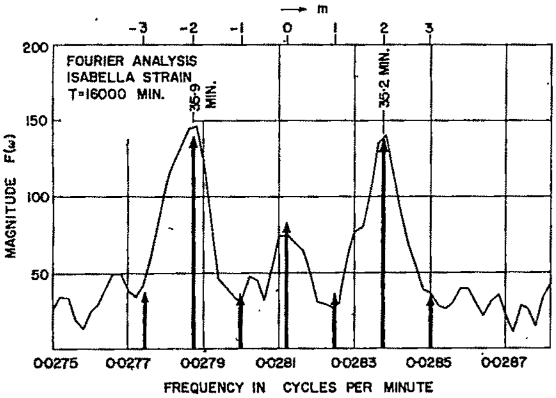

Fig. 2. $n = 3$. Arrows show positions and amplitudes of multiplet. ——, Spectral intensity of Chilean earthquake observed at Isabella by Benioff, Press and Smith (ref. 5)

In addition to the doublets in the fundamental $n = 2$ and $n = 3$ modes, the first overtone of the $n = 3$ mode was also reported[4] as a gravitational doublet: $T = 17{\cdot}88$ min. and $17{\cdot}68$ min. Theoretically, the relative amplitudes within a multiplet for the overtones should be the same as for the fundamental, as given in Table 1. The strongest lines in all the overtones of $n = 3$ should therefore be again the pair $m = \pm 2$. The period-interval for the pair comes out 0·19 min., in agreement with the observed value of 0·2 min.

(2) *Seismic*. Two doublets were reported by Benioff, Press and Smith[5] from a Fourier analysis of the strain seismogram at Isabella, California. In Fig. 1 is reproduced their spectral intensity curve in the vicinity of the $n = 2-$ pair of 54·7 min. and 53·1 min. In the same diagram are shown, by arrows, the theoretical separations and amplitudes of the five lines making up the $n = 2$ quintet, in accordance with the results given in Table 1. The central $m = 0$ line should be extremely weak, and is indeed below the background noise. The $m = 2$ line is not far from the observed intensity-level. On the other hand, the $m = -2$ line is definitely above the observed intensity in its vicinity. One would expect a rise in intensity immediately to the left of this line, which is outside the limit of the figure.

In Fig. 2 are shown the observed spectral curve for $n = 3$, and the theoretical positions and amplitudes of the $n = 3$ septet. The central $m = 0$ line is defined, and its theoretical intensity is close to the observed value. The $m = \pm 1$ and ± 3 pairs are close to the general noise-level in their respective neighbourhoods.

It is seen that, in general, the doublets observed are those lines of the multiplet which are strongest theoretically, and that the missing lines would not be expected to stand out above the observed noise-level.

This investigation was supported by the Office of Naval Research and Project VELA-UNIFORM of the Advanced Research Projects Agency.

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FLUORESCENCE OF TETRACYCLINES IN BONE TUMOURS, NORMAL BONE AND TEETH

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FOLLOWING the observations on the localization of the tetracyclines in bone by Milch, Rall and Tobie[1], other workers have used the tetracyclines as bone markers and have also studied under ultra-violet light the fluorescence produced by these compounds in various pathological lesions, including bone tumours and other neoplasms. Some recent investigations on this subject are described in this communication and will be published in detail elsewhere.

The source of ultra-violet light was a Wood's mercury vapour lamp as used in the diagnosis of ringworm. Photographs were made on high-speed Ektachrome film using a Wrayflex camera, with a Wratten 2*E* filter, with the object at about 30 cm. distant from both the light source and the camera. Exposure times were usually about 4 min. A Leitz fluorescence microscope with a Philips 150-watt mercury vapour lamp was used for the histological examinations.

A group of rats, seven weeks old, was subjected to fracture of the left tibia with the intact fibula acting as a splint during repair. Animals were killed at intervals of 3 days. 24 hr. before death they were injected intraperitoneally with chlortetracycline. Yellow orange fluorescence could be detected in the fracture callus from the ninth day onwards. When young rats with deep yellow-orange fluorescent bones due to previous administration of chlortetracycline were subjected to fracture of the tibia and no more chlortetracycline administered the resulting callus, in contrast to the fracture fragments, showed no fluorescence. Following the injection of chlortetracycline into older rats with osteomyelitis of the femur the new bone fluoresced yellow while the older normal bone showed only blue autofluorescence. Normal bone laid down in drill holes made in the femur a year previously did not fluoresce.

Sarcomata produced 8 months after implantation of methylcholanthrene crystals into drill holes made in the femur of rats showed no fluorescence, but roughened areas of new bone adjacent to the original drill holes fluoresced bright yellow. Osteosarcomata *D*177 showed yellow fluorescence, but the bones of these animals showed only normal blue autofluorescence. Sarcomata produced by the sub-cutaneous implantation of plastic disks 2 cm. in diameter did not fluoresce.

Normal rats about 19 days pregnant were injected intramuscularly with chlortetracycline and killed 24 hr. later. Gross examination of the fœtuses showed marked yellow fluorescence in both 'membrane bone' and 'cartilage bone' demonstrating crossing of the placental barrier by the drug. Young female rats dosed heavily with chlortetracycline prior to mating and the bones of which fluoresced bright orange-yellow under ultra-violet light produced fœtuses the bones of which showed no fluorescence.

A normal dog, eight weeks old, was dosed regularly with 750 mgm. chlortetracycline on six days a week for a month. Following eruption of the permanent teeth these appeared pale yellow in daylight as compared with the teeth of normal litter mates, and there was intense yellow fluorescence under ultra-violet light. On histological examination both dentine and enamel were found to fluoresce. Four narrow bands of non-fluorescent dentine occurred in the wide band of fluorescent dentine and probably correspond to the Sundays when no chlortetracycline was administered (Fig. 1).

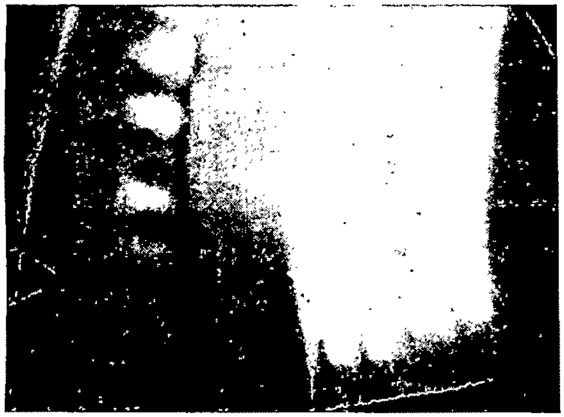

Fig. 1. Section of incisor tooth of dog showing fluorescence of Schreger's lines of the enamel (left) and fluorescent bands in the dentine (right), following chlortetracycline administration (× *c*. 30)

Chlortetracycline was administered to a 5-month old Great Dane with a condition indistinguishable from osteogenesis imperfecta. Examination of the bones at death three days later showed the very thin cortices to fluoresce in a similar manner and intensity to that seen in young dogs with normal thickness of cortex. The epiphyseal line showed normal blue autofluorescence.

A Great Dane weighing 60 kgm. and with spontaneous osteosarcomata of the distal radius and ulna in both fore limbs had previously had these limbs perfused with nitrogen mustard, using an extracorporeal circulation[2]. An increase in size of the tumour in the right radius was noted 5 months later and the animal was killed. About 18 hr. prior to killing 1 gm. of chlortetracycline hydrochloride was given intravenously and 1 gm. orally but an unknown quantity of the oral dose was later lost by vomiting. On post-mortem examination brilliant yellow fluorescence was seen under ultra-violet light in the area of new bone in the tumours of the fore limbs both in the medullary cavity and as extensions from the periosteum (Fig. 2). This fluorescence was much brighter than the orange fluorescence which has been observed in fracture callus of dogs following administration of tetracyclines. Areas of non-osteogenic tumour tissue, which were of a rubber-like consistency, did not fluoresce. Normal bone adjacent to the tumour showed only blue autofluorescence. Brilliant yellow fluorescence also occurred in the osteogenic areas of a metastasis in the right axillary lymph node (Fig. 3) and in a small and previously unsuspected area of malignant new bone in the left tibia. This latter area was difficult to detect in daylight but under ultra-violet light the narrow band of yellow fluorescence was very striking. Two small metastases in the lungs, undetectable by radiography, did not fluoresce, and on microscopical examination were found to consist solely of sarcoma cells with no osteogenic tissue. The only normal soft tissues showing appreciable fluorescence were the testicles, which showed a dull yellow fluorescence tinged with green.

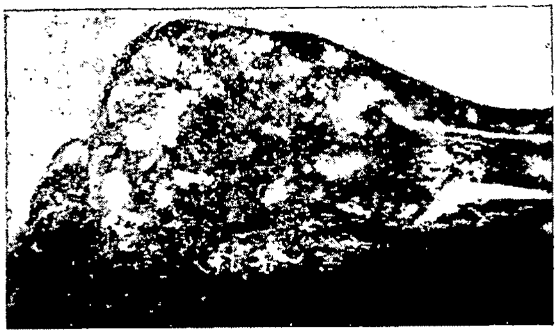

Fig. 2. Longitudinal section of the right distal radius of a Great Dane showing fluorescent areas of new bone in the osteosarcoma. The adjacent normal bone showed no yellow fluorescence but is clearly visible on the photograph because of its normal blue autofluorescence. (× *c*. 1/3)

A greyhound with an osteosarcoma of the proximal tibia was injected intravenously with tetracycline hydrochloride (20 mgm./kgm.) and four days later was killed. Yellow-orange fluorescence was particularly noticeable on the large new areas of bone attached to the tibia but also occurred in smaller osteogenic areas in the tumour tissue. Other tissues showed no similar fluorescence and no metastases were discovered in this dog.

Fluorescence of tetracyclines in new bone as opposed to normal soft tissues in dogs persists for

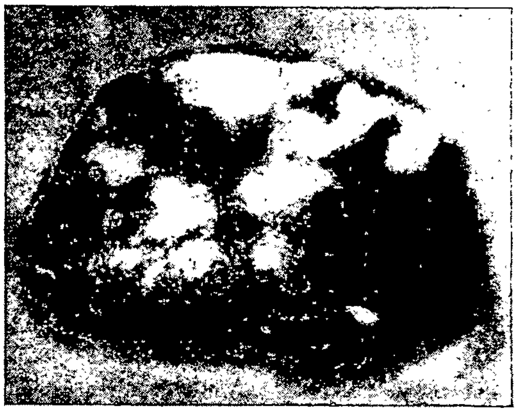

Fig. 3. Section through the right axillary lymph node of the same dog showing fluorescent areas of tumour bone. (× *c*. 3)

long periods of time. Older normal bone shows only blue autofluorescence. It would therefore seem possible that if tetracycline labelled with iodine-131 had been administered to the Great Dane with multiple osteosarcomata confirmation of a metastasis in the axillary lymph node would have been obtained. The scintillation counter would also have discovered the new focus of malignancy in the tibia but not the small non-osteogenic areas in the lungs.

Further work is planned to develop the therapeutic possibilities of radioactive or other destructive agents attached to the tetracycline molecule.

Rats bearing osteosarcoma $D177$ were generously supplied by Prof. Alexander Haddow from the Chester Beatty Cancer Research Institute. Lederle Laboratories, Ltd., gave the chlortetracycline ('Aureomycin') and tetracycline ('Achromycin'), and I am particularly grateful to Dr. Ruth Porter for arranging this and for her help in this investigation. Mr. A. R. Jennings kindly confirmed the diagnosis of the tumours.

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EFFECTS OF OLIGOMYCIN ON RESPIRATION AND SWELLING OF ISOLATED LIVER MITOCHONDRIA

By Dr. J. B. CHAPPELL* and Dr. G. D. GREVILLE†

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INTACT liver mitochondria oxidize substrate rapidly only when in the presence of adenosine diphosphate (ADP) or of an uncoupling agent such as 2 : 4-dinitrophenol (DNP). Since oligomycin inhibits oxidations of pyridine nucleotide-linked substrates in presence of the former and the inhibition is reversed by the latter, Lardy and his colleagues[1] have concluded that this antibiotic interferes with the enzyme system responsible for respiratory-chain phosphorylation. This is strengthened by the finding that it inhibits mitochondrial DNP-stimulated adenosine triphosphatase and the adenosine triphosphate – inorganic phosphate exchange, both of which are thought to involve enzymes of the phosphorylation system of the respiratory chain; it also prevents the loss of oxygen-18 from labelled inorganic phosphate. These further observations indicate that oligomycin acts on that part of the phosphorylation system which is involved in the uptake of inorganic phosphate.

Oligomycin has also been found to prevent the swelling of rat-liver mitochondria induced by inorganic phosphate, and DNP reversed this effect[2]. This seemed to be in conformity with the proposal[3,4] that swelling of this type occurs only when some measure of electron transport is proceeding. Since, however, the observations on swelling were made with media containing sucrose (0·3 M), which markedly inhibits the oxidation of some substrates[5], it was desirable that these latter effects of oligomycin should be re-investigated.

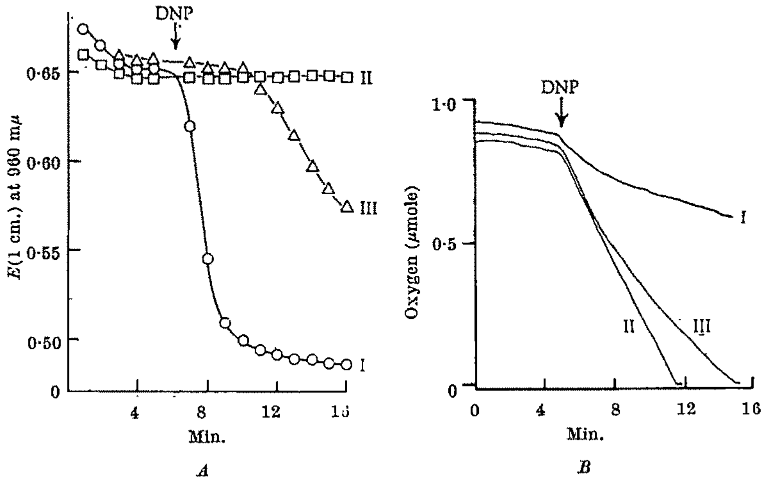

Fig. 1. Swelling (*A*) and respiration (*B*) of rat-liver mitochondria in presence of L-glutamate. In all runs, oligomycin (0·6 μgm./ml.) was present throughout and 2 : 4-dinitrophenol (0·04 mM) was added 5 min. after mitochondria (arrow). I, no adenine nucleotide added; II, 0·33 mM adenosine diphosphate added before mitochondria; III, 0·33 mM adenosine triphosphate added before mitochondria. Mitochondrial nitrogen, 0·11 mgm./ml. Other constituents of medium as in text. Total volume, *A*, 3 ml.; *B*, 4 ml. Temp. 25° C.

In a medium (pH 7·4) containing 80 mM potassium ions, 6 mM magnesium ions, 27 mM 2-amino-2-hydroxymethylpropane-1 : 3-diol, 92 mM chloride ions, 16 mM inorganic phosphate and 10 mM L-glutamate, and with the sucrose reduced to about 5 mM, oligomycin again prevented swelling, and subsequent addition of DNP initiated it. However, in the presence of ADP this effect of DNP was not seen; that is, with oligomycin present, a rapid swelling was caused by DNP alone but not by DNP together with ADP (Fig. 1*A*). Simultaneous measurements of respiration with the Clark oxygen electrode (technique to be described by J. B. C.) under identical conditions showed that, in the absence of adenine nucleotide and presence of oligomycin, respiration was not maintained after addition of DNP, but that addition of ADP before or shortly after the DNP prevented this decline in respiratory-rate (Fig. 1*B*). Adenosine triphosphate temporarily prevented the swelling induced by DNP in the presence of oligomycin and, also for a short period, the fall in respiration; we judge these effects to be due to ADP derived from the added triphosphate. If a very small amount of ADP is added after the DNP, the decline in respiratory-rate is delayed for only a short time: repeated small additions of ADP result in a stepwise curve (Fig. 2). Thus mitochondria treated with oligomycin

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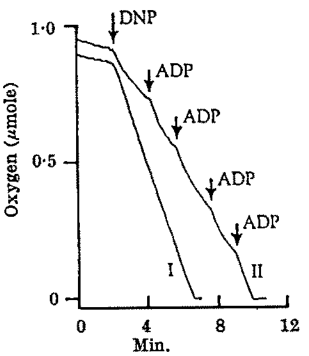

Fig. 2. Respiration of rat-liver mitochondria in presence of L-glutamate. 2 : 4-Dinitrophenol (0·04 mM) added at time shown by arrow. I, no addition of oligomycin or adenine nucleotide; II, oligomycin (1·2 μgm./ml.) present throughout, and additions of adenosine diphosphate as shown (12 μM in the first three and 24 μM in the last). In both runs, mitochondria equivalent to 0·2 gm. of liver in 4 ml. total volume: 1 mM ethylenediamine tetraacetate; other constituents of medium as in text. Temp., 25° C.

show some measure of respiratory control despite the presence of DNP. Results similar to those in Fig. 1*A*, *B* were obtained with ferricyanide instead of oxygen as terminal acceptor. The reduction of ferricyanide was followed by means of a smooth platinum electrode and automatic titrator (Radiometer, Copenhagen) (unpublished work by G. D. G. and J. B. C.).

Thus, in presence of oligomycin and DNP and with glutamate as substrate, ADP both maintains respiration and prevents swelling. The decline in respiratory-rate in the absence of adenine nucleotide appears to result from failure of the oxidation of the α-ketoglutarate formed from the glutamate, insufficient ADP being available for the obligatory phosphorylation at the substrate-level. The requirement for ADP when DNP is present is most likely to be related to this stage in the tricarboxylic acid cycle, since when the substrate was changed to β-hydroxybutyrate, L-malate, L-proline or succinate, addition of ADP was no longer necessary for the maintenance of rapid respiration. In fact, of the substrates tested, only glutamate, $L_s(+)$-*iso*citrate and α-ketoglutarate showed the requirement for ADP. When succinate was substrate, in order to obtain rapid oxidation uninhibited by accumulation of oxaloacetate, amytal was added to inhibit oxidation of malate. When malate was substrate, oxaloacetate was removed by transamination with glutamate, β-chlorovinyl arsenious oxide (lewisite oxide) being added to stop oxidation of the glutamate and α-ketoglutarate (see later).

It can be deduced therefore that, with DNP present, ADP is not required for the oxidation of mitochondrial reduced pyridine nucleotide. The oxidation of α-ketoglutarate, on the other hand, requires ADP for the associated substrate-level phosphorylation. The ADP requirement for the oxidation of glutamate and *iso*citrate may be ascribed to the circumstance that these give rise immediately to an α-ketoglutarate. This implies that oxidation of glutamate and *iso*citrate cannot take place when α-ketoglutarate oxidation is prevented. This is indeed so, since low concentrations (for example, 0·5 μM) of β-chlorovinyl arsenious oxide, which specifically interfere with the dehydrogenation of α-keto-acids, prevent the oxidation of glutamate and *iso*citrate as well as of α-ketoglutarate. Presumably the site of action of the arsenical is the lipoate involved in the dehydrogenation of α-ketoglutarate[6]. β-Chlorovinyl arsenious oxide does not inhibit glutamate dehydrogenase since, on addition of diphosphopyridine nucleotide together with cytochrome c and sufficient of the detergent 'Triton' to cause lysis of the mitochondria, oxidation of glutamate proceeds rapidly in the presence of the arsenical. It would appear that the inhibition of glutamate oxidation observed with intact mitochondria is due to accumulation of α-ketoglutarate, and that with particles treated with detergent glutamate oxidation is not dependent on the subsequent enzymatic removal of the keto-compound.

Krebs and Bellamy[7] have recently demonstrated a mechanism for the utilization of glutamate which involves transamination :

$$\begin{array}{l}\text{oxaloacetate} + \text{glutamate} \rightarrow \text{aspartate} + \\ \qquad\qquad\qquad\qquad\qquad\qquad \alpha\text{-ketoglutarate} \; ; \\ \alpha\text{-ketoglutarate} + 1\tfrac{1}{2}O_2 \rightarrow \text{oxaloacetate} + CO_2 \\ \hline \text{sum} : \; \text{glutamate} + 1\tfrac{1}{2}O_2 \rightarrow \text{aspartate} + CO_2\end{array}$$

In this, the oxidation of glutamate to α-ketoglutarate is at most needed as a priming reaction. It is clear that any mitochondrial oxidation of glutamate by the mechanism of Krebs and Bellamy will likewise be inhibited by β-chlorovinyl arsenious oxide, since the reaction sequence is completely dependent on the oxidation of α-ketoglutarate. In presence of the arsenical, however, glutamate could still undergo oxidation to α-ketoglutarate were it not that this is prevented by accumulation of the product, as already indicated.

With *iso*citrate as substrate a further factor is involved. Oxidation of this substance depends on re-oxidation at the expense of oxaloacetate of the reduced triphosphopyridine nucleotide which is generated by the *iso*citrate dehydrogenase. In the presence of β-chlorovinyl arsenious oxide, the level of oxaloacetate is insufficient, but the further addition of catalytic amounts of malate leads to restoration of *iso*citrate oxidation[8].

Since ADP is required for the oxidation of α-ketoglutarate, glutamate or *iso*citrate, in the presence of DNP, only when oligomycin is also added, it must be inferred that (*a*) in absence of oligomycin, DNP ensures a supply of ADP from the endogenous adenine nucleotide adequate for the needs of the succinyl coenzyme A synthetase (*P*-enzyme), and (*b*) oligomycin reduces the production of ADP until this becomes the limiting factor. Presumably its inhibitory effect on DNP-stimulated mitochondrial adenosine triphosphatase[1] would account for this. The requirement for inorganic phosphate for DNP-stimulated oxidation of glutamate[9], and the similar requirement for *iso*citrate oxidation (unpublished work by J. B. C.), are seen to result from the demands of the *P*-enzyme. It was found that no added phosphate was required for DNP-stimulated oxidation of proline, succinate, malate or β-hydroxybutyrate.

It seems clear, therefore, that oligomycin, which inhibits respiratory-chain phosphorylation[1], does not affect substrate-level phosphorylation. It is interesting to inquire whether this antibiotic inhibits at each of the three possible sites of respiratory-chain phosphorylation. It is possible to isolate single phosphorylation steps corresponding to two of these. Thus (i) with succinate as substrate and ferricyanide as electron acceptor, and (ii) with *p*-phenylenediamine as substrate and oxygen as acceptor, ADP does not stimulate electron transport in presence of oligomycin, although DNP does. Whether oligomycin exerts an inhibitory effect at the remaining phosphorylation site, namely, that adjacent to diphosphopyridine nucleotide, is at present unknown, although it is likely that it does, for the following reason. Oligomycin reduces the ADP-stimulated respiration with DPN-linked substrates to the corresponding state-4 rate (ref. 10), which is lower than the state-4 rate or the rate obtained in presence of ADP together with oligomycin in both cases (i) and (ii) mentioned above.

The decline in respiration in absence of adenine nucleotide (Fig. 1*B*), although due ultimately to failure of oxidation of α-ketoglutarate, is probably accentuated by loss of cofactors or other damage to the integrated mitochondrial respiratory system. Some evidence for this is as follows. If the concentration of the swelling agent, inorganic phosphate, was lowered from 16 m*M* to 2·5 m*M* or less, then, with oligomycin and DNP but no adenine nucleotide, addition of ADP after the respiratory-rate had fallen off re-instated the respiration ; but this did not occur with the higher concentration of phosphate. Furthermore, with some mitochondrial preparations, after swelling had occurred in the presence of DNP, oligomycin and 16 m*M* inorganic phosphate and absence of adenine nucleotide, addition of diphosphopyridine nucleotide and cytochrome *c* restored respiration, whereas with unswollen mitochondria a similar addition did not significantly increase the rate of DNP- or ADP-stimulated respiration.

It remains to be considered why, in presence of oligomycin, glutamate, DNP and a high concentration of phosphate, swelling occurs in the absence but not in the presence of ADP. It emerges from what has been said that in the absence of ADP an accumulation of α-ketoglutarate is to be expected. This would not occur in the absence of oligomycin, or in the presence of oligomycin together with ADP, since under these conditions α-ketoglutarate is oxidized. One is tempted to speculate that swelling in media which pre-dispose to this effect, for example, those containing high concentrations of phosphate, is connected with this accumulation of α-keto-acid.

To summarize, in a salt medium containing 16 m*M* phosphate and of low sucrose content, the respiration of rat-liver mitochondria in the presence of oligomycin and dinitrophenol is not maintained unless ADP is added as well. This phenomenon is observed with α-ketoglutarate, and with glutamate and *iso*citrate which give rise immediately to this keto-acid, but not with other substrates, and is ascribed to the requirement for ADP for substrate-level phosphorylation. Under these conditions the mitochondria swell rapidly in the absence but not in the presence of ADP, and damage to the respiratory system consequent on swelling may be a contributory cause of the decline in respiration.

We thank Miss Freda Johnson for expert technical assistance and Dr. A. S. Mildvan for criticizing the manuscript.

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OSMOTIC PRESSURE EFFECT OF THE RED BLOOD CELLS—POSSIBLE PHYSIOLOGICAL SIGNIFICANCE

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THE present concept of the net fluid volume transfer across the capillary wall rests mainly on the balance between the hydrostatic and colloid osmotic pressures as expressed in Starling's hypothesis[1]. The osmotic forces demonstrated in the conspicuous and well-known behaviour of the red blood cells as perfect osmometers[2–6] have not been invoked in attempts to explain the obvious inconsistencies between Starling's hypothesis and a series of common clinical observations related to œdema formation.

Osmotic Pressure Effect of the Red Blood Cells

During attempts to measure the colloid osmotic pressure of whole blood it became apparent that the red blood cells could influence the osmotic pressure under certain conditions in an electronic osmometer[7–9]. Fig. 1 shows the results of a single experiment. The first upward deflexion is due to the osmotic pressure generated by plasma when the reference fluid in the osmometer is 0·5 per cent sodium chloride. The very much higher pressure that follows develops when a sample of red blood cells concentrated by centrifugation in the same plasma is placed on the semi-permeable membrane, the reference fluid here also being 0·5 per cent sodium chloride. With plasma the pressure settles at a level of about 33 mm. mercury. When red blood cells form the sample the pressure decreases slowly after a maximum of about 400 mm. mercury has been reached. This is in accordance with the fact that the initial salt concentration difference between the reference fluid and the plasma remaining between the red blood cells is being eliminated by diffusion. The abrupt drop of pressure to the far right is caused by flushing with a 0·5 per cent sodium chloride solution. The effect of the salt concentration difference in the sample of the red blood cells is depending on the permeability of the membrane of the red blood cell, whereas the tiny and rapidly eliminated overshot in the plasma curve is the osmotic effect of the salt concentration difference across the semi-permeable membrane of the osmometer.

In Fig. 2 are shown semi-quantitatively a few principal experiments. The diagram can be interpreted as follows:

When the red blood cells are in close apposition to the semi-permeable membrane of the osmometer (*C*, *D*, *F*, *G*) but otherwise not (*E*), they exert an osmotic effect as if the semi-permeable membrane were replaced by a membrane having the osmotic characteristics of the membrane of the red blood cell. The difference between the osmolar concentration within the red blood cells and that of the reference fluid therefore determines the osmotic flow out of the measuring chamber and the resulting negative sub-atmospheric) equilibrium pressure (*C*, *D*, *F*, *G*). Differences in concentration of substances (as, for example, urea) which penetrate the red cell membrane exert no osmotic effect of their own and do not interfere with that of the salt (*G*). No hæmolysis was observed and hæmoglobin inside the red blood cell was found to be without colloid osmotic effect in the osmometer (*F* 0·9). The plasma protein in the space between the red blood cells exerts its usual colloid osmotic pressure (*B*) across the semi-permeable membrane (the low pressure in all the instances except *A* and *F*). The colloid osmotic pressure itself is not influenced by the various salt concentrations used (*B*). However, by restricting the amount of water available to the red blood cells outside the measuring chamber (*C* 0·5 as compared to *F* 0·5), the protein enhances the osmotic effect of the salt concentration differences.

The maximum osmotic pressure that can be developed by a sample of red blood cells equilibrated with a 0·9 per cent sodium chloride solution (310 milliosmolar) when the reference fluid is a 0·5 per cent sodium chloride solution (162 milliosmolar) is :

$$(310-162)\ 17 = 2{,}516 \text{ mm. mercury}$$

where 17 is the factor used for the conversion of concentration into pressure $\left(\frac{22\cdot4 \times 760}{1{,}000} = 17\right)$, when concentration is expressed in milliosmoles.

A volume transfer from the measuring chamber into the sample creates a negative pressure of 1 mm. mercury per 4×10^{-4} mm.^{3}.

The volume of one single layer of red blood cells on the semi-permeable membrane (area 40 mm.^{3}) is 4×10^{-2} mm.^{3}. The water phase is 0·6.

If all the water (V) is taken up from the measuring chamber we have the following equation at equilibrium pressure, where the subscript to V denotes the number of layers of red blood cells:

$$\frac{V_1}{4 \times 10^{-4}} = 2{,}516 - \frac{V_1 \times 310}{0\cdot6 \times 4 \times 10^{-2}}\ 17 \sim 30 \text{ mm. mercury}$$

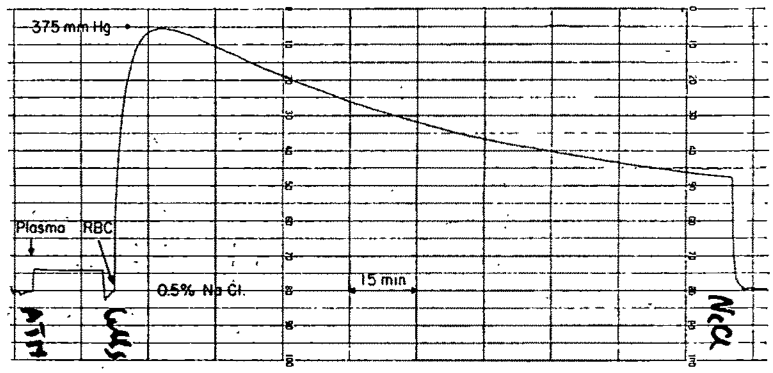
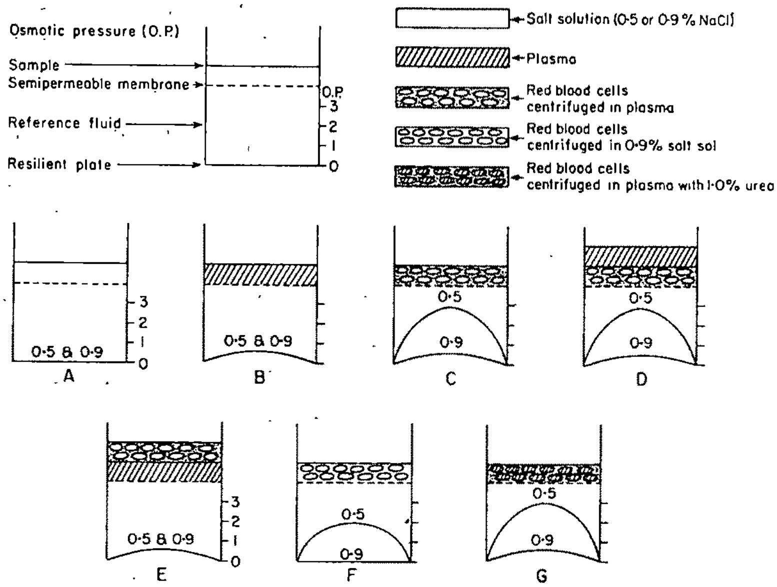


Fig. 1



Osmotic pressure (O.P.)
Sample
Semipermeable membrane
Reference fluid
Resilient plate
O.P
3
2
1
0
Salt solution (0·5 or 0·9 % NaCl)
Plasma
Red blood cells centrifuged in plasma
Red blood cells centrifuged in 0·9% salt sol
Red blood cells centrifuged in plasma with 1·0% urea
0·5 & 0·9
0·5
0·9
A
B
C
D
E
F
G

Fig. 2. The resilient plate is part of an electric capacitor. Its deflexion is detected by the change in capacitance to which it gives rise. The graduation shown on the pressure chamber of the osmometer is related to the order of magnitude of the actual pressures obtained rather than to exact values. The deflexion of the resilient plate is grossly exaggerated for the sake of distinction. The actual volume displacement is 4×10^{-4} mm.^{3}/mm. mercury

With 500 layers of red blood cells roughly equivalent to the usual samples the equation is:

$$\frac{V_{500}}{4 \times 10^{-4}} = 2{,}516 - \frac{V_{500} \times 310}{0\cdot6 \times 500 \times 4 \times 10^{-2}}\ 17 \sim 2{,}160 \text{ mm. mercury}$$

Considering that some water is available outside the osmometer, and that diffusion, as mentioned, tends to eliminate the original concentration difference, the actual maximum pressure values of 400–1,000 mm. mercury are acceptable as being of the same order of magnitude.

Red Blood Cells in the Capillaries

It has been tacitly accepted that the osmotic conditions in the capillaries are determined solely by the plasma and the capillary wall, leaving no role for the red blood cells, as already mentioned. The validity of this concept is not obvious when it is realized that the red blood cells often pass capillaries in close contact with the wall and tightly squeezed (Fig. 3),

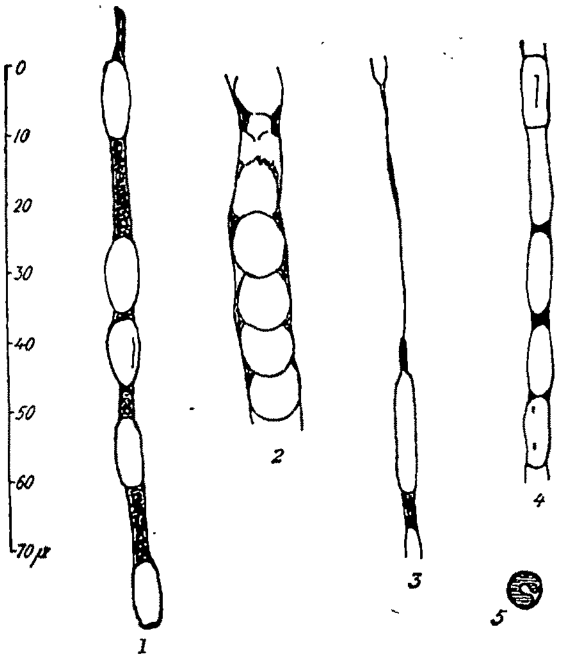


Fig. 3. Muscle capillaries from guinea pig, vitally injected with Indian ink. Walls of capillaries not shown. (From A. Krogh, ref. 25)

and that the capillary wall and the tissue fluid are analogous to the semi-permeable membrane and the reference fluid of the osmometer respectively. In Fig. 4, (*a*) represents the present concept and (*b*) the situation in which the red blood cell may exert an osmotic effect across the capillary membrane. The dilatation of the capillary in case (*a*) is meant as symbolic only. However, the acceptance of the mechanism postulated in (*b*) would require it to be real in order that the same effect be obtained.

As an osmolar concentration difference between the red blood cell and the tissue fluid makes it easier to explain the transfer mechanism, blood is assumed to enter the capillary with a slightly higher salt concentration ($C + \Delta C$) than that existing in the tissue fluid (C). Osmotic equilibrium between plasma and red blood cells prevails at both ends of the capillary. For the sake of simplicity it is further assumed that: (1) the volume of the tissue fluid is equal to the water phase of the blood in the capillary; (2) the colloid osmotic pressure is balanced by the hydrostatic pressure throughout the capillary. In (*a*) any exchange across the capillary membrane is diffusional, and consequently no net volume transfer takes place. The protein is concentrated by a factor :

$$\frac{C + \Delta C}{C + \frac{\Delta C}{2}}$$

which is immaterial as ΔC is small. The volume change of the red cell is accomplished by a redistribution of water entirely within the capillary. In (*b*), however, the water is taken up from the tissue fluid by the red blood cells adjoining the capillary wall, presumably with little interference from this structure, which is known to offer less resistance to water flow than the membrane of the red blood cell itself[10]. In the plasma section of (*b*) diffusion equilibrium obtains as in (*a*). The plasma protein may enhance the effect of the red blood cells as in the osmometer. Its concentration remains unchanged.

Salt concentration differences between plasma and tissue fluid as assumed here are physiologically very probable as a result of resorption and excretion of salt and water. They are inherent in a concept of dynamic equilibrium and will lead to water transfer via the red blood cells in or out of the capillaries dependent on the direction of the concentration difference.

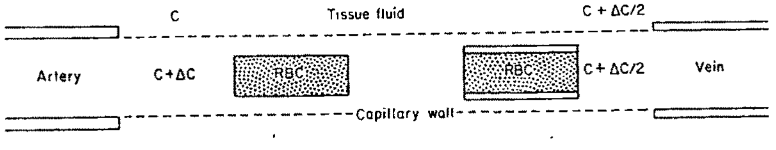
Even less hypothetical is the osmolar concentration gradient between the red blood cells and the tissue fluid that results from the respiratory function. The difference is a consequence of the increase of the osmolar concentration in the red blood cells as they pass along the capillaries releasing oxygen and taking up carbon dioxide. This is observable as a swelling of the red blood cells. Such a swelling of the red blood cells was discovered by Nasse[11] and observed both *in vitro* and *in vivo*, and has been studied among others by Hamburger[12], Warburg[13], Van Slyke[14] and Henderson[15]. Apart from the very minute effect it might have on the protein concentration[16,17] it has not been associated with important physiological functions, although Nasse felt that it was bound to be important. The osmotic effects of the phenomenon have been imitated by means of my technique (Fig. 5), and an estimate of the water transfer capacity is possible.

If Henderson's figures for the increase in volume of the red blood cell are used, we shall have for the resting person with an average arterio-venous oxygen deficit: measured volume increase of red blood cell 1 per cent, water phase 0·6. The total osmolar concentration is about 300 milliosmoles, the increase consequently 4·5 milliosmoles.

The total amount of water taken up by the red blood cells in 24 hr. at rest, assuming a hæmatocrit of 50 per cent and a cardiac output of 5 litres per min., will be:

$$\frac{5 \text{ litres} \times 24 \times 60 \times 50 \times 1}{100 \times 100} = 36 \text{ litres}$$

a) Blood homogeneous



b) Blood inhomogeneous

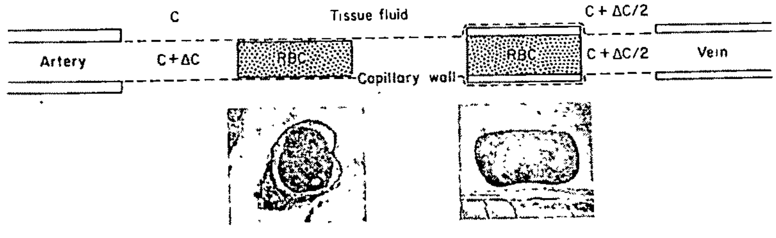


Fig. 4. The inserted photoelectron micrographs are from the heart of a rat. The completely solid appearance of the squeezed red blood cells is noteworthy. (The micrographs were kindly placed at my disposal by Dr. George E. Palade, Rockefeller Institute)

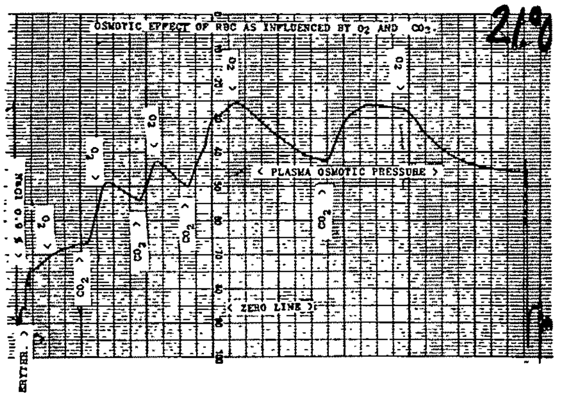


Fig. 5. Variation of the osmotic pressure of red blood cells under influence of carbon dioxide and oxygen. The aeration takes place from the surface of the sample not facing the semi-permeable membrane. This causes the effect to be relatively small and slowly developing

If all the capillaries were dilated as in Fig. 4*a*, all this water would be taken up from the plasma inside the capillary. If they were contracted as in Fig. 4*b* and we assign a length of 7μ and a radius of 1·5μ to the cylindrically deformed blood cell (Fig. 3), 8 litres would be taken up from the plasma across the two end surfaces and 28 litres across the capillary wall. This is still an amount that would seem to be abundant for any demand on water transfer across the capillary membrane, and certainly sufficient to remove the surplus of water formed metabolically in the tissue, which amounts to about 300 ml. per day. The mechanism is particularly suited for this task because the water-removing capacity of the red blood cells is proportionate to the oxygen metabolism, locally as well as totally. It should also be remembered that although the maximum capacity for water transfer seems very high indeed, any intermediate exploitation of the capacity between zero and maximum may be attained depending on the state of dilatation of the capillaries at any time and at any site.

The water taken up by the red blood cells from inside or outside the capillaries is in turn released in the lung capillaries. Any adverse effect of water transfer across the capillary wall into the tissue is prevented by the colloid osmotic pressure of the plasma, which is here in normal cases always in excess of the hydrostatic pressure—the only place in the organism where this is true. A small part, about 350 ml. in 24 hr., is excreted here as water vapour. In the glomeruli there is no deoxygenation of the red blood cells and, therefore, no interference with the filtration.

The red blood cells may take up water against a hydrostatic pressure in excess of the plasma colloid osmotic pressure, provided the wall supports it as the semi-permeable membrane does in the osmometer. The apparent effect would be that of a higher colloid osmotic pressure than the one measured *in vitro*. In the example in which the osmotic concentration rose 4·5 milliosmoles the equivalent pressure would be:

$$17 \times 4 \cdot 5 = 77 \text{ mm. mercury}$$

The suction effect is in addition to that of the colloid osmotic pressure, but the effect diminishes as water is taken up. If, therefore, the plasma section and the red blood cell section are of equal volume, the maximum excess of hydrostatic pressure that the red blood cells will be able to counteract is that which they can exert after they have taken up the water which has left the capillary in the plasma section. In my example it is about:

$$\frac{28}{36} \times \frac{77}{2} \text{ mm. mercury}$$

in excess of the colloid osmotic pressure. This pressure effect could easily be doubled within the physiological limits of deoxygenation.

Discussion

One of the reasons for this attempt to evaluate the hypothetical function of the red blood cells in some detail is its relation to net volume translocation across the capillary wall, which is now not satisfactorily understood[18–20].

In contrast to the exchange of solutes across the capillary wall, which can be accounted for by diffusion[10,21], net volume transfer requires hydrostatic or osmotic forces.

Starling's hypothesis provides for osmotic forces, and physiological experimentation has shown that fluid movement across the capillary wall can be brought about in agreement with the colloid osmotic pressure[1,22–24]. However, strictly speaking, such results only show that the capillary wall by and large is impermeable to proteins and freely penetrable for water and crystalloids, and that the colloid osmotic pressure is about the same as the hydrostatic average pressure in the capillaries. It does not seem permissible to infer that the hypothesis represents the sole mechanism by which net volume transfer between the vascular and the tissue fluid compartments takes place, and clinical observations, as mentioned in the introduction, make it clear that this is not likely.

A mechanism as the one discussed, based on an osmotic effect of electrolytes and closely associated with the capillary function (no matter how this function is conceived[25,26]) as well as with renal, respiratory and metabolic functions, would supplement the present concept of volume translocation in a very helpful way.

By invoking the surplus of suction effect of the red blood cells in contracted capillaries it would be easier to understand that a normal person does not accumulate œdema in the legs in spite of a capillary pressure here much higher than the colloid osmotic pressure[27]. In cardiac failure dilatation of the capillaries[28] as secondary to the well-known increase in vascular volume[29] would reduce the transcapillary osmotic effect of the red blood cells and thus explain why œdema develops, although the capillary pressure is not materially higher than in normal people.

Nephrotic œdema would be the result of an enhanced transcapillary filtration due to the low colloid osmotic pressure but with normal plasma–red blood cell ratio. Anæmic œdema[30] would develop as a result of increased transcapillary filtration due to an enlarged plasma section relative to the red blood cell section but with normal colloid osmotic pressure. In both cases the capacity of the red blood cells to remove water from the tissue fluid would have been exceeded. The final result would be influenced by the state of contraction of the capillaries, and this could be the explanation for the very puzzling fact that nephrotic œdema may be excreted without change of colloid osmotic pressure (ref. 31 and my observations), and, as it must be surmised, with an adverse change of tissue pressure and extracapillary colloid osmotic pressure—the other forces that play a part in Starling's hypothesis.

The cases of severe chronic cor pulmonale with œdema that vanishes with treatment which improves the low arterial oxygenation and the carbonic dioxide retention, but otherwise has no direct effect, may represent an interference with the osmotic function of the red blood cells because of faulty gas exchange.

These are all examples of clinical facts hitherto unexplained. The demonstration of negative tissue pressure, by Guyton *et al.*[32], the fact that the joint cavities are kept 'dry', and Mannery's estimate[33] that the tissue fluid is normally only sufficient to cover the structures with a thin layer of tissue fluid, fit in well with the surplus of suction effect that the red blood cells might be able to generate. An effect would be self-limiting by the increase in surface tension forces in the tissue and the increase in colloid osmotic pressure of the tissue fluid that it would bring about.

Finally, the observation repeatedly made in perfusion experiments, that blood is superior to plasma in preventing œdema[34-36], and that oxygenated blood is better than deoxygenated[37], would seem less controversial[38-40] in the light of a red blood cell effect than it does now.

The argument and the examples presented here are not meant as a substitute for experimental scrutiny, as it is all too obvious that our present knowledge about the capillary circulation is too meagre to permit any solid deductions. It might, however, be worth while to take the suggested possibility into consideration in future work in the many fields where the capillary circulation plays a significant part.

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COMPARISON OF AN ATTENUATED AND VIRULENT LOUPING ILL STRAIN

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OUR work on the 'attenuated strain of louping ill virus' began in 1957 [1] during experiments on the transmission of tick-borne encephalitis viruses by milk of domestic animals. Besides the Czechoslovak strain *Hypr* of tick-borne encephalitis a virus strain labelled by the Institute of Epidemiology and Microbiology in Prague as louping ill was included in our investigation so as to compare the properties of both strains. It was found that the strain *Hypr* of tick-borne encephalitis (TBE) when inoculated subcutaneously to sheep caused regular viræmia and excretion of virus into milk in large quantities (Table 1). After intracerebral infection it caused death 5–6 days later. In sheep infected with the louping ill strain, viræmia was of short duration and excretion of the virus by milk was observed only in two out of six animals (Table 2).

Therefore, we decided to investigate more carefully the properties of the aforementioned louping ill strain, which was originally obtained by Dr. Gallia, Institute of Epidemiology and Microbiology, Prague, and passed to our Institute in 1956. Further, we shall call this strain 'attenuated louping ill strain'. For comparison, another louping ill strain was chosen isolated recently from Nature and kindly supplied by Dr. MacCallum, London. This strain was used in its second to fourth intracerebral passage on mice and will be called 'virulent louping ill strain'.

The pathogenicity of the two louping ill strains was examined in white mice from two breeds with higher (Děčín) and lower (Dobrá Voda) susceptibility, in suckling rats, hamsters, monkeys and sheep (Table 3).

The virulent louping ill strain was pathogenic after intracerebral inoculation for all animals listed. After intracerebral infection in sheep, it caused regular encephalitis and death.

The 'attenuated louping ill strain' killed mice both after intracerebral and peripheral inoculations; suckling rats and young hamsters died after intra-

Table 1. RESULTS OF INOCULATING SHEEP WITH TICK-BORNE ENCEPHALITIS VIRUS, *Hypr*

No. of sheep	LD_{50} inoculation	Route of inoculation	Clinical symptoms	Virus in blood and milk (days after infection) 1	2	3	4	5	6	7	8	Neutralization index†
45	6·66 × 10^8	subcutaneously	–	△	△ ○	△ ○	○	○	○	○		100
46	6·66 × 10^8	,,	–		△		○	○	○	○		1,000
868	1·04 × 10^9	,,	–	△	△	△	△					12,000
869	1·04 × 10^9	,,	+	△	△ ○	△ ○	△ ○	△ ○	○	○		126,000
38	6·66 × 10^8	,,	–			△ ○	△ ○	○	○			10,000
867	1·04 × 10^9	,,	–		△ ○	△ ○	○	○				251,000
1*	6·66 × 10^9	intracerebrally	+		△							Death after 6 days
16*	6·66 × 10^9	,,	+			△						6 days
50*	6·66 × 10^9	,,	+				△	△				5 days

* Lamb; △, isolation of virus from blood; ○, isolation of virus from milk; † neutralization index for mice inoculated intracerebrally.

Table 2. RESULTS OF INOCULATING SHEEP WITH ATTENUATED LOUPING ILL VIRUS

No of sheep	LD_{50} inoculation	Route of inoculation	Clinical symptoms	Virus in blood and in milk (days after inoculation) 1	2	3	4	5	6	7	8	Virus in brain
23	6·66 × 10^3	Subcutaneously	–	–	–	–	–	–	–	–	–	–
24	6·66 × 10^3	,,	–	–	–	–	–	–	–	–	–	–
27*	3·33 × 10^5	,,	–	–	–	–	–	–	–	–	–	–
49*	3·33 × 10^5	,,	–	–	–	(△)	–	–	–	(△)○	○	–
5	6·66 × 10^9	,,	–	–	–	–	–	–	–	–	–	–
15	6·66 × 10^9	,,	–	△	–	–	–	○	○	○	–	–
25†	6·66 × 10^3	intracerebrally	–	–	–	–	–	–	–	–	–	–
26†	6·66 × 10^3	,,	–	–	–	–	–	–	–	–	–	–
30†	3·33 × 10^8	,,	–	–	–	–	–	–	–	–	–	–
31†	3·33 × 10^8	,,	–	–	–	–	–	–	–	–	–	–

*, Infected by ticks; △, virus in blood; ○, virus in milk; –, negative results; †, lamb.

Table 3. EXPERIMENTAL PATHOGENICITY OF ATTENUATED AND VIRULENT LOUPING ILL VIRUS

Animals	Route of of inoculation	Virulent louping ill virus				Attenuated louping ill virus			
		Clinical symptoms	Death	– log. LD_{50}*	Encephalitis (histology)	Clinical symptoms	Death	– log. LD_{50}*	Encephalitis (histology)
Mice, 'Děčín' breed	intracerebrally	+	+	6·0	+	+	+	7·5	+
	subcutaneously	+	+	4·1	+	+	+	6·0	+
Mice, 'Dobrá Voda' breed	intracerebrally	+	+	5·5	+	+	+	6·6	+
	subcutaneously	+	+	4·0	+	+	+	6·0	+
Suckling rats	intracerebrally	+	+	5·0	+	+	+	7·0	–
Suckling hamsters	intracerebrally	+	+	–	+	+	+	–	–
Monkey	intracerebrally	+	0	–	+	0	0	–	0
Sheep	intracerebrally	+	+	–	+	0	0	–	×
	subcutaneously	±	0	–	±	0	0	–	0

+, Positive result; 0, negative result; ±, irregular result; –, not tested; ×, vascular changes only; *, in brain.

cerebral inoculation. It produced, however, no clinical symptoms or death in monkeys and sheep. Even after massive intracerebral inoculation with a 10 per cent suspension containing $10^{8.52}$ LD_{50}, no signs of illness occurred.

The titres for the two strains are shown in Table 3.

The differences in pathogenicity of the two louping ill strains led us to examine the course of infection in their closest and most susceptible natural host, namely, in sheep.

After infection with the virulent louping ill strain, elevation of temperature ensued 3–4 days later, reaching 41·7° C. On the fifth day tremor, ataxia and paralysis developed. Death occurred 5–7 days after inoculation. On section, a marked hyperæmia and small hæmorrhages were present in the brain and spinal cord. From animals killed at various intervals after inoculation nineteen tissue samples were taken for virological and morphological examinations. After intracerebral inoculation, no virus was present at the 24-hr. interval; 3 days after inoculation it was present only in tissues of the reticulo-endothelial system and in the central nervous system. At the same time, encephalitis could be demonstrated histologically. Five days after inoculation, virus could be demonstrated in high titres (up to 10^6) in brain both virologically and by the fluorescent antibody methods. Inflammatory lesions and damage of neurones were present.

After subcutaneous infection of sheep with the virulent strain of louping ill, a long-lasting viræmia developed, reaching considerable values. In tissues measurable amounts of virus were present not earlier than 3 days after inoculation and again in the lymph nodes and spleen. Five days after inoculation, virus was present in reticulo-endothelial tissues and also in the ganglia of the peripheral nervous system. Slight perivascular changes in the central nervous system 7 days after inoculation pointed to incipient invasion of the central nervous system, although no virus could be demonstrated virologically.

Under similar conditions, experiments were performed with the 'attenuated louping ill strain'. No rise in temperature occurred after intracerebral or subcutaneous inoculation even with a high dose of virus. Attempts to isolate virus from organs of these animals were unsuccessful. Histological examination of animals inoculated intracerebrally showed perivascular infiltrations, although no damage of nerve cells could be demonstrated, as was the case with the virulent strain. No viral antigen could be demonstrated in nerve cells with the method of fluorescent antibodies. Animals inoculated intracerebrally with normal mouse brain or with a suspension of attenuated louping ill virus inactivated at 56° C. for 1 hr., showed no histological changes at all.

To determine whether, in spite of these unusual pathogenic features, the 'attenuated louping ill strain' really belongs to the tick-borne encephalitis group, the neutralizing capacity of sera against different tick-borne encephalitis viruses (Czechoslovak tick-borne encephalitis virus, virulent louping ill and 'attenuated louping ill strains') was tested with the 'attenuated louping ill virus'. The louping ill virus was neutralized in the intraperitoneal neutralization test by all three sera used.

To test the immunogenicity of the two louping ill strains, two sheep were inoculated subcutaneously with the virulent and two with the attenuated strains. Both viruses led to the formation of neutralizing antibodies, the neutralization index reaching values of more than 3 logarithms.

In a protection test, sheep were inoculated with a 10 per cent suspension of the attenuated louping ill strain. Ten and 14 days later, they were challenged with the virulent louping ill strain. Control animals which received only the virulent strain died 7 days after inoculation. Sheep inoculated first with the attenuated strain, remained well for the following 14 days of observation.

Preliminary results only have been reported here. Perhaps these findings could form a basis for a possible use of this strain for the preparation of a vaccine against viruses of the tick-borne encephalitis complex.

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TWO PHOTOCHEMICAL SYSTEMS IN PHOTOSYNTHESIS

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BY sensitive differential absorption spectroscopy it was demonstrated[1] that illumination causes oxidation of an *f*- or *c*-like cytochrome in *Porphyridium cruentum*. The action spectrum for cytochrome oxidation showed a maximum at 680 mμ, apparently due to chlorophyll *a*, and a much lower maximum at 560 mμ, probably due to phycoerythrin[2]. This was surprising, since light of 680 mμ is known to be far less efficient in bringing about photosynthesis and chlorophyll *a* fluorescence than equally intense light of 560 mμ (ref. 3, cf. ref. 4). From this, it followed that at least two photochemical pigment systems are present in *Porphyridium*. The system which is mainly responsible for the action spectrum of chlorophyll *a* fluorescence is called system 2. System 2 contains fluorescent chlorophyll *a* and a high proportion of phycobilins, mainly phycoerythrin; light quanta absorbed by phycoerythrin in this system are transferred to the fluorescent chlorophyll *a* and are thus photochemically active (cf. ref. 3). The other system, 1, contains non- or weakly fluorescent chlorophyll *a* and less phycobilins, and is active in cytochrome oxidation. We will assume that only these two pigment systems are present in *Porphyridium*; this assumption will prove sufficient for a satisfactory interpretation of our experiments.

It has also been observed[2] that the kinetics of cytochrome oxidation were different in light of 560 mμ from those in light of 680 mμ. This phenomenon shows that at least two pigment systems, which according to the preceding assumption must be the systems 1 and 2, interact in the cytochrome reaction. The kind of interaction is shown in Fig. 1. The decrease in absorption at its maximum, 420 mμ, which was shown to be a measure of the oxidation of the cytochrome, was recorded. The recorder tracing shows that actinic light of 680 mμ causes oxidation of the cytochrome; additional illumination with actinic light of 560 mμ causes a reduction of this cytochrome. Addition of the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to the very low concentration of $1 \cdot 1 \times 10^{-6}$ *M* inhibited the reduction at 560 mμ; an oxidation is observed instead. A similar effect was observed in the presence of $4 \cdot 3 \times 10^{-2}$ *M* N-ethylurethane. The simplest interpretation of these experiments is that system 1, with a predominant activity at 680 mμ, directly or indirectly oxidizes, and that system 2, with predominant activity at 560 mμ, reduces the cytochrome; DCMU prevents system 2 in some way from reducing the cytochrome.

The action spectrum of cytochrome oxidation in the presence of DCMU or N-ethylurethane is shown in Fig. 2. According to the previous interpretation, this action spectrum should be equal to that of system 1.

It was found that the action spectrum of systems 1 and 2 varied for different cultures. We estimated the ratio of activities at 560 and 680 mμ to vary for system 2 between at least 2·4 and 6, and the corresponding ratio for system 1 from 0·8 to 1·2. The ratio for system 2 was calculated from earlier measurements[3a,4].

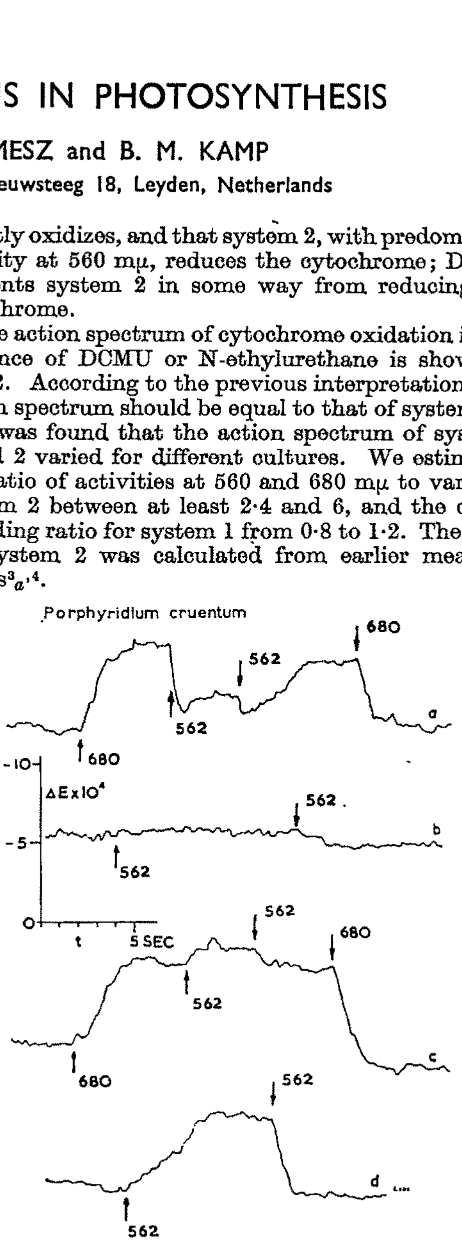

Fig. 1. Recorder tracings of absorption changes of *Porphyridium cruentum* at 420 mμ in the absence (*a*, *b*) and in the presence (*c*, *d*) of DCMU. Upward deflexions are decreases in absorption and reflect the oxidation of cytochrome. Upward and downward pointing arrows indicate the switching on and off of the actinic light. (The intensities are $4 \cdot 4 \times 10^{-10}$ Einstein/(cm.^{2} sec.) at 680 mμ and $5 \cdot 3 \times 10^{-10}$ Einstein/(cm.^{2} sec.) at 562 mμ.) Tracing (*a*) shows a reduction of the cytochrome by light of 562 mμ

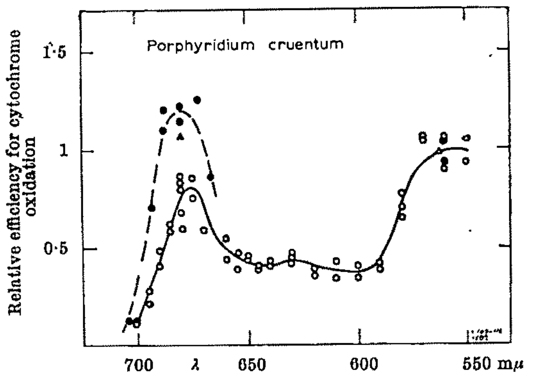

Fig. 2. Action spectrum for cytochrome oxidation in the presence of N-ethylurethane (open circles) and of DCMU (solid circles and triangles) for three different cultures of *Porphyridium cruentum*

Because of the approximate proportionality between the action spectrum of photosynthesis and that for excitation of chlorophyll *a* fluorescence, it had been concluded[3,5] that the pigment system responsible for the chlorophyll *a* fluorescence (system 2) was active in photosynthesis. The non- or weakly fluorescent chlorophyll *a* was believed to be inactive. However, the fact that both systems react with the same cytochrome indicates that system 1 also participates in photosynthesis and supplements in some way system 2. The relatively good quantum efficiency for cytochrome oxidation (about 5 quanta absorbed at 680 mμ per cytochrome molecule oxidized[2]) supports this view.

Further support for the hypothesis that systems 1 and 2 interact in photosynthesis was obtained from photosynthesis measurements by means of an oxygen polarograph, similar to one described recently[6]. The relative rates of photosynthesis calculated per equal number of incident quanta per sec., for light of 570 mμ and 680 mμ, were 83·5 and 20·6 (ratio, 4·0) and, with $2{\cdot}1 \times 10^{-3}$ *M* N-ethylurethane, for another sample of the same culture, 59·5 and 9·6 (ratio, 6·2). It is interesting to see that the percentage inhibition, which at a given wave-length is the same for various intensities, is smaller in actinic light of 570 mμ than in that of 680 mμ. This phenomenon was observed with both DCMU and N-ethylurethane with four different cultures. An experiment with DCMU gave a ratio 560/680 mμ of 7·0, as compared with a ratio of 4·4 without inhibitor.

When photosynthesis of unpoisoned *Porphyridium* was measured at 680 mμ with a background of strong light of 562 mμ, the rate of photosynthesis was found to be enhanced considerably (by a factor of 2). A similar enhancement effect has been observed for species of algae of different groups by Emerson and co-workers[7], and more recently by other authors, and has been called the 'Emerson effect'. The enhancement effect earlier observed[8,9] in *Porphyridium* occurred to a smaller extent and under conditions different from those reported here.

Our experiments can be interpreted as follows: In order to bring about photosynthesis, systems 1 and 2 must both be excited. If system 2 is strongly inhibited, the rate of photosynthesis, and thus the action spectrum of photosynthesis, will be mainly determined by system 2 at all wave-lengths. In uninhibited algae, however, system 2 is more active than system 1 at 560 mμ, and less active at 680 mμ. If the algae are illuminated with both wave-lengths simultaneously, then the rate of photosynthesis will be higher than the sum of the rates at each wave-length separately, because by simultaneous illumination the excess absorptions supplement each other.

The 'Emerson effect' has been observed for a variety of algae, belonging to different classes. This indicates that the scheme of Fig. 3, which summarizes and generalizes our hypothesis, applies also for other algae and probably for higher plants too.

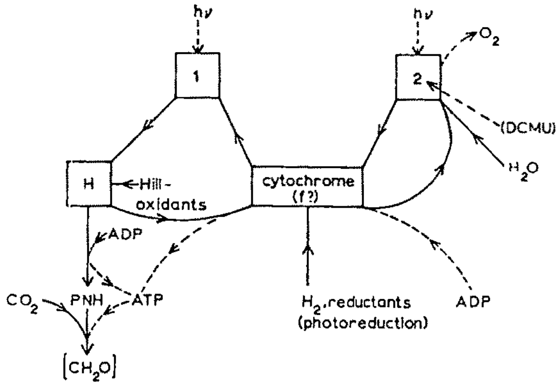

Fig. 3. Hypothetical scheme showing functions of systems 1 and 2 in photosynthesis

Further evidence for this scheme is as follows. DCMU, which strongly inhibits photosynthesis, does not inhibit the photoreduction of carbon dioxide with hydrogen gas in *Scenedesmus*[10] and the photoreduction of triphosphopyridine nucleotide (TPN) by spinach chloroplasts in the presence of ascorbic acid and a catalytic amount of 2,6-dichlorophenol indophenol[11]. This indicates that these photoreductions proceed via system 1 solely. The action spectrum for the reduction of phosphopyridine nucleotide (PN) in the blue alga *Anacystis nidulans* in the presence of DCMU was found to be proportional to the action spectrum for cytochrome oxidation, also measured in the presence of DCMU[12]. Thus reduction of PN is presumably closely linked to system 1.

The observation[13] that also the Hill reaction of *Chlorella* with quinone shows an 'Emerson effect', indicates that both systems 1 and 2 participate in the Hill reaction.

Our scheme is intended as a working hypothesis, which is certainly incomplete, and may require modification. At present it explains most experiments known to us in a simple and plausible way. A more detailed report is in preparation, in which we will discuss partly similar but less detailed and experimentally less supported hypotheses concerning the role of the photosynthetic pigments which have been proposed recently by other authors.

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WOOD-BORING ACTIVITY OF *LIMNORIA* (ISOPODA) IN RELATION TO OXYGEN TENSION

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CONFLICTING reports are found in the literature concerning the effect of pollution on the wood-boring activity of the marine isopod *Limnoria*. Some authors[1–3] consider it has a deleterious effect, others[4–7] that it has no effect, while Fraser[8] maintains that a moderate degree of pollution is beneficial to the borer. The confusion probably arises from the various forms the pollution can take, for example, chemical, oil or organic. The toxicity of chemical effluents will depend on their concentrations; but it was thought that organic pollution affected *Limnoria* to the degree by which it reduced the dissolved oxygen content through its decomposition. It was observed that *Limnoria* abandon their burrows in stagnant sea-water, and this reaction has been made the basis of an experiment to evaluate the effect of the oxygen tension on the boring activity. The possibility was also considered that changes in *p*H of the water, caused by the decomposition of organic matter on the wood, were responsible for the evacuation of the burrows.

The experimental procedure was as follows: a large piece of piling, showing about 260 sq. cm. of *Limnoria*-attacked surface, was scraped down to remove debris and attached organisms and rinsed several times in clean sea-water. The water-logged wood was immersed in sea-water which had previously been well aerated, and was allowed to stand for a period of five days. Initial measurements were made of the *p*H and oxygen tension of a sample of water taken from near the timber surface. These readings were repeated at intervals during the experiment. Immediately after each set of readings, the wood was taken from the water and all *Limnoria* on the surface were counted and removed. At the end of the experiment the wood was split open and all animals remaining in the wood were counted. Thus the original total population was known, and the percentage of animals leaving the wood at intervals was calculated. A correlation was then sought between these figures and the *p*H and oxygen tension records.

The oxygen tensions were measured by the Whitney syringe pipette method[9], which allowed a sample of water to be taken under the surface adjacent to the burrow entrances. Accurate measurements could not be made below an oxygen content of 0·5 per cent saturation. The readings are given as percentages of the oxygen present in saturated sea-water corrected for temperature and salinity, using the solubility tables of Truesdale *et al.*[10]. The *p*H values were estimated colorimetrically, using a B.D.H. small comparator with phenol red indicator. The salinity of the water was 35·8 per mille and its temperature during the experiment varied between 16° and 17° C.

The timber, which was taken from pine piling at Calshot Pier in Southampton Water, contained three species of *Limnoria*, namely, *L. lignorum* (Rathké), *L. quadripunctata* Holthuis and *L. tripunctata* Menzies. In order that any differences in behaviour could be assessed, the specific composition of a sample of 50 individuals was noted each time a group of *Limnoria* was removed from the wood.

The results of the experiment are given in Table 1. The rate at which *Limnoria* left the wood is compared with the *p*H and oxygen tension readings in Fig. 1. This shows that the desertion of the timber by *Limnoria* was accompanied by a progressive reduction in the oxygen content of the water. There was little variation in the *p*H (8·0–7·6) over the course of the experiment and this small change can have had little effect as *Limnoria* is said[11] to be tolerant of a *p*H range between 4·5 and 9·6. It is a reasonable assumption, therefore, that the exodus of the *Limnoria* was caused by the fall in oxygen tension. From these results an estimate can be made of the percentage saturation value which limits the boring activity.

The plots of the percentage of *Limnoria* leaving the wood fall on a sigmoid curve, the steep slope of which corresponds approximately to the time interval between 25 and 85 hr. During this period of maximum desertion, the oxygen tension fell from

Table 1. RATE AT WHICH *Limnoria* LEFT THE TIMBER UNDER REDUCED OXYGEN CONDITIONS
Each percentage shows the proportion of *Limnoria* which had left the timber since the beginning of the experiment

Time (hr. from beginning of experiment)	*p*H	O_2 tension (per cent saturation)	No. and progressive percentage of *Limnoria* leaving the timber:							
			Combined species		*L. lignorum*		*L. quadripunctata*		*L. tripunctata*	
			No.	per cent	No.	per cent	No.	per cent	No.	per cent
Zero	8·0	90·5	0	—	0	—	0	—	0	—
18·5	7·8	34·4	221	6·4	13	7·7	199	7·5	9	1·5
32·5	7·8	24·7	169	11·3	3	9·5	140	13·0	17	4·2
42·5	7·8	12·8	425	23·6	9	14·8	357	26·4	59	13·8
48·0	7·8	5·9	235	30·4	5	17·7	207	34·2	23	17·5
55·5	7·8	4·1	266	38·1	11	24·3	234	42·9	21	20·9
69·0	7·6	2·8	606	55·7	12	31·4	485	61·1	109	38·6
95·0	7·6	0·7	761	77·7	107	94·7	441	77·7	213	73·2
126·0	7·6	< 0·5	337	87·5	0	94·7	243	86·8	94	88·5
143·0	7·6	< 0·5	79	89·7	2	95·9	63	89·1	14	90·7
No. remaining in timber			354	(10·3)	7	(4·1)	290	(10·9)	57	(9·3)
No. initially in timber			3,453	(100·0)	169	(100·0)	2,668	(100·0)	616	(100·0)

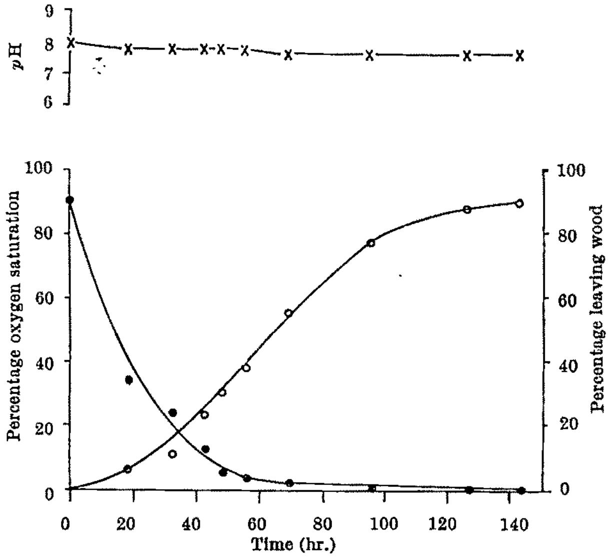

Fig. 1. Relation between the oxygen tension of sea-water and the rate at which *Limnoria* abandoned the timber
o, percentage of *Limnoria* leaving timber ; [●, percentage oxygen saturation; ×, *p*H

about 17 to 1 per cent. Below about 17 per cent saturation, therefore, the amount of dissolved oxygen must have begun to exert a limiting effect on the boring activity. The actual values for the water within the burrows were probably somewhat less than those given above. These results show a considerable toleration of low oxygen conditions, for 50 per cent of the *Limnoria* had not left the wood at oxygen tensions as low as 3 or 4 per cent saturation, and even at the end of the experiment, when the oxygen content was too low to be measured, 10 per cent of the *Limnoria* still remained in the wood. There is no certainty, however, that these animals were still able to bore. It is difficult to assess a limiting value for the oxygen tension but it seems probable, from Fig. 1, that the critical value had been reached at 2 per cent saturation.

The figures for the separate species, derived from the analyses of samples each of 50 *Limnoria*, show a difference between *L. quadripunctata* and *L. tripunctata*. A higher proportion of the former species left their burrows earlier, and therefore at a higher oxygen tension, than was the case with *L. tripunctata*. However, the final percentage of either species remaining in the wood at the end of the experiment was the same. The more erratic rate of emergence of *L. lignorum* from the burrows may be an artefact, due to the small numbers of this species present in the total population. However, its behaviour resembled that of *L. tripunctata* in that the majority of the specimens did not leave the wood until very low oxygen conditions (less than 3 per cent saturation) obtained. It seems, therefore, that *L. quadripunctata* is more sensitive than the other species to low oxygen concentrations.

The conclusions of this experiment in relation to pollution apply to those cases in which the pollution causes a reduction in the oxygen tension of the water. It is suggested that if the oxygen dissolved in the water at the surface of submerged timber falls to a level below 2 per cent saturation, the boring activity of *Limnoria* within the wood will cease. This conclusion could have practical applications in problems of *Limnoria* infestations.

I am grateful to Prof. J. E. G. Raymont of the University of Southampton for facilities in the Department of Zoology. The work was carried out during the tenure of a Department of Scientific and Industrial Research maintenance grant.

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THE EFFECT OF DEPTH OF TRANSPLANTING ON TILLERING AND SEED PRODUCTION IN WHEAT

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SEVERAL strand plants, namely members of the Gramineae, are known to thrive on wind-blown sand. These are *Agropyron junceum* L., *Elymus arenarius* L. and especially *Ammophila arenaria* Link. The latter species reacts favourably to periodical burial under fresh layers of sand by producing tall robust shoots from the lower leaf axils[1].

Pot experiments with transplanted *Ammophila* culms showed that increased depth of planting tended to produce more stout shoots, although the over-all yield was not greatly affected (Halwagy, unpublished work).

The present work aimed at exploring the possibility of a similar response in a cultivated member of the Gramineae. Wheat (*Triticum vulgare*) was chosen as the study material.

Culms of *Triticum vulgare* (Giza 139) were obtained from the University of Alexandria farm on February 23, 1957. They had been grown from seed on November 17, 1956. The culms were cut back to a uniform length of 50 cm. Of this, 8–10 cm. had been underground, consisting of a few internodes and carrying buds at the nodes. Culms were well mixed before being transplanted in 30-cm. pots, in clayey loam. Each pot had 25 culms; culms and pots being allotted to treatments at random. The pots were arranged in a Latin square of 5 replicates and 5 treatments. The 5 treatments were: culms transplanted at a

Table 1

Treatment	Total No. of shoots	No. of flowering shoots	Dry weight of shoots (gm.)	Dry weight of spikes (gm.)	Dry weight of crop (shoots + spikes) (gm.)
A (transplanted 10 cm. deep)	76 ± 10·02	15 ± 7.64	97·4 ± 26·52	25·59 ± 16·71	123·0 ± 40·74
B (transplanted 15 cm. deep)	92 ± 10·02	52 ± 7·64	185·6 ± 26·52	86·58 ± 16·71	272·2 ± 40·74
C (transplanted 20 cm. deep)	48 ± 10·02	12 ± 7·64	58·7 ± 26·52	19·03 ± 16·71	77·7 ± 40·74
D (transplanted 25 cm. deep)	11 ± 10·02	5 ± 7·64	13·3 ± 26·52	5·48 ± 16·71	18·7 ± 40·74
E (transplanted 28 cm. deep)	16 ± 10·02	10 ± 7·64	16·6 ± 26·52	7·39 ± 16·71	23·9 ± 40·74

depth of 10, 15, 20, 25 and 28 cm. below the soil surface.

On May 13, 1957, the height of all new shoots was measured. On July 4, 1957, the crop was harvested by clipping at soil-level. The following quantitative estimates were made: the total number of shoots, the number of flowering shoots, dry weight of shoots (oven-dried at 105° C.), dry weight of spikes (excluding the spike-bearing stalks, oven-dried at 105° C.) and the mean shoot weight. The results were analysed statistically and the 5 per cent level of significance adopted.

The effect of the depth of transplanting on the yield, as measured by different characteristics, is shown in Fig. 1. The analysis of variance (Table 1) showed that transplanting at a depth of 15 cm. produced a larger number of shoots than all other treatments, transplanting 10 cm. deep excepted. Further, that the number of flowering shoots, dry weight of shoots, spikes and total weight of crop were significantly greater in culms transplanted 15 cm. deep than in any other treatment. The better growth is due to the production of more shoots, vegetative and flowering, rather than producing heavier individual shoots.

The reaction of transplanted wheat culms to various depths of planting, as revealed by this work, is at variance with that of *Ammophila*. For wheat, the optimum depth appears to be 15 cm. In *Ammophila*, the quality rather than the quantity of new growth was influenced by the depth of transplanting. Increased depth (between 10 and 25 cm.) caused a slight increase in the over-all weight of shoots, but had a more pronounced effect on the mean shoot weight. Culms planted at greater depths produced fewer but taller and stouter shoots.

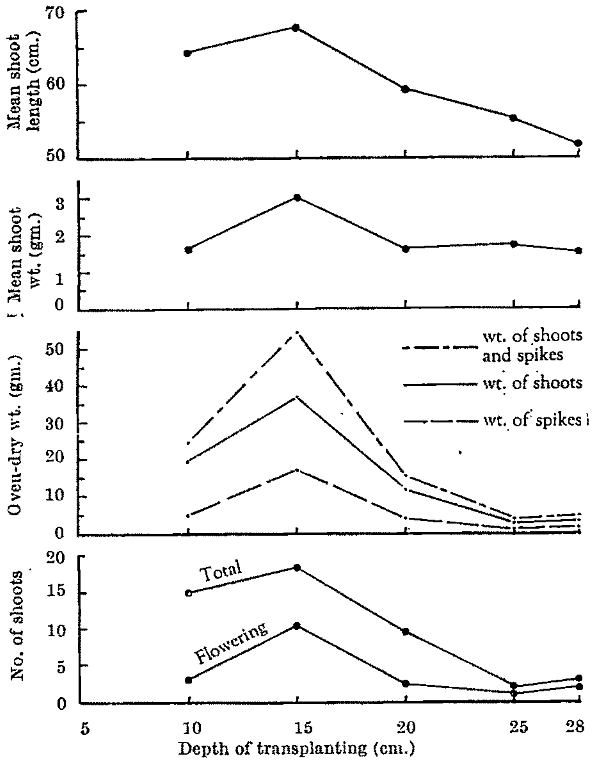

Fig. 1. Effect of depth of transplanting on yield of wheat culms

The nature of the stimulus is not fully understood. It seems likely that by increasing the depth of burial of wheat culms from 10 to 15 cm. the buds are pushed a few centimetres in the soil, where the temperature is more or less constant and large fluctuations in temperature are thus avoided. Moreover, deeper buds become less subject to desiccation when the top centimetres of the soil dry out. These buds will therefore have a better chance of tillering, than the more shallowly placed ones nearer the soil surface.

It is not clear, however, why further burial (at 20, 25 and 28 cm.) does not induce still better tillering. It should be recalled that the yield data are based on the aerial portion of the crop, so that the number and weight of shoots recorded refer to those shoots which emerged above the soil surface. It might have been desirable to investigate the possibility of production, from the deeply buried buds, of tillers which failed to appear above the soil surface and were therefore not taken into consideration.

The results of this experiment are more suggestive than conclusive, and may well prove rewarding if further work is carried out along similar lines. Also, the economic implications of the problem have not been considered. It should be noted that in the field, the usual depth of the underground part of the culm is 8–10 cm. It has been shown that better vegetative growth and seed production may be induced by burying the culms to a depth of 15 cm. Before the advantages of these results are utilized, it is necessary to find out the effect of the mere pulling out and transplanting of wheat culms. Comparison must be made in the field, between the yield of plots on which wheat culms have been pulled and later transplanted 15 cm. deep, and control plots. It is very probable that pulling has an adverse effect on the subsequent growth and seeding of wheat culms, so that any gain in yield obtained through transplanting at a depth of 15 cm. will be counterbalanced by this inimical effect and the additional cost of labour.

Deep burial of seed at the time of sowing may seem an easier and more practicable alternative. But the limitations of this method are obvious since seedlings produced from seeds which had been sown too deeply will fail to emerge above the soil surface.

I am indebted to Prof. A. H. Nasr for various working facilities during the course of this work and to Prof. A. el Khishin for providing wheat culms. Thanks are also due to Dr. S. Tolba and Dr. S. Nasr for statistical advice.

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SEX AND STRAIN DIFFERENCES IN ELECTROSHOCK CONVULSIONS OF THE RAT

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OUR objectives were to determine whether differences in the threshold and pattern of electroshock seizures exist between male and female, and maze-bright and maze-dull rats.

Our interest in this problem developed from the following considerations: (1) Woolley and Timiras[1] have shown that the administration of various sex steroids to rats alters brain excitability as assessed by the electroshock seizure threshold test. The striking excitatory action of œstradiol suggested that female rats might have a lower convulsive threshold than males. (2) Rosenzweig *et al.*[2] have demonstrated that in rats selectively bred for differences in maze-learning ability, the superior learners (S_1 strain) have a higher brain acetylcholine (ACh) content and cholinesterase (ChE) activity than the inferior learners (S_3 strain). They have proposed that the level of central ACh–ChE activity is related to efficiency of neural transmission and of learning. This, together with reports[3] that drugs which lower the convulsive threshold also increase ChE activity in some parts of the cat brain, and with the finding[4] that subconvulsive doses of central excitatory drugs increase the learning ability of the rat, suggested that the S_1 strain might have lower convulsive thresholds than the S_3 strain.

In the present work male and female S_1 and S_3 rats, 125 days of age, were used. Electroshock seizures were produced with the apparatus described in ref. 5; this apparatus has a constant current circuit and can be pre-set to give the desired value of current independently of the resistance of the animal. The electroshock seizure threshold (EST) is defined as the minimal amount of alternating current in milliamperes delivered through corneal electrodes for 0·2 sec. which will elicit a barely detectable clonic convulsion of the ears, jaws and forepaws without loss of equilibrium. A lower threshold is assumed to indicate greater brain excitability.

In Table 1 are shown the EST values for the four sub-groups. In both strains, female rats have a significantly lower value than males. For both sexes, the S_1 rats have a lower EST than the S_3. (The P-values in the table are based on t-tests.) These sex and strain differences are what we expected.

Table 1. ELECTROSHOCK SEIZURE THRESHOLD DIFFERENCES IN MALE AND FEMALE MAZE-BRIGHT AND MAZE-DULL RATS

Strain of Rats*	$\bar{x}$ and σ of electroshock seizure thresholds (m.amp.)		Male vs. female P
	Male	Female	
Maze-bright (S_1)	23·41 ± 0·23	21·15 ± 0·20	< 0·01
Maze-dull (S_3)	24·32 ± 0·33	22·02 ± 0·27	< 0·01
S_1 vs. S_3 P	< 0·05	< 0·02	

* Eighteen rats were used in each of the four sub-groups, for a total of 72; all were about 125 days old.

Because the females were lighter than the males, it was desirable to determine whether EST was related to body weight. Therefore, correlations between body weight and EST were determined for each sex-strain sub-group separately and were then combined by Snedecor's method to give an average correlation. The following product–moment correlation coefficients were found: S_1 ♂, 0·76; S_1 ♀, 0·15; S_3 ♂, 0·51; S_3 ♀, 0·46; average 0·47 ($P < 0·01$). These positive correlations might be explained by either or both of two hypotheses: (1) variations in EST are consequences of the influence of body mass on current spread, and thus on current density in the brain. Therefore, the heavier the animal the greater the current that must be applied to elicit a seizure; (2) body weight and EST are independent expressions of the hormonal and metabolic balance of the animal.

To obtain further information on the effects of tissue mass on current density in the brain, we examined the correlation between brain weight and EST for the various sub-groups. These correlations ranged from − 0·12 to 0·01, none being significantly greater than zero. This may be interpreted as inconsistent with the 'tissue mass' hypothesis. Also, contrary to this hypothesis, is the fact that, although the S_1 and S_3 strains of rats differed significantly in EST, their body weights were equal.

It is known that œstradiol decreases body weight and EST. In support of the 'metabolic' hypothesis is the finding[6] that œstradiol can lower EST before body weight is decreased.

Although we tend to interpret the covariance of body weight and EST as reflecting two relatively independent effects of the hormonal state of the animal, we cannot eliminate completely the 'tissue mass' hypothesis. However, in our second test of brain activity, the maximal electroshock seizure (MES) pattern, the response to the supramaximal stimulus, is independent of current density, as demonstrated in ref. 7.

A maximal electroshock seizure consists of both tonic and clonic components, and in the rat the tonic phase is divided into tonic flexion followed by tonic extension of the limbs. The tonic extensor phase represents the maximal seizure activity of which the nervous system is capable, and its duration is taken as a measure of the spread or reverberation of impulses involved in convulsions. The duration of tonic flexion is proportional to the intensity of the inhibitory state which must be overcome before appearance of the tonic extension. Thus, the longer the tonic extension and/or the shorter the tonic flexion the greater the convulsability.

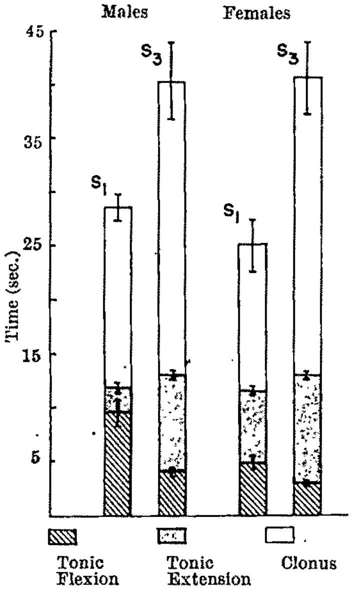

Fig. 1. Maximal electroshock seizure (MES) patterns in male and female maze-bright (S_1) and maze-dull (S_3) rats following a shock of 150 m.amp. for 0·2 sec. Eighteen rats were used in each of the four sub-groups, for a total of 72 rats; all were about 125 days old. Vertical bracketed lines show standard errors of the means for durations of each phase

The MES patterns for the different groups are given in Fig. 1. Again strain and sex differences were observed. 72 per cent of the S_1 males and 8 per cent of the S_1 females failed to exhibit tonic extension, whereas all the S_3 rats showed this phase. Both S_1 males and females showed significantly shorter total tonic and tonic extensor phases and significantly longer tonic flexor phase than the S_3. Duration of clonus was shorter in the S_1 strain than in the S_3.

Thus the maze-bright animals have a seizure pattern which suggests a lesser reverberation of impulses in the brain as compared with the maze-dull, although excitation occurs more readily in the maze-bright. This interpretation is in accord with conclusions[4] derived from investigations of behaviour of the S_1 and S_3 strains.

In both strains the males have a significantly shorter extension and longer flexion than the females. This sex difference can be ascribed to œstradiol, which has been shown[1] to lengthen tonic extension and shorten tonic flexion. Both the longer tonic extensor phase and the lower EST of the females support the view that they have a greater convulsability than males.

In conclusion, it seems to us that female rats have a greater convulsability than males, and both male and female rats of a maze-bright strain have lower seizure thresholds and shorter extensor phases during maximal seizures than maze-dull rats. It is suggested that these sex and strain differences are due to differences in hormonal balance (sex steroids) and brain chemistry (acetylcholine–cholinesterase levels).

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* U.S. Public Health Service predoctoral fellow.

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EFFECT OF *STAPHYLOCOCCI* ON LEUCOCYTE PYRIDINE NUCLEOTIDES

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THE presence of a normally inactive diphosphopyridine nucleotide (DPN) pyrophosphatase in *Staphylococcus aureus* has been shown[1] recently. The presence of this enzyme can be demonstrated in whole organisms or in sonic extracts only after certain manipulations such as heat treatment. Because in some circumstances *Staphylococci* may survive in leucocytes after phagocytosis[2], the possibility was entertained that intraleucocytic activation of this staphylococcal enzyme might decrease the pyridine nucleotide-level of the white cell sufficiently to interfere with its metabolism and with its eventual killing of the engulfed micro-organisms.

Changes in glycolysis, and in the monophosphate shunt, as well as in the electron transport system (all involving some pyridine nucleotide-linked reactions) have been reported as occurring in association with phagocytosis. Specifically, studies employing inert polystyrene particles as the foreign material have shown, during the phagocytic event, rather striking metabolic changes, such as increased oxygen uptake, increased lactate production, and increased activity of the monophosphate shunt as evidenced by an increased appearance of C-1 of glucose as carbon dioxide relative to C-6 of glucose[3]. The importance of glycolysis in the successful phagocytosis of heat-killed yeast cells by guinea pig polymorphonuclear leucocytes was further shown in the work of Fisher and Ginsberg[4]. They demonstrated reduced phagocytosis by polymorphonuclear leucocytes which had previously been allowed to react with influenza virus. However, their evidence suggested that the influenza-induced block in metabolism that resulted in decreased phagocytosis was probably at the glucose-6-phosphate to fructose-6-phosphate step.

Studies of oxygen uptake by leucocytes that had phagocytosed heat-killed *Staphylococci* have shown an increased uptake with a coagulase-negative strain of *Staphylococcus epidermidis* and decreased uptake by a coagulase-positive strain of *Staphylococcus*[5]. Similar investigations with viable coagulase-positive *Staphylococci* by the same workers have shown increased uptake of oxygen with one strain and decreased uptake with a second strain. This work, particularly the decreased uptake of oxygen by white cells which had phagocytosed heat-killed coagulase-positive *Staphylococci*, suggested the possibility that the 'heat-activated' DPN pyrophosphatase of the micro-organism might have been active within the leucocyte, since DPN has a well-established role in electron transport. To study possible evidence for activation within the white cell of the normally inhibited DPN pyrophosphatase of *Staphylococcus aureus*, direct measurements of leucocytic pyridine nucleotides following interaction with various types of bacteria were undertaken.

In the work to be described, the polymorphonuclear leucocytes were prepared from exudates produced by the intraperitoneal injection of a sterile 12 per cent solution of neutralized sodium caseinate. The method of collection with heparinized saline, centrifugation, and washing was essentially that described by Sbarra and Karnovsky[5]. Cells were suspended in a Krebs-Ringer phosphate medium buffered at *p*H 7·4. Cells were enumerated in an improved Neubauer chamber, and there was regularly less than 1 per cent contamination with red blood cells. About 85 per cent of the white blood cells were polymorphonuclear leucocytes. The bacteria used were 16-hr. cultures grown with aeration in 2 per cent trypticase broth, except the *Streptococci*, which were grown under relatively anaerobic conditions in the medium of Bernheimer[6]. The number of viable organisms was established by colony counts. The bacteria were then centrifuged and washed twice in 0·9 per cent potassium chloride. Afterwards, they were thoroughly dispersed and suspended in either 0·9 per cent potassium chloride solution or in Krebs-Ringer phosphate buffer adjusted to *p*H 7·4. Incubation mixtures consisted of approximately 4 × 10^8 white blood cells, fresh guinea pig serum in a final concentration of 20 per cent, glucose (28 μmoles), and *Staphylococci* at average multiplicities of 2, 5, 10, 25, or 50 per white blood cell, all in a final volume of 6 ml. The mixtures, in polyethylene tubes, were shaken at 90 cycles/min. in 37° C. water-bath. In later experiments, incubation mixtures of 3 ml. volumes in small silicone-treated Erlenmeyer flasks were shaken under the same conditions. All experiments were carried out aerobically for 40 min. At the conclusion of the experiment the contents of the flasks were chilled, transferred to polyethylene tubes, and centrifuged in the cold at 11,000 *g*. The sedimented cells were then extracted with 5 per cent trichloroacetic acid and analysed for DPN by the methyl ethyl ketone fluorescence method[7]. For the estimation of the reduced form of DPN and TPN, hot 0·1 *N* sodium carbonate extracts were prepared[7].

The DPN content of freshly isolated guinea pig leucocytes was found to be 7·82 ± 0·38 μgm./10^8 white cells (20 determinations). The value for total pyridine nucleotides, that is, including the oxidized and reduced forms of both TPN and DPN, was 14·56 μgm./10^8 white cells. A somewhat lower value (9·22 μgm./10^8 white blood cells) for total pyridine nucleotides of human leucocytes has previously been reported[8].

Within three minutes of interaction with an average multiplicity of fifty viable *Staphylococci* (var. *aureus*, strain No. 241) per leucocyte, the white cell DPN-level began to decrease and continued to decrease over the first 15 min. of incubation (Fig. 1). After this time, for as long as 1 hr. the levels remained stationary. At this same average multiplicity even more striking decreases in the leucocyte DPN-level were produced when older cultures of *Staphylococci* were used. As can be seen in Fig. 2, cells well along in the stationary phase of growth (14–36 hr.) were more effective in lowering the DPN-levels than cells collected in the logarithmic phase of growth. That this enhancement of loss of DPN was not due to an increase in activity of the 'heat-activated' DPN pyrophosphatase of the organism can also be seen in Fig. 2. There was no significant variation in the specific activity of sonic extracts of *Staphylococcus aureus* No. 241 in cells harvested between ten and thirty-six hours of growth. Similar decreases in leucocyte DPNH and TPNH were observed in comparable experiments. The normal level of TPN in the cells was so low as to make measurements of change in its level subject to question.

In order to ascertain whether the decrease of DPN in the white cells was due to intracellular destruction or 'leakage' from the white cells, attempts were made to determine if DPN could be recovered from the medium after 40 min. of interaction of *Staphylococci* with leucocytes. None was recovered following lyophilization of the medium under conditions in which 5 μgm. of added DPN could readily be detected. However, inasmuch as intact guinea pig leucocytes have a potent DPNase which rapidly cleaves exogenous DPN, this experiment has not ruled out the possibility of DPN leakage and subsequent cleavage.

To evaluate further any possible role of the 'heat-activated' DPN pyrophosphatase of *Staphylococcus aureus* in the decrease in pyridine nucleotides of white blood cells, experiments were carried out with boiled *Staphylococci* (pyrophosphatase fully activated) at an average multiplicity of 50 per leucocyte. The decrease with boiled *Staphylococci* proved to be only about 60 per cent of that obtained with unboiled organisms. Although the original

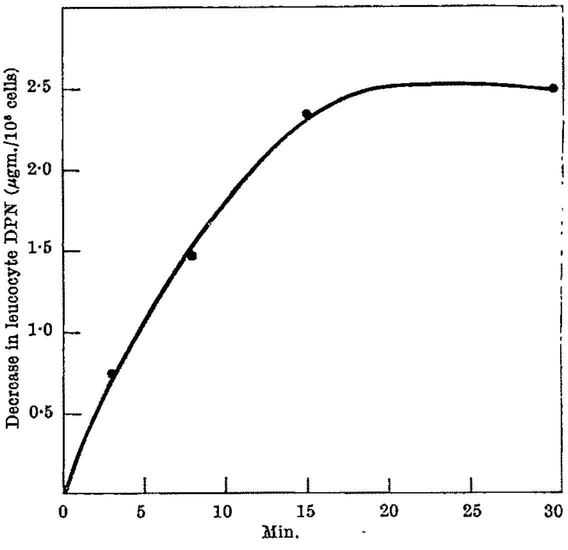

Fig. 1. Time-course of loss of DPN in white cells exposed to *Staphylococcus aureus* strain No. 241, at an average multiplicity of 50 *Staphylococci* per leucocyte. Time is in minutes after the addition of *Staphylococci* to white cell suspension in Krebs-Ringer phosphate solution containing 20 per cent fresh guinea pig serum and 28 μ mol. of glucose in a final volume of 6 ml.

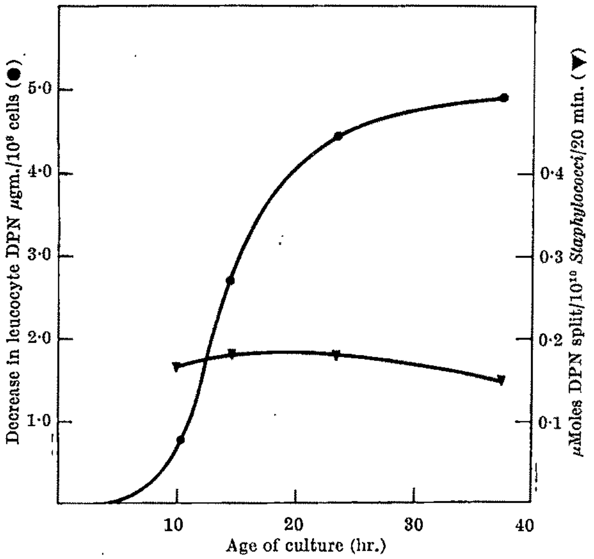

Fig. 2. Effect of age of *Staphylococci* (strain No. 241) in producing DPN decrease in white blood cells (average multiplicity of fifty). ● decrease in leucocyte DPN-levels induced by *Staphylococci* grown for varying number of hours ; ▼, specific activity of 'heat-activated' DPN pyrophosphatase obtained from *Staphylococci* grown for varying number of hours

observations were made at high multiplicities (50), significant effects were noted with boiled and unboiled organisms at multiplicities of 10 and 25 (Table 1). It became possible to demonstrate decreases in leucocyte DPN-levels with multiplicities as low as 2 or 5 when incubation was carried out in siliconized flasks in which mixing was more efficient than in polyethylene tubes. Under these conditions, about 75 per cent of the white cells contained *Staphylococci* on stained smear.

Of five strains of coagulase-positive *Staphylococcus aureus*, all possessing the 'heat-activated' DPN pyrophosphatase, four produced distinct drops in the DPN content of leucocytes, but one strain (No. 754) did not (Table 2). One strain of *Staphylococcus albus* possessing the enzyme produced a decrease in DPN content, whereas two other strains lacking the enzyme failed to do so. The number of strains tested is too small as yet to draw any general conclusions.

Table 1. EFFECT OF NUMBER OF *Staphylococci* AND METHOD OF INCUBATION ON LEUCOCYTE DPN-LEVELS

	Per cent decrease in white blood cell DPN-levels		
Average multiplicity	Unboiled*	Boiled†	Improved shaking (unboiled)‡
2	—	—	8·5
5	0	—	8
10	11	8	20
25	16	20	30
50	42	28	44·5

* *Staphylococci* not heated ; shaken in polyethylene tubes.

† *Staphylococci* heated at 100° C. for 1 min. ; shaken in polyethylene tubes.

‡ *Staphylococci* not heated ; shaken in siliconized Erlenmeyer flasks.

Table 2. EFFECT OF VARIOUS BACTERIA* ON LEUCOCYTE DPN-LEVELS

Organism	'Heat-activated' DPN pyrophosphatase†	Per cent decrease in leucocyte DPN-level
Staph. aureus No. 241	+	30
,, ,, No. 9196	+	24
,, ,, No. 754	+	0
,, ,, No. 9894	+	40
,, ,, No. 193	+	10
Staph. albus No. 410	−	0
,, ,, No. 331	−	0
,, ,, No. 32	+	24
	Any type of DPN-cleaving enzyme†	Per cent decrease in leucocyte DPN-level
E. coli Strain *B*	−	0
,, ,, Strain No. 93	−	0
,, ,, Strain *X*	−	0
B. subtilis Strain 'MGH'	not tested	0
,, ,, Strain 'Brandeis'	+	0
Proteus vulgaris (ATCC No. 9484)	+	0
Beta hæmolytic *Streptococcus* No. 29	+	57
,, ,, ,, No. 30	+	76

* Average multiplicity of fifty bacteria per leucocyte.

† +, present strain ; −, absent in strain.

The loss of pyridine nucleotides on interaction with *Staphylococci* is not a non-specific one resulting from interaction of any bacterium with leucocytes, as can be seen in Table 2. Three different strains of *Escherichia coli* did not lower the DPN-level of leucocytes. These organisms had no DPNase or DPN pyrophosphatase. Two strains of *B. subtilis* similarly had no effect, one of these, strain 'Brandeis' (kindly supplied by Prof. N. O. Kaplan), having a 'heat-activated' DPNase. *Proteus vulgaris* (ATCC No. 9484) had a DPN pyrophosphatase which was not 'heat-activated', but it failed to cause a drop in DPN-levels. Both strains of group A *Streptococci* tested produced profound drops in white blood cell DPN-levels. It is of interest to note that strain No. 30 that caused the greater decrease did not produce the extracellular enzyme DPNase, whereas strain No. 29 produced large amounts of this enzyme. Thus, it would appear that the presence of a DPN-cleaving enzyme in a micro-organism is not necessarily correlated with its ability to induce DPN loss in leucocytes. Bernheimer *et al.* have shown a rather striking correlation between DPNase production and leucotoxicity of streptococcal strains. However, they were unable to demonstrate injury to the leucocytes by a purified preparation of the extracellular DPNase. However, this did not eliminate the possibility that DPNase might be responsible for the leucotoxicity, since it was not known whether the enzyme had actually penetrated into the leucocytes.

The significance of the loss of DPN in white blood cells initiated by *Staphylococci* and its possible relation to the virulence of the organism remains to be elucidated. Work on the survival of intracellular *Staphylococci* in such depleted white blood cells as well as further work on uptake of oxygen, etc., should help to elucidate the significance of this biochemical lesion.

This investigation was supported (in part) by a U.S. Public Health Service research grant (*E*–1884) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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LETTERS TO THE EDITORS

RADIOASTRONOMY

A New Determination of the Solar Parallax by Means of Radar Echoes from Venus

During the recent close approach of Venus the Jodrell Bank radio telescope has been used to obtain radar echoes from the planet. The radar equipment, which operated at a frequency of 408 Mc./s., had a peak-power of 60 kW. and gave 30-m.sec. pulses at one pulse per second. The transmitter and receiver were operated alternately for periods of approximately five minutes, the light-time to Venus and back. To eliminate fading caused by Faraday rotation of the line of polarization of the signals in the Earth's ionosphere, an aerial system transmitting right circular polarization and receiving left circular was used. This was achieved by means of a feed aerial consisting of crossed coplanar dipoles and reflectors fed in quadrature from a hybrid ring, which also provided 40 db. of isolation between the receiver and transmitter. An electron-beam parametric amplifier (Adler tube) with a noise figure of 2 db. formed the input to the receiver.

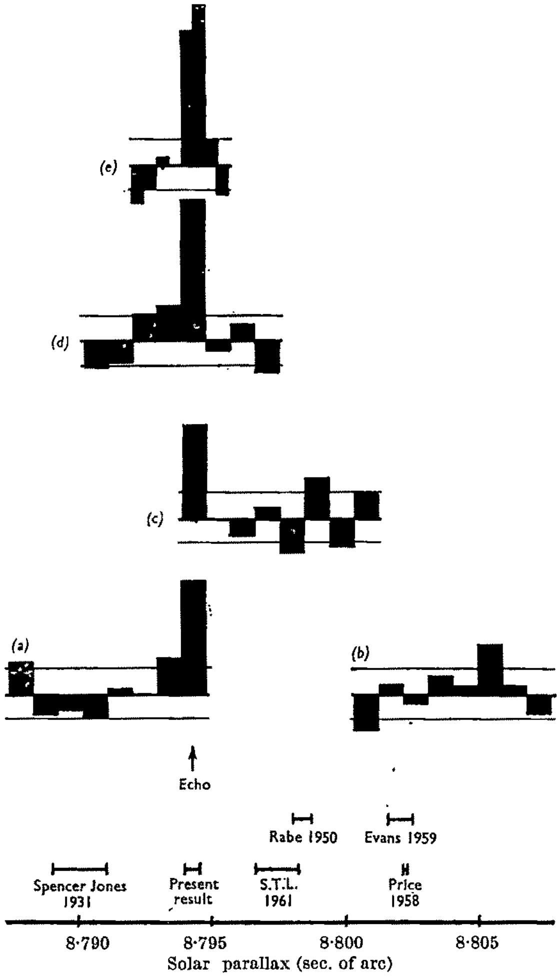

Fig. 1. Some recent determinations of the solar parallax are shown in relation to the present result. At (*a*), (*b*), (*c*) and (*d*) are shown histograms representing deviations from the mean of the eight integrator channels after twenty 5-min. periods of integration. The fine lines in each case represent one standard deviation. The range of Venus assumed on each occasion corresponded to the value of the solar parallax below the centre of each histogram; the echo always stayed in the channel corresponding to the present value. At (*e*) is shown the effect of halving the integrator channel width: the echo now divides equally between the two channels, confirming its central position

As the signal to noise ratio was expected to be less than unity a real-time integrator was used to detect the echo. The output of the final intermediate frequency amplifier, which had a band-width of 67 c./s., was applied to a linear detector; a variable frequency pulse output was then produced, linearly dependent in frequency on the detected voltage. The integrator sampled this pulse output at eight adjacent positions on the time base, each sampling period being 30 m.sec. The outputs from corresponding sampling periods on successive sweeps were counted in eight separate counting channels. Thus if only noise is present the counts in the eight channels will show random fluctuations about a mean, but if an echo is present the count in one channel will show a significant rise from the mean.

During the observations the range of Venus was changing by as much as a sampling period within a few minutes, so an automatic system was employed to alter the position of the sampling channels to keep the echo always within one channel. This system was accurate to within ±2 m.sec. The Doppler shift of the returned signals was changing by an amount equal to the receiver band-width every few minutes, due to the rate of change of the relative line of sight velocity, and therefore the receiver was tuned manually to keep it within ±3 c./s. of the predicted frequency. The whole spread of likely values of range was covered by three different settings of the sampling channels. Echoes were first detected on April 8, as soon as the equipment was operating correctly, and continued to be detected until observations ceased on April 25.

Fig. 1 shows some of the results, together with a chart of some other recent determinations of the astronomical unit, the values corresponding to the possible ranges of Venus explored. Each of the sections *a*, *b*, *c* and *d* shows the result of integrating for 20 receive periods assuming the value of the astronomical unit below the centre of the integration channel system. The lines on each side of the means represent one standard deviation. Also shown at *e* is the result of halving the sampling period in order to confirm the position of the echo more precisely; it will be seen that the echo is now divided equally to within a standard deviation between channels 3 and 4. The present result lies about half-way between the values of Rabe[1] and Spencer Jones[2], and is significantly lower than the recent Space Technology Laboratories[3] value.

Our result is:

Light-time for one A.U. = 499·011 ± 0·017 sec.
or
Solar parallax = 8·7943 ± 0·0003 sec. of arc

The computations of azimuth, elevation, range and Doppler shift needed for this work were made on the *Mercury* Computer in the University of Manchester. They were based on the *Astronomical Ephemeris* and the following values of constants were assumed:

Velocity of light	299,792·5 km./sec.
Equatorial radius of Earth	6,378·388 km.
Jodrell Bank radius of Earth	6,365 km.
Radius of Venus (ref. 4)	6,200 km.

The most likely cause of systematic error in the result is uncertainty as to the distance of the reflecting surface from the centre of Venus; it has been assumed that this is the same as the planet's radius. However, if the effective reflecting surface were closer to the centre by 7 per cent it would only make an increase of one part in 10^5 in the above value for the parallax, so that such an effect would be small compared with the limits of error.

A previous attempt to determine the solar parallax was made at Jodrell Bank during the close approach of Venus in September 1959[5]. After 350 receive periods—equivalent to 58¾ hr. of integration—a value of the solar parallax of 8·8020 sec. of arc was determined on the basis of an echo of 2½ standard deviations. This agreed with the Millstone Hill value obtained in 1958[6], but there was an 8 per cent chance that noise alone produced the rise in the counter channel which would appear to be a probable explanation of these earlier results.

A fuller account of this work, together with a discussion of the radio albedo of Venus, will be reported elsewhere when the tape recordings made during the observations have been analysed.

We wish to thank Sir Bernard Lovell for his interest and encouragement. Our technical assistant, A. C. Prior, designed and constructed the automatic ranging circuits and gave valuable assistance at all stages of the programme. This work was supported in part by United States Air Force Contract No. *AF*61 (052)–172.

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ASTROPHYSICS

Observations of Satellite 1960 ζ1, and a Comparison with Satellite 1958 δ1

Since July, 1960, I have made observations of satellite 1960 ζ1 (*Midas* 2), as an exercise in prediction and visual tracking, and to study the damping of its spin. The following data and comments may be of interest to those engaged in precise analysis.

This satellite has given characteristic flashes, which are sharply defined when the satellite is between the Sun and the observer. This is in keeping with the now familiar experience that in 'crescent' illumination, the intensity of sunlight reflected from a satellite is most sensitive to change of profile, and superimposed specular reflexions. *Midas* 2 is normally a magnitude +3 object at close range, with infrequent flashes to magnitude +1; while at long range it is very much fainter.

Table 1 contains the data from my observations at this location, 34° 43′ S., 138° 42′ E. Timing the flashes by a simple stop-watch and binocular technique, the interval T containing N flash cycles can be determined usually to within 1 sec. The two observations made at long range, and therefore smaller angular velocity of transit, suggest a slight reduction of the observed flash period. It may be tentatively assumed that the cylinder is rotating in a direct sense about a transverse axis.

Table 1. Flash Period of Satellite 1960 ζ1, observed from Elizabeth, South Australia

Time of observation		Interval T (sec. ± 1)	No. of flash cycles, N	Mean flash period (sec. ± $1/N$)	Approximate orientation
S.A. time	G.M.T.				
1960					
July 18.781	18.385	27·0	5	5·40	65° N.N.E.
Oct. 21.826	21.430	31·6	5	6·32	60° N.N.E.
Oct. 22.808	22.412	38·7	6	6·45	60° N.
Nov. 23.128	22.732	33·8	5	6·75	50° N.N.E.
Dec. 7.868	7.472	13·8	2	6·9	60° N.N.E.
Dec. 8.851	8.455	34·5	5	6·90	60° N.N.E.
Dec. 9.833	9.437	96·2	14	6·87	55° N.E.→E.
1961					
Jan. 9.157	8.761	22·0	3	7·3	55° N.E.
Jan. 10.140	9.744	36·0	5	7·20	55° N.N.E.
		43·0	6	7·17	45° N.E.→E.

If the 'tumbling' period ($\frac{1}{2}P$) can be stated closely by the relation: $\frac{1}{2}P = T/(N + \theta/180)$, the deviation of the observed flash period from the true tumbling period is about +0·2 sec. in transits at 1°/sec. In the above equation, θ is the angle of movement of the line of sight during an observation.

The rotation period P has increased at a constant rate of 0·020 sec./day, from 10·5 sec. on July 18, 1960, to 14·0 sec. on January 8, 1961. During this interval, the orbital period has decreased only from 94·40 to 94·32 min.

It is of particular interest to compare the rate of decrease of angular momentum of spin for 1960 ζ1, with that for 1958 δ1 (*Sputnik* 3 rocket). Relevant details are set out in Table 2. The critical comparisons are between the respective values of the retarding couple $d(I\omega)/dt$ and the factors considered likely to determine these, principally the air density at perigee, and the length and profile area of each satellite.

Table 2. Factors relating to the Rotation of Satellites 1960 ζ1 and 1958 δ1

Physical data assumed	1960 ζ1	1958 δ1
Length, L (cm.) Diameter, D (cm.) Mass, M (gm.)	670 150 $2 \cdot 27 \times 10^6$	1,800 250 $4 \cdot 5 \times 10^6$
Moment of inertia (gm.-cm.^{2}) $I = M(D^2/16 + L^2/12)$	$8 \cdot 8 \times 10^{10}$	150×10^{10}
Interval considered	18/7/60 – 8/1/61	17/8/58 – 27/10/58
Angular velocity ω (rad./sec.) initial final	 0·60 0·45	 0·349 0·334
Angular momentum $I\omega$ (gm.-cm.^{2}/sec.) initial final extrapolated to $\omega = 0 \cdot 35$	 $5 \cdot 28 \times 10^{10}$ $3 \cdot 96 \times 10^{10}$ $3 \cdot 08 \times 10^{10}$	 $52 \cdot 35 \times 10^{10}$ $50 \cdot 10 \times 10^{10}$ —
Fraction of time spent near perigee, f	1·0	0·1
$d(I\omega)/dt$ (dyne-cm.) initial final extrapolated to $\omega = 0 \cdot 35$	 1,200 650 400	37,000 at $f = 0 \cdot 1$ 3,700 at $f = 1 \cdot 0$
Mean air density at perigee (gm.-cm.$^{-3}$)	$2 \cdot 6 \times 10^{-15}$	$2 \cdot 5 \times 10^{-13}$

The dimensions of 1958 δ1 are not accurately known. Notni and Oleak[1] derive from data by King-Hele[2] the relation $\rho DL/M = 2 \cdot 86 \times 10^{-14}$ cm.$^{-1}$, where ρ is the air density at perigee (approximately 3×10^{-13} gm.cm.$^{-3}$). The rocket may have been heavier than the *Atlas* satellite (1958ζ), so M may have been as high as 5,000 kgm. From the observed brightness and large fluctuations of brightness, it is reasonable to expect $L \sim 20$ m., with $L \gg D$, while consideration of mechanical design suggests $L < 10D$. Assuming that the rocket body tapered to the base of the instrumented cone

(1958 $\delta 2$), (diameter 170 cm.), the data used in Table 2 are: $D=250$ cm., $L=1{,}800$ cm., $M=4\cdot 5\times 10^5$ gm.

The retarding couple acting on 1958 $\delta 1$, concentrated in the transits through perigee ($f=0\cdot 1$), is of the order of 100 times greater than that acting on 1960 $\zeta 1$ at comparable rate of spin. This would explain satisfactorily the relative changes in terms of air friction, if the two satellites were known to be similar in weight and size. However, because 1958 $\delta 1$ was of such lower density and greater length, it is possible that the couple on it was much too small to permit this explanation, unless gross errors are present in the physical data assumed in Table 2.

If damping of satellite spin by the Earth's magnetic field is the controlling factor, even for the large empty rocket at a height of 220 km., the couple on 1958 $\delta 1$, averaged over the orbit ($f=1\cdot 0$), would be only about 10 times greater than that on 1960 $\zeta 1$. It is this possibility which I offer for critical analysis, in the light of the recent discussion[3] of the rotation of 1958 $\delta 1$.

The relative values of $d(I\omega)/dt$ would be even closer if either the weight or the moment of inertia of 1958 $\delta 1$ were appreciably less than assumed in Table 2.

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PHYSICS

Thermal Shock Behaviour of Brittle Materials

WE have been investigating the thermal shock behaviour of some glasses and other materials, using an argon plasma jet, as a preliminary part of an investigation into the thermal shock behaviour of brittle materials. The plasma jet was used in this investigation since it affords an easy and reliable method of producing very high heat transfer-rates of the order of 400–500 cal./cm.^{2}sec. In the present experiments the samples, in the form of disks $\frac{1}{2}$ in. thick and $\frac{7}{8}$ in. diameter, could be shocked every 2 sec. for up to 1,000 times, the shock itself lasting about $0\cdot 16$ sec. Under these conditions it was possible to obtain surface melting on such materials as soda glass, borosilicate glass and an electrical porcelain.

It was found, as a general rule, that where no surface cracking occurred during a single shock no cracking would be produced at all however many times the shock was repeated up to the maximum tried of 1,000 shocks. At low heat loadings, however, often nothing would be apparent on visual examination but cracks would appear later, sometimes weeks later, which were of a different form from those produced during a single shock. For example, with soda glass no cracks were observed by eye at heat loadings of 75 cal./cm.^{2}sec. until several days after the experiment, when patterns of the type in Fig. 1 or Fig. 2 were produced. Fig. 1 indicates a shearing of the heated glass from the remainder and Fig. 2 shows cracking, in tension, of the surface during cooling. At higher heat loadings cracking occurred immediately (Fig. 3). Fig. 3 shows a series of shallow cracks generally radiating out from the central hot zone whereas Figs. 1 and 2 show only one or a few deep cracks. The surface completely disintegrated at the highest heat loading tried, 400 cal./cm.^{2}sec., into a fused cracked mass, but again none of the cracks extended into the body of the disk.

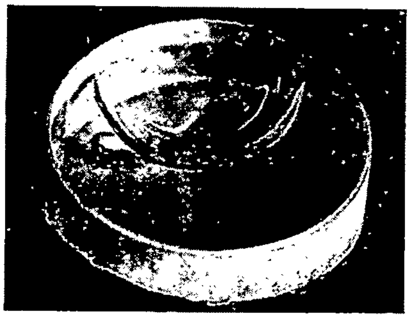

Fig. 1. Soda glass after 500 shocks at 75 cal./cm.^{2}sec. Sheer crack during cooling

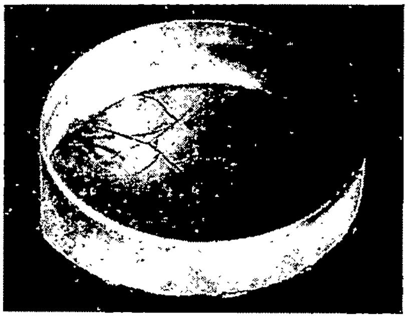

Fig. 2. Soda glass after 500 shocks at 75 cal./cm.^{2}sec. Tensile cracking during cooling

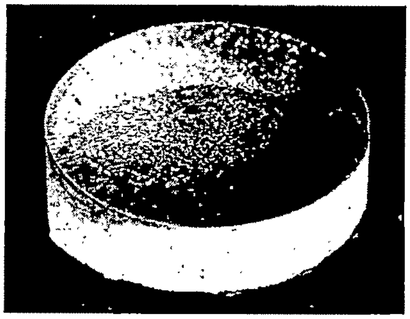

Fig. 3. Soda glass after 1 shock at 400 cal./cm.^{2}sec.

The results obtained with a borosilicate glass were similar to those observed with soda glass, except that a greater heat loading was required to produce similar effects. No effects were observed at all with 75 cal./cm.^{2}sec. but at 150 cal./cm.^{2}sec. cracking of the types shown in Figs. 1 and 2 was found. At the higher heat loadings surface cracking and melting occurred. No surface cracks could be produced on silica with heat loadings in the range 75–400 cal./cm.^{2}sec. but it was found that, on leaving for several months, the two samples which had received 100 shocks at 400 cal./cm.^{2}sec. were cracked, one as in Fig. 1 and the other as in Fig. 2.

Electrical porcelain behaved similarly to the borosilicate glass. Typical samples are shown in Figs. 4 and 5. One unusual feature of these results was that the electrical porcelain often cracked into two halves, the crack following the path of the jet across the surface, whoreas the only glass to crack bodily was the

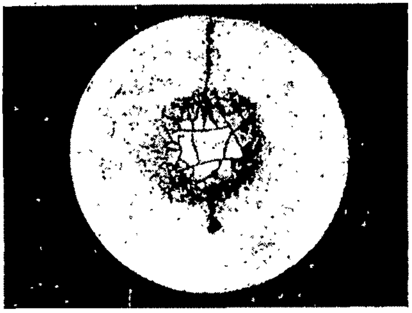

Fig. 4. Electrical porcelain after 1,000 shocks at 150 cal./cm.²sec. Note major cleavage crack in path of jet across surface

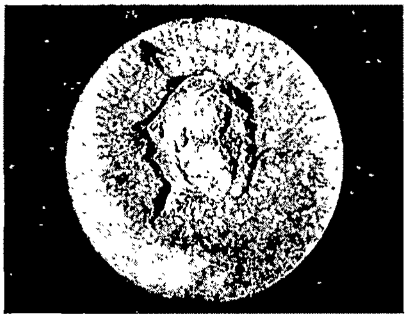

Fig. 5. Electrical porcelain after 10 shocks at 400 cal./cm.²sec.

soda glass and that did so normal to the path of the jet.

This work forms the initial stage of a fundamental study of the thermal shock behaviour of ceramic materials. I wish to thank Mr. Dugdale of the Metallurgy Division, United Kingdom Atomic Energy Authority, Harwell, for his advice and help during the earlier part of this investigation.

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The Parametric Line: a Photon and Gravitational Field Analogue

Let E and v be the energy and velocity of a pulse in a 'parametric line'[1], denoting the input and output ends by the subscripts 1 and 2 respectively. It has been shown[1] that the pulse energy amplification factor has the form:

$$E_2/E_1 = v_2/v_1 \qquad (1)$$

If this factor does not differ very much from unity, it can be written in the form:

$$E_2/E_1 = [\{(L/T)(v_2 - v_1)\}/\{\tfrac{1}{2}(v_1 + v_2)\}^2] + 1 \qquad (2)$$

where L is the length of the line, T is the time necessary for traversing the line and $\frac{1}{2}(v_1 + v_2)$ is the average speed of the pulse along it.

According to the General Theory of Relativity, an electromagnetic wave packet running over a length l in the direction of a gravitational field of intensity g (units of acceleration) gains frequency and hence energy[2]. The initial and final energies (ε_1 and ε_2) are related according to:

$$\varepsilon_2/\varepsilon_1 = 1 + \{gl/c^2\} \qquad (3)$$

where c is the velocity of light.

An analogy between equations (2) and (3) is established by substituting c for $\frac{1}{2}(v_1 + v_2)$, g for $(v_2 - v_1)/T$, l for L and ε for E. It will be noted that in each case the substitutions have the same dimensions.

Another analogy can be derived by considering the frequency spectrum of the pulse. Denoting the spread of the frequency by f and the duration of the pulse by τ, it follows from Fourier's theorem that:

$$\tau_1 f_1 = \tau_2 f_2 \simeq 1 \qquad (4)$$

It has been shown that in a parametric line (ref. 1):

$$\tau_1/\tau_2 = v_1/v_2 \qquad (5)$$

It follows from (4), (5) and (1) that:

$$E_1/f_1 = E_2/f_2$$

This equation resembles the energy frequency (ε, ν) relation

$$\varepsilon/\nu = h$$

where h is Planck's constant.

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[1] Physical Society Exhibition (demonstration 1960), further details to be published (*Phys. Soc. Handbook 1960*, p. 104).

[2] Wilson, H. A., *Modern Physics*, 6, 332 (Blackie and Sons). Equation (3) may be derived by considering a small distance dr in which the field is considered uniform and by substituting ordinary units for the special units used. dt'/dt is taken to be equal to $v_2/v_1 = \varepsilon_2/\varepsilon_1$. See also *Electronic Technology*, 39, No. 7, 267.

Standard Unit of Pressure in Vacuum Physics

It is regrettable that spokesmen for the Bureau International des Poids et Mesures[1] and the Standards Division of the National Physical Laboratory[2] have failed to understand my recent communication[3].

Previous work on standardization has unfortunately resulted in the use of two units in the vacuum field, not related decimally to each other: millibar and torr, the latter being not equal to mm. of mercury[4]. My endeavour was to propose a practical solution to this problem much discussed by vacuum physicists and technologists in recent years. An attempt, therefore, was made to define a new unit which, being decimally related to both mm. mercury and C.G.S. (and M.K.S.) unit of pressure, could be considered as the standard unit of pressure in vacuum physics. The unit proposed, which was conveniently termed 'vac', should not be confused with millibar, as was done by the commentators mentioned above, the millibar invariably being nothing else than a multiple of the absolute unit of pressure, while the vac is defined in terms of the height of the mercury column under specified conditions. The new unit does not bring great implications in practical use. It represents an acceptable compromise to satisfy the international systems of units, and to avoid the replacement of mm. mercury by an arbitrary unit, like the torr.

I may mention that a detailed discussion of this matter has been reported elsewhere[5].

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[1] Volet, Ch., *Nature*, 188, 1017 (1960).

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In order to obtain a unit of pressure in terms of the height of a column in a liquid manometer, a unit which is also related decimally to absolute units of pressure, Dr. Florescu proposes to elevate the pressure due to a 0·75-mm. column of liquid of density 13·5951 gm./cm.^{3}, g being 980·7455 cm./sec.^{2}, to the status of a new unit, and call it the 'vac'. But as Dr. Florescu admitted originally, this pressure is effectively 10^3 dynes/cm.^{2}, called internationally the millibar, and for the practical purposes of vacuum technology the pressure due to ¾ mm. of a standard mercury column (density 13·5951 gm./cm.^{3}, standard gravity 980·665 cm./sec.^{2}) is one millibar (more closely it is 0·999 92 mb.). Quite apart, therefore, from Dr. Volet's point, with which I entirely agree, I see no justification for introducing a new unit, the 'vac', the definition of which involves a new, arbitrary reference gravity, when the scale it would provide would in vacuum practice be indistinguishable from a scale of millibars.

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METALLURGY

Anomalous Magnetostriction of the 'Grain-orientated' Silicon-Iron Sheet

This communication is concerned with the anomalous phenomenon occasionally found in examining the magnetostriction of the 'grain-orientated' silicon-iron sheets, and it was believed to be a prime cause which gives rise to a transformer noise. The method of measurement is the static one which has been adopted by us for a long time[1], and by this method the mean value of magnetostriction can be obtained. The experiments were carefully made with respect to a thin plate-like specimen.

The specimen contains 4·35 per cent silicon, and is used by a trade name of 'T-90' in Japan. The specimen is a rectangular plate, 0·37 mm. thick, 6 mm. wide and 10 cm. long, and the direction of length corresponds to that of measurement and rolling. Fig. 1 shows a result for the specimen annealed at 600° C. for 1 hr. after cutting down the specimen from a large sheet.

As shown in Fig. 1, the magnetostriction does not appear up to 100 c.g.s. and then increases gradually, showing a minimum just before saturation with the increasing intensity of magnetization, and lastly, increases abruptly to a saturation value. It will be due to the 180° wall displacements that the magnetostriction does not appear in the first step. Adequate interpretation of this phenomenon may be very difficult, but we reasoned as follows.

In general, in iron-base alloys, including the 'grain-oriented' iron-silicon sheets, the magnetostriction is positive in an easy direction, and is negative in a hard direction. Therefore, the magnetostriction of polycrystal specimen is the sum of both values mentioned above, so that it is ordinarily encountered that the magnetostriction curve is either parabolic or exponential.

Now, in the range of continuous rotation of domain in the magnetization process, the mechanism of rotation may be greatly affected not only by the intrinsic crystal magnetic anisotropy but also by the additive restraint force or uniaxial anisotropy induced by rolling.

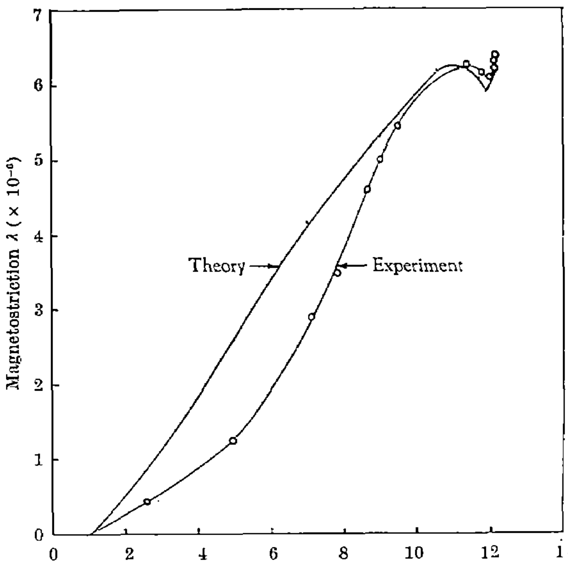

Fig. 1. Anomalous magnetostriction curves obtained experimentally and theoretically in the 'grain-orientated' silicon-iron sheet

Some amount of the restraint force mentioned above may coincide with a special direction near an easy one. If so, in this direction, the domain rotations may be restrained, hence the magnetostriction does not appear below a critical magnetic field the energy of which surpasses the restraint force.

In such a way, the anomalous curve shown in Fig. 1 may be explained. Next, the simple theoretical expression may be given according to Heisenberg's theory of magnetostriction in the unsaturated magnetization region[2].

Using the above results and the magnetostriction constants obtained by Shturkin[3], and assuming that the fractions of orientation <100>, <110> and <111> are 0·3, 0·6 and 0·1 respectively, the theoretical magnetostriction can be calculated as shown in Fig. 1. A good agreement exists between the theoretical and the experimental results.

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Formation of Silicon Nitride Whiskers on a Ferro-Silicon Alloy at Room Temperature

After approximately ten years of operation, a calcium carbide furnace at Odda Smelteverk A/S, Norway, was temporarily stopped. At the bottom of the furnace a phase containing a ferro-silicon alloy was found. A sample taken from this bottom phase was kept in contact with air at room temperature. After a few weeks, formation of greyish-brown whiskers on the surface of the sample was observed.

These whiskers were removed, but after an additional few weeks needle-shaped crystals were again formed. This process has been repeated up to now, about one and a half years after the sample was taken out of the furnace. At present, however, the crystal growth seems to have almost ceased.

Chemical analysis of the sample on which the crystals grew gave the following result (weight per cent): iron, 82·4; silicon, 14·5; carbon, 2·85; calcium, 0; nickel, manganese, cobalt, vanadium, copper, titanium—minor impurities.

A metallographic investigation showed clearly that this substance contained only one ferro-silicon phase, the α-phase. The carbon appeared as a separate graphite phase.

The crystals forming the whiskers were investigated by X-ray analysis. From the powder diagrams, *d*-spacings were calculated. They were in almost perfect agreement with the X-ray lines given by Popper and Ruddlesden[1] for α- and β-Si_3N_4. Both allotropic forms were present in the crystals investigated.

The results of a quantitative chemical analysis of the crystals correspond to the composition $Si_{3\cdot 04}N_4$.

Earlier investigations[2,3] describe cases where Si_3N_4 was formed during, for example, the nitriding process for case-hardening of steels containing small amounts of silicon. But in all cases the nitride was formed at temperatures not lower than 250° C.[4]

The results reported here, however, clearly demonstrate the formation of α- and β-Si_3N_4 on a lump of a ferro-silicon alloy even at room temperature. The kinetics of this process will be discussed elsewhere.

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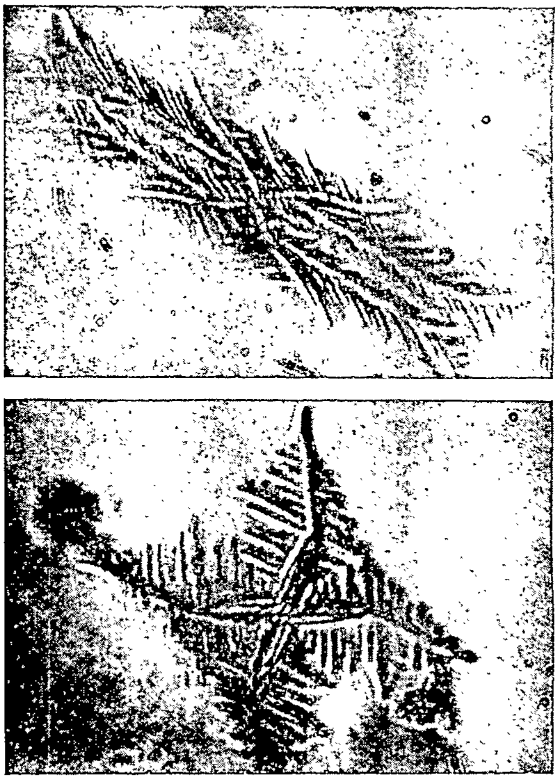
CRYSTALLOGRAPHY

Some Features of the Precipitation of Barium Chromate by an Extra-slow Technique

In the course of experiments concerning the preparation of very pure compounds, the precipitation of barium chromate was chosen as a model. S. Rykowski and I found[1] that a very slow precipitation with dilute solutions produces a precipitate which contains very minute crystals known as 'crystal skeletts'.

Continuing this work I have developed a technique of extremely slow precipitation, and I have observed some interesting features as regards the shapes of the crystals (see Fig. 1). A precipitate was obtained by addition of a dilute barium chloride solution to a large volume of potassium chromate solution and steady and vigorous mixing. After some hours minute crystals could be seen under the microscope (× 720–2,000). These crystals showed strikingly perfect symmetry, and some had regularly curved shapes. Besides the X-shaped crystals, very regular hexagonal plates and other more complicated forms appeared.


Figs. 1 and 2

When ammonium chromate instead of potassium chromate solution was used, crystals of similar shapes were formed. Addition of potassium chloride (150 gm./l.) to the solution of potassium chromate produced exclusively crystals of the hexagonal type.

When a mixture of barium nitrate and silver nitrate (10: 1) was added to the solution of potassium chromate X-shaped crystals were formed. These crystals consisted of two parts of different shape and colour. It is assumed that the larger one is formed of barium chromate (pale yellow) the other one being silver chromate (dark brown) (see Fig. 2).

Barium sulphate and barium phosphate precipitates also showed interesting shapes of similar character.

Details of this work, which is being continued, will be published elsewhere. I wish to thank Mr. L. Trojanowski for his assistance.

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Retention of Carbon Dioxide Bubbles on Calcite Etch-Peaks

The etch-patterns on cleavage faces of calcite which have been reported[1–3] bear some interesting similarities and dissimilarities to etch-patterns obtained in this Laboratory. Watts etched calcite for 30 sec. in 1 per cent nitric acid and obtained fivesided figures which he called pits. We immersed calcite in 0·001 N hydrochloric acid for one week and obtained essentially six-sided peaks (Fig. 1) with irregular valleys between them. The sixth side has a rougher surface than the other sides and it seldom extends to the apex of the peak. It may constitute an incompletely formed figure. Stanley used 10 per cent hydrochloric acid for 10–60 sec. and obtained figures which he describes as four-sided, although from his published photographs we detect a tendency of these figures toward the six-sided etch-patterns observed in our study. The Pandyas, using ammonium chloride, obtained parallelograms.

Another curious phenomenon was observed in this investigation. After the acid-carbonate reaction had proceeded long enough to etch the calcite surface, new carbon dioxide bubbles were nucleated on peaks of the etch-figures and remained there during their periods of growth (Fig. 2). A bubble would remain on a peak for a few seconds to many minutes, depending on the rapidity of the reaction. Escape of the bubble was followed quickly by the appearance of a new one on the same site. When such bubbles are viewed in silhouette, a gaseous stem is seen holding the bubble to the solid surface (Fig. 3). The end attached to the calcite remains stationary while the bubble is wafted to and fro by gentle currents in the acid solution. The remarkable 'contact angle' contrasts sharply with the attitude of carbon dioxide bubbles which form on uncleaned cleavage surfaces promptly after immersion in the dilute hydrochloric acid (Fig. 4).

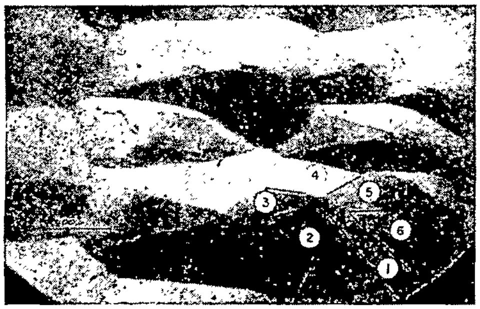

Fig. 1. Etch figures on calcite, magnification, × 87 ; illumination from top of page

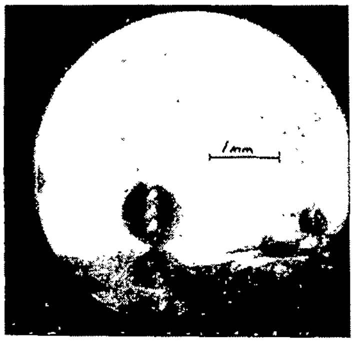

Fig. 2. Bubbles formed after etching, magnification, × 9

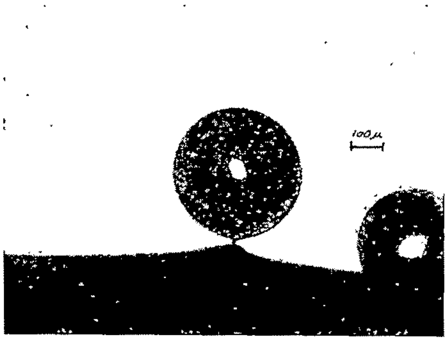

Fig. 3. Silhouette view of bubble on etch-peak, magnification, × 40

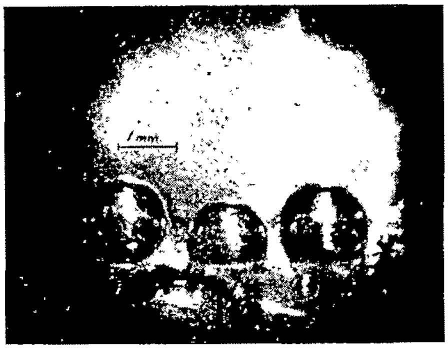

Fig. 4. Bubbles formed upon first contact of acid with calcite, magnification, × 7·5

The etch-peaks in Fig. 1 show a pinched appearance. It is suggested that this added erosion occurs during the up-sweep of comparatively strong acid that follows the escape of a bubble from the peak.

We thank Socony Mobil Oil Company, Inc., for sponsoring this work.

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GEOLOGY

Stilpnomelane in North Wales

THE occurrence of stilpnomelane in Great Britain was first recorded by Hallimond[1]. He described the mineral from the Pen-y-rallt mine in Merionethshire, where it occurs in a pisolitic ironstone bed at the contact with "a mass of igneous rock". The mineral occurs in association with chalybite in roughly parallel asbestiform veins up to one inch wide.

Present investigations in the region between Arthog and Dolgelley, Merionethshire, have shown that stilpnomelane is very common in certain basic marginal phases of the post Lower Llanvirn Cregennen granophyre sheet and in the associated 'markfieldites'. The mineral also occurs in some vitric (? welded) acid tuffs of the Lower Llanvirn Bryn Brith Series[2].

The analysed stilpnomelane-bearing granophyre from the upper contact zone consists essentially of quartz (generally granophyric), a little orthoclase, plagioclase (albite-oligoclase), and iron-rich chlorite mainly replacing actinolitic amphibole. Apatite is abundant together with ilmenite and sphene.

Stilpnomelane occurs as sharply terminated laths which are commonly arranged in either radiating sheafs or as 'bow-tie' aggregates. It appears to be the last mineral formed in the rock and replaces chlorite and feldspar. It is also a constituent of small veins and fills occasional druses in the rock. The mineral is intensely pleochroic from pale yellow to a deep golden-brown or black and often shows varying degrees of alteration to a sub-isotropic reddish-brown vermiculite. The form and general characters of the mineral are identical with those of stilpnomelane from California[3].

A chemical analysis of the stilpnomelane-bearing granophyre gave the following: SiO_2 58·97, TiO_2 1·93, Al_2O_3 14·31, Fe_2O_3 2·10, FeO 9·21, MnO 0·21, MgO 2·37, CaO 2·76, Na_2O 4·60, K_2O 0·14, H_2O^+ 3·08, H_2O^- 0·14, P_2O_5 0·78, CO_2 0·00. Total 100·60 (analyst T. W. Bloxam). Detailed optical and chemical analyses of the stilpnomelane will be given in a later communication.

The mode of occurrence and relationships of the mineral are very similar to those of the stilpnomelane[4] from the Skaergaard intrusion, east Greenland. It is probable that the 'biotite' from the Cregennen granophyre[2] is in fact stilpnomelane—a conclusion which is supported by the low K_2O-content of the analysed rock.

Stilpnomelane in the acid tuffs has the same form as that in the basic granophyres, but shows less alteration to vermiculite. Frequently adjacent fibres are orientated at right angles to each other producing a type of 'mesh-structure' in thin sections[5]. The mineral replaces chlorite infillings of small vesicular aggregates and also fine-grained ashy material in the ground-mass.

We suggest tentatively that the stilpnomelane is a product of post-granophyre metasomatism accompanied by low-grade metamorphism equivalent to the chlorite sub-zone one[6]. Woodland[7] has already described the Cambrian (Harlech Series) north of the present area, and has noted the progressive metamorphism of these rocks.

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Lower Purbeck Beds of Swindon Facies in Dorset

IN the course of a yet unpublished study of the pseudomorphs after gypsum prevalent in the 'Purbeck Caps' and 'Broken Beds' of Lower Purbeckian age in Dorset, a marl has been discovered with a fauna resembling that of the freshwater facies of the 'Swindon Series'. In the well-known Portisham quarry with the 'fossil elephant' (a silicified tree trunk[1]), the three-foot "impure marls with seams of chert" listed by Woodward[1] as occurring 9 ft. 6 in. above the Portland Stone have been found to contain the Swindon ostracod *Ulwellia papulata* Anderson, and well-preserved charophytes in abundance. Gastropods are also abundant and perhaps have Swindon affinities. None of these has been recorded before in British strata of indisputably Lower Purbeck age, and a full palæontological investigation of the fauna is at present proceeding.

Lithologically the similarity in facies between the Dorset and Swindon beds is also well marked, and it is only with difficulty that the carbonaceous conglomeratic marl of Portisham can be distinguished from that of Swindon. At the former locality, however, there has occurred at places within this marl, the penecontemporaneous introduction of epigenetic lenticular gypsum. The geochemical conditions seem to have favoured silicification, which must here have been largely selective. The freshwater fossils are well preserved, and their presence in a rock full of pseudomorphs would seem at first sight to be anomalous. This appears to be due to the intercalation of the freshwater marl in an evaporitic succession marginal to the main gypsum deposits. These main gypsum deposits were rarely less than 6 ft. thick in the Broken Beds of the Lulworth region, where the 'Caps' were also very gypsiferous; at Portsdown and Henfield, anhydrite is dominant in the lower part of the Lower Purbeck[2].

At Swindon the freshwater nature of the greater part of the 'Swindon Series' has long been clear and they can be correlated with the Purbeck Beds of the Aylesbury district; but their correlation with the Purbeck Beds of Dorset has not previously been possible. A very thorough and painstaking investigation of these beds by Sylvester-Bradley[3] led to the realization that the fauna and flora, although differing in detail, were of a facies which at that time was known from Dorset only in the Middle Purbeck Beds. Twenty years ago Arkell and Sylvester-Bradley[4] discussed the age of the 'Swindon Series'. Arkell was convinced the evidence pointed to a Middle Purbeck age, but the view that the new discoveries strikingly support was that put forward by Sylvester-Bradley, who considered that the absence of *Cypridea granulosa* (Sow.) and *Cypridea fasciculata* (Forbes) suggested Lower Purbeck age.

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CHEMISTRY

Activity-based Diffusion Coefficients for Liquid Solutions

WHETHER an equilibrium concept is applied to a consideration for diffusion in liquids or whether the methods of irreversible thermodynamics[1,2] are used, it is clearly established that the driving force for molecular diffusion in liquids, such as occurs in the well-known diaphragm cell, is the gradient of the chemical potential. However, it has been the common practice to report diffusion coefficients calculated on the basis of concentration gradients as the driving force rather than the gradients of the chemical potential.

For the case of a binary (components A and B) non-ideal liquid system, the usual barycentric diffusion coefficient, D (that is, taken with respect to the mass average velocity) is defined as follows[2,3] :

$$D = \mathfrak{D} \cdot \frac{\partial \ln a}{\partial \ln x} \qquad (1)$$

where $\mathfrak{D}$ is the binary activity-based diffusion coefficient, a is the activity of either component, and x is the corresponding mole fraction. The coefficient, $\partial \ln a/\partial \ln x$, is a measure of the deviation from ideal solution behaviour with a value of unity for ideal solutions. With $\partial \ln a/\partial \ln x$ accounting for such deviation, it would appear that $\mathfrak{D}(x)$ should not be affected by non-ideality since, being essentially a proportionality constant, it may be expected to be independent of the driving force associated with it. On this basis, it appears reasonable that the form of the function $\mathfrak{D}(x)$ should be the same for all binary systems, that is, the same as observed for ideal solutions. Data on some very nearly ideal solutions[4] do indicate that the form of the function $\mathfrak{D}(x)$ is a straight line or very nearly so.

Recent work by us[5] has provided extensive data on the ethanol–water system at 25° C. in agreement with earlier results[6]. The true or differential diffusion coefficients reported in both cases are D-values based on concentrations, and thus include corrections for non-ideality. Following equation (1) these results have been combined with the coefficients $\partial \ln a/\partial \ln x$ as computed by Guggenheim[7], using the vapour-pressure data of Dobson[8], on the assumption that

$$\frac{\partial \ln a}{\partial \ln x} = \frac{\partial \ln p}{\partial \ln x} \qquad (2)$$

where p is the partial pressure of the component with mole fraction x. Fig. 1 presents the results. The peculiar shape of this curve suggests the possibility of errors resulting from the difficulty of finding accurate shapes of vapour-pressure curves based on ten experimental points distributed over the total concentration range. This has been checked by integrating equation (1), using equation (2), starting from the pure component, and assuming that $\mathfrak{D}(x)$ is linear. This latter assumption, applied to the ethanol–water system, means that $\mathfrak{D} = 1{\cdot}22$ is constant. Using tabular integration, the partial pressures of ethanol and of water were determined at a series of compositions. The calculated pressures are shown on Fig. 2 by the continuous curves, together with the experimental points obtained by Dobson. The Duhem–Margules equation was used. Agreement with the experimental values being quite good, the use of a straight line for $\mathfrak{D}(x)$ does seem valid for this case.

Johnson and Babb[9], however, by applying similar corrections to their diffusivity data on the systems methanol–benzene and ethanol–benzene, obtained curves for $\mathfrak{D}(x)$ that vary by more than 100 per cent and in no way approximate linearity. For the case of the methanol–benzene system they used the data by Scatchard *et al.*[10] at 35° C., which they corrected to 25° C. by an unspecified method.

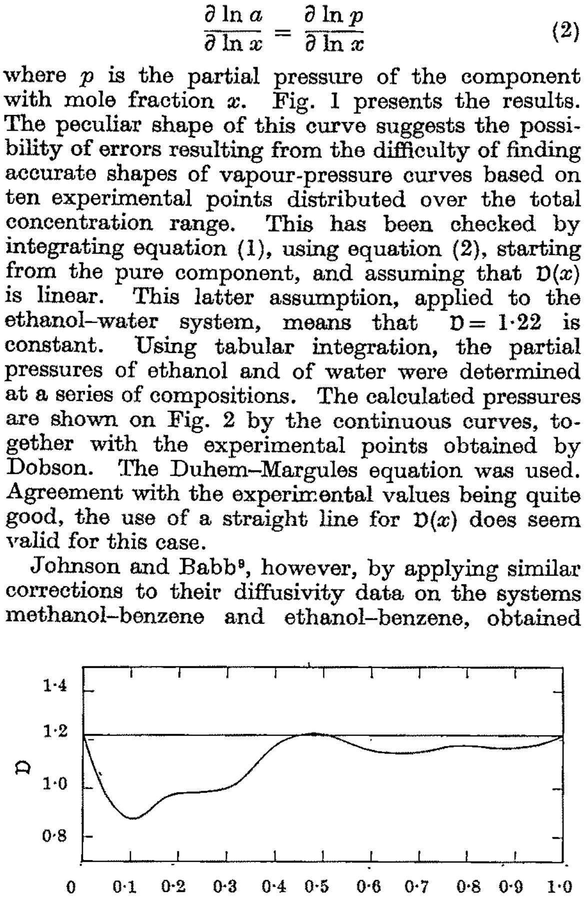

Fig. 1. Activity-based diffusivities in the ethanol – water system at 25° C., based on $\partial \ln p/\partial \ln x$ data as given by Guggenheim, and on measured values of D presented in ref. 5

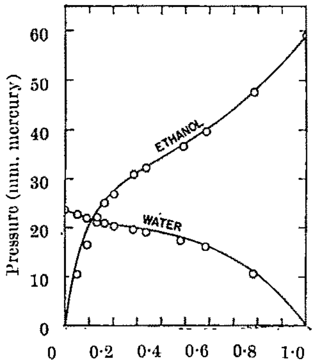

Fig. 2. Equilibrium vapour pressures (fugacities) in the ethanol – water system at 25° C. Continuous curves calculated by the equations,

$$D = \mathfrak{D}\,\frac{\partial \ln f_{\text{ethanol}}}{\partial \ln x_{\text{ethanol}}} = \mathfrak{D}\,\frac{\partial \ln f_{\text{water}}}{\partial \ln x_{\text{water}}}$$

using measured values of D, and $\mathfrak{D} = 1{\cdot}22$ cm.^{2}/sec., and assuming that the pure vapours behave as ideal gases. The circles denote experimental partial pressure values by Dobson (ref. 8)

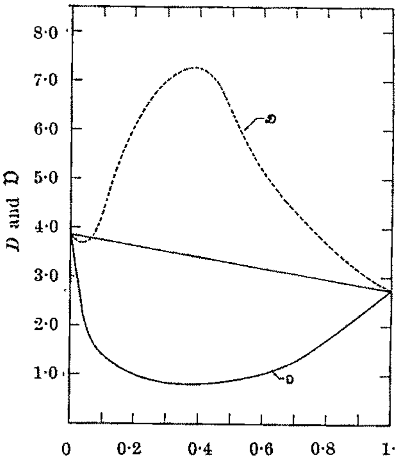

Fig. 3 Measured diffusivities D, and activity-based diffusivities, $\mathfrak{D}$ in the methanol – benzene system at 25° C., after Babb and Johnson (ref. 9)

This result, if correct, would certainly negate any speculation concerning the linearity of the function $\mathfrak{D}(x)$. There is little doubt that the vapour–liquid equilibrium data, as well as the measured diffusion coefficients, are essentially correct. There is a possibility of error in correcting the activity data to 25° C. To check this, the same integration procedure as used for the ethanol–water system was applied, using equation (1) for the measured diffusivities and the $\mathfrak{D}(x)$ curve by Johnson and Babb (Fig. 6, *loc. cit.*, and reproduced in Fig. 3). The curves then obtained are plotted in Fig. 4 as broken lines. It is noted that these curves do not pass through one point at 25° C. reported by Scatchard *et al.* The conclusion can be reached, however, that had Johnson and Babb used measured vapour-pressure curves at 25° C. to calculate $\mathfrak{D}(x)$, they would have obtained an even higher peak for this latter curve. In our opinion, the

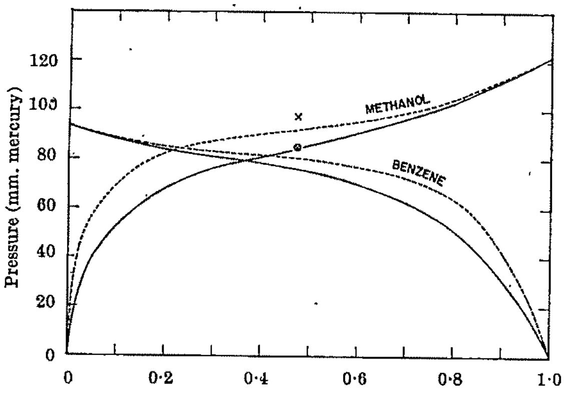

Fig. 4. Full lines are the fugacities in the methanol – benzene system at 25° C., as computed by the equations,

$$D = \mathfrak{D}\,\frac{\partial \ln f_{\text{methanol}}}{\partial \ln x_{\text{methanol}}} = \frac{\partial \ln f_{\text{benzene}}}{\partial \ln x_{\text{benzene}}}$$

using diffusivities given by Babb and Johnson (ref. 9) and a straight-line relationship for $\mathfrak{D}$. Broken lines show apparent values obtained by using the dashed curve on Fig. 3. × and ⊗ are measured partial pressures of methanol and benzene, respectively, after Scatchard *et al.* (ref. 10)

reason for the anomalous behaviour of the functions $\mathfrak{D}(x)$ as computed by Johnson and Babb, is the fact that benzene vapours do not form ideal mixtures with ethanol or methanol vapours. In the case of methanol at least, this has been experimentally verified by the observation of large heats of mixing in the system methanol vapour–benzene vapour[11]. Under such conditions equation (2) should be replaced with the more general equation :

$$\frac{\partial \ln a}{\partial \ln x} = \frac{\partial \ln f}{\partial \ln x} \qquad (3)$$

where f is the fugacity of the component with mole fraction x. In such a case experimental vapour-pressure data obviously cannot be used for the purpose of making activity corrections. The calculated curve $\mathfrak{D}(x)$ of Johnson and Babb, based on an erroneous assumption, cannot be considered as an argument against the suggested linearity of the function $\mathfrak{D}(x)$. It is of interest to calculate the fugacities of the two components over methanol–benzene mixtures at 25° C. using the experimental diffusivities of Johnson and Babb and a straight-line relationship for $\mathfrak{D}(x)$ between the two end-points of the diffusivity-mole fraction curve. The relationship :

$$D = \mathfrak{D} \cdot \frac{\partial \ln f}{\partial \ln x} \qquad (4)$$

was used together with the vapour-pressures of the pure components. The curves obtained are plotted also on Fig. 4 as continuous lines. As might be expected, the relative deviation between the two curves increases monotonically from the pure component end and, eventually, stays constant at a maximum 'saturation' value.

It may thus be proposed tentatively that the measured diffusion coefficients, in conjunction with a straight-line relationship for the activity-based diffusion coefficient be used to calculate the coefficient $\partial \ln a/\partial \ln x$, and hence the activities in the liquid solution, and the fugacities in the equilibrium vapour, for binary solutions of non-electrolytes. This procedure would be simpler than existing methods, and would also eliminate the necessity for differentiation, which should result in a considerable improvement in accuracy. It is hoped that by making isothermal diffusivity and vapour–liquid equilibrium determinations on a number of non-ideal systems, along with investigations of the vapour-phase for possible deviations from ideal solution behaviour, it will be possible to subject the above suggestion to more extensive testing.

We thank the National Research Council of Canada for a grant-in-aid.

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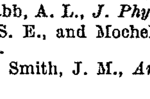
Complex Hydrides of Platinum

FOLLOWING a recent investigation of the structure of some rhodium(III) and iridium(III) tertiary arsine complexes[1], which were shown to contain a metal-hydrogen bond, we have examined certain tertiary phosphine compounds of platinum and suggest that these also are complex metal hydrides.

These related compounds, which have the formulæ $[(PPh_3)_4PtH_2]^0$ and $[(PPh_3)_3PtH_2]^0$, where PPh_3 is triphenylphosphine, were first prepared by Malatesta and Cariello[2a]. In the absence of evidence for the presence of metal-hydrogen bonds, they were originally formulated as tertiary phosphine complexes of zero-valent platinum. We have now shown that a third derivative $[(Ph_3P)_2PtH_2]^0$ (see ref. 2*b*) can be isolated from a benzene solution of either of the above hydrides, the other product of dissociation being triphenylphosphine. The compound $[(\text{diarsine})_2\text{-}PtCl_2]^0$, where diarsine has the formula I



(I)

has been prepared by treating $[(Ph_3P)_4PtH_2]^0$ with two moles of the diarsine in methylene chloride solution, the intermediate diarsine hydride reacting rapidly with the solvent. The properties of the various complex hydrides are given in Table 1.

The *tetrakis*-triphenylphosphine complex was prepared[2] by adding an aqueous solution of potassium tetra-chloroplatinate(II) to a solution of triphenyl-

Table 1. TYPICAL ANALYTICAL DATA

Compound	Colour	Melting point (° C.)	Calculated	Molecular weights In benzene	In nitrobenzene	In methylene chloride
$(PPh_3)_4PtH_2$	Ivory-yellow	116–118	1,242	400	915	—
$(PPh_3)_3PtH_2$	Yellow	125–130	982	420	800	—
$(PPh_3)_2PtH_2$*	Yellow-white	175–178	724	—	—	644

* Found: C, 60·8; H, 5·02 per cent; calculated for $C_{36}H_{32}P_2Pt$: C, 59·6; H, 4·4 per cent. Infra-red bands at 1,670 and 815 cm.$^{-1}$.

phosphine in alcohol containing potassium hydroxide. The reaction appears to be:

$$[PtCl_4]^{2-} + 2OH^- + 2C_2H_5OH + 4PPh_3 \rightarrow [(PPh_3)_4PtH_2]^0 + 2CH_3CHO + 4Cl^- + 2H_2O$$

The *tris*-triphenylphosphine complex was prepared[2] conveniently by adding hydrazine to a suspension of $[(Ph_3P)_2PtI_2]^0$ in ethanol.

The molecular weight in benzene found for the *tetrakis*- and *tris*-triphenylphosphine complexes may be interpreted in terms of a step-wise dissociation, namely:

$$[(PPh_3)_4PtH_2] \rightleftarrows [(PPh_3)_3PtH_2] + PPh_3$$
$$[(PPh_3)_3PtH_2] \rightleftarrows [(PPh_3)_2PtH_2] + PPh_3$$

The molecular weight in benzene indicates complete dissociation of both the *tetrakis*- and *tris*-compounds. This marked dissociation may, as suggested by Malatesta and Cariello[2], be due to some interaction of the complex with the solvent. This seems to be supported by the fact that the molecular weights in benzene are dependent on time; also, the benzene solutions show new infra-red bands which are not attributable to the solvent, the ligand or the complexes already mentioned. The dissociation is clearly much less in nitrobenzene (see Table 1).

The infra-red spectra have been studied, and some platinum–hydrogen active frequencies are shown in Table 1. As in other metal hydrides[3], the bands are characteristic of the metal-hydrogen stretching in regard both to frequency and shape. It is of interest to note that no Pt—H stretching frequencies in the usual region have been observed for the compounds $[(PPh_3)_4PtH_2]^0$ or $[(PPh_3)_3PtH_2]^0$. This is confirmed by deuteration studies. In contrast, however, the deuterium analogue of $[(PPh_3)_2PtH_2]^0$, prepared from a deuterated sample of $[(PPh_3)_3PtH_2]^0$, does show the presence of a Pt—D stretching frequency.

The number of hydride ligands in the *tetrakis*- and *tris*-compounds was determined by studying spectrophotometrically the amount of bromine consumed by these compounds. Known amounts of the solid complex were added to a standard solution of bromine in methylene chloride, and the disappearance of bromine studied by observing the absorption at 500 mμ. In both cases a sharp end-point occurred after 2 moles of bromine had been added; extraction of the resulting solution with water and titration of this with alkali revealed that 2 equivalents of hydrogen bromide had been produced:

$$[(PPh_3)_3 \text{ or } {}_4PtH_2]^0 + 2Br_2 \rightarrow [(PPh_3)_2PtBr_2]^0 + 2HBr + 1 \text{ or } 2\ PPh_3$$

The infra-red spectrum of the $[(PPh_3)_2PtBr_2]^0$ produced shows no Pt—H bands.

Further evidence for a Pt—H bond in the *tetrakis*- and *tris*-complexes is also provided by the vigorous reaction which they undergo with carbon tetrachloride to yield chloroform—a reaction which is characteristic of metal hydrides[3]. The composition of the products was established by vapour phase chromatography.

The fact that these triphenylphosphine adducts are considered to be hydrides of bivalent platinum raises the interesting question of whether any truly zerovalent complexes of platinum have yet been prepared; especially as the above hydrides have often been used as the intermediates in the preparation of certain Pt^0 compounds.

Thus, in the above compounds, in which the platinum atom is regarded as bivalent with a d^8 non-bonding configuration, we have examples of the metal atom with co-ordination numbers of 4 (cf. $[PtCl_4]^{2-}$), 5 (cf. $[Pt(\text{diarsine})_2Br]ClO_4$[5]) and 6 (cf. $[Pt(\text{triarsine})_2][ClO_4]_2$[6], $[Au(\text{diarsine})_2I_2]ClO_4$[7]).

We thank Dr. G. K. N. Reddy and Mr. K. Khaleeluddin for their assistance. One of us (J. A. C.) gratefully acknowledges the award of a Fulbright fellowship, during the tenure of which this work was carried out.

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A Sealed-edge Theory of Graphite 'Compounds'

IN the stepwise formation and decomposition of certain graphite compounds, notably the bisulphate and the potassium compounds, surprise has often been expressed that one layer of carbon atoms can influence the behaviour of other layers that are several times the spacing of 3·35 Å. away.

The following tentative theory explains this by centering attention on the carbon atoms at the edges of the layers. It is generally agreed that these edge atoms are bonded to oxygen, hydrogen, hydroxyl or hydrocarbon residues and perhaps to similar atoms in adjacent layers. Now if such bonds between edge atoms do exist, either directly or through oxygen or hydrocarbon residues, then it is reasonable that not all of them from a given layer are directed to the same adjacent layer. Openings will therefore be available through which adducts can diffuse into the central volume, the sheathing layers of which are thereby forced out to two or three times their normal separation. If, however, the edge atom separations throughout the crystal are unaffected, the displaced layers will decrease the spacing to the next layers and may transmit the effect to several successive layers. This contraction is probably most significant near the edges, and indeed need be significant only there to influence diffusion to the central region. If great enough, it will prevent the occupation of one or more layers adjacent to a filled one. The theory therefore explains the non-filling of blocks of spaces in the formation of the compound and the emptying of blocks of spaces during decomposition. Various isotherms, kinetic studies, and other data will shortly be published to support the theory.

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Field-Emission Microscopic Investigation of Catalysed Synthesis of Ammonia on Tungsten

Field-emission microscopy was applied to the investigation of the mechanism of the catalysed synthesis of ammonia on tungsten. It was concluded from the result that (1) the dissociative adsorption of nitrogen is considerably accelerated by the presence of hydrogen, and that (2) the rate-determining step of the synthesis is either the formation of adsorbed imino-group NH(a) from adsorbed nitrogen atom N(a) and hydrogen atom H(a), or that of adsorbed amino-group NH_2(a) from NH(a) and H(a) but not the dissociative adsorption of nitrogen, as is generally accepted.

Nitrogen, hydrogen, a 1 : 5 mixture of nitrogen and hydrogen, and ammonia were allowed, in turn, to be adsorbed on the clean tungsten tip of a field-emission microscope, while pressure was increased gradually from 10^{-9} to 10^{-7} mm. mercury and then decreased by evacuation. After evacuation was begun, the tip was subjected to intermittent heat treatments at temperatures raised successively from 500° to 3,000° K. Field-emission patterns and the associated changes of average work function were observed at liquid-nitrogen temperature for each series of gases along with the initial increase followed by decrease of pressure as well as after every heat treatment.

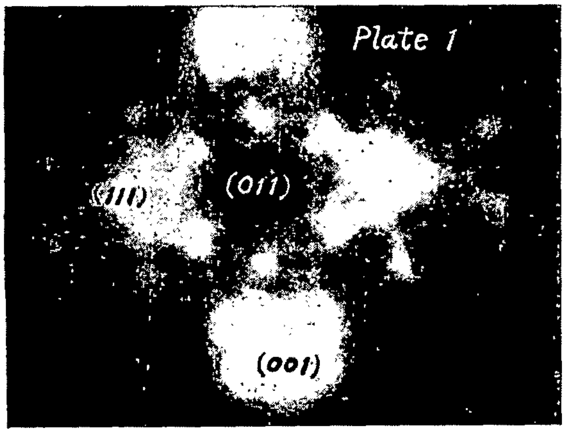

Fig. 1. The N(a) pattern has characteristics of the bright surroundings of the (001)-region and of the bright (111)-region. The increase of average work function $e\Delta\varphi$ is about 0·35 eV. from the initial value of the clean tungsten surface

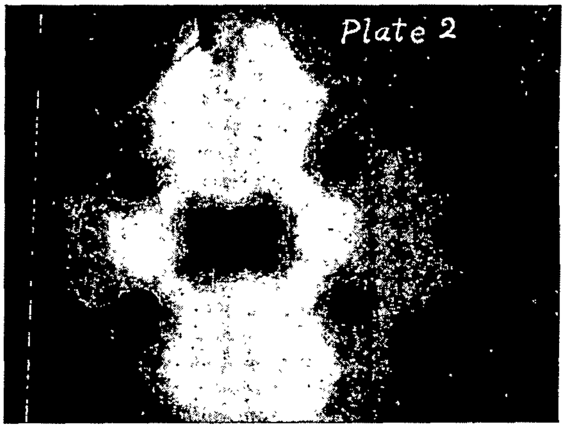

Fig. 2. The characteristics of the H(a) pattern are shown by the bright region spread toward the centre of the dark (011)-region along the (111)-(011) zone and the relatively darker (111)-region along the zone. $e\Delta\varphi$ is about 0·05 eV.

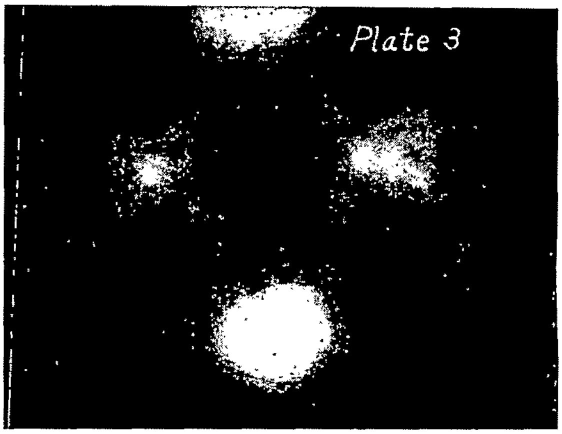

Fig. 3. The pattern of the nitrogen–hydrogen mixture is the superposition of the N(a) and H(a) patterns. $e\Delta\varphi$ is about 0·5 eV.

Figs. 1 and 2 show the patterns observed steadily at the later stage of the nitrogen and the hydrogen series. These patterns, along with associated changes of the average work function, were judged to be characteristic of N(a) and H(a). Fig. 3 is a pattern observed for a nitrogen–hydrogen mixture, which is identified as a superposition of the N(a) and H(a) patterns. Conclusion (1) was arrived at from the early appearance of a N(a) pattern in the latter series as compared with that in the nitrogen series. Fig. 4 for ammonia gives a pattern which is distinguishable from the patterns characteristic of N(a) and of H(a). The latter characteristic was attributed to NH(a) and/or NH_2(a) in agreement with an associated decrease of average work function.

The characteristic pattern of N(a) was observed with ammonia, just after the heat treatments at elevated temperatures. Similarly, patterns characteristic of NH(a) and/or NH_2(a) were observed with a nitrogen–hydrogen mixture after the heat treatments at elevated temperatures, but they were unstable.

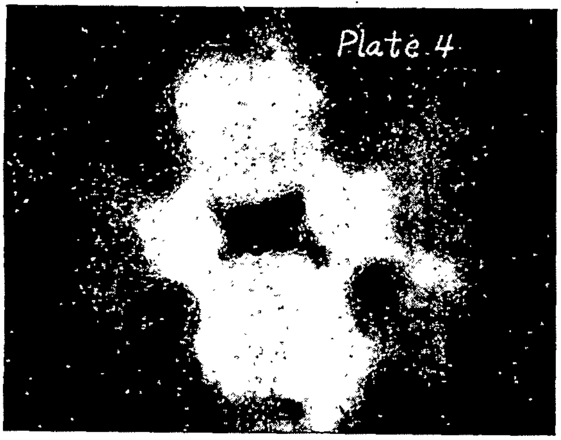

Fig. 4. The pattern of ammonia has the characteristically bright and dark regions along the (001)–(011) zone. $e\Delta\varphi$ is about −0·1 eV.

These results indicate that N(a) on one hand and NH(a) and/or NH_2(a) on the other hand are walled off from each other by a slow step, which is just surmounted in either direction by the later heat treatments; hence the conclusion (2) is arrived at, suggesting that catalysed ammonia synthesis proceeds through the sequence of steps: $N_2 \rightarrow 2N(a)$, $H_2 \rightarrow 2H(a)$, $N(a) + H(a) \rightarrow NH(a)$, $NH(a) + H(a) \rightarrow NH_2(a)$ and $NH_2(a) + H(a) \rightarrow NH_3$.

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Detection of Trace Quantities of Acenaphthene by Gas Chromatography

In the course of investigations into the c-mitotic effect we wished to determine the acenaphthene uptake in the root tips of onions grown in a saturated water solution of acenaphthene. As the quantities expected were of the order of μ, the very sensitive flame ionization detector described by McWilliam and Dewar[1] was used. A 6-in. long ⅛-in. bore column packed with 60–80 mesh brick dust carrying 15 per cent w/w silicone oil was used for the analysis, and pure hydrogen was used as the carrier gas.

Because of the small amounts of material being handled it was necessary to introduce the samples on to the column in a solvent. For this purpose it was found that redistilled commercial 'Decalin' (b.p. 184–187° C.) was suitable. At the temperature of the column (183° C.) the main peaks of the 'Decalin' had been eluted before the appearance of the acenaphthene peak, and, although the sample used 'tailed' badly, the tailing was not sufficiently serious to interfere badly with the detection of acenaphthene.

More volatile solvents or solvent mixtures had, of course, very short retention times, and tended to give a sooty flame which interfered with the operation of the detector.

Tests with prepared solutions showed that we could detect quantities as small as 0·2 μgm. acenaphthene.

Onion roots were treated with ether and the extract, after evaporation to dryness at room temperature, was redissolved in 0·25 ml. decalin. 20-μl. samples of decalin solution were injected on to the head of the column. The detector sensitivity was turned down to minimum until the main decalin peaks had been eluted and was then increased until the 'noise-level' was about 2 per cent of full scale.

Chromatograms made with extract from onions grown in pure water showed no peaks in the region about the retention time of acenaphthene. The extract from onions grown in acenaphthene solution and showing c-mitotic activity gave peaks which on comparison with the test chromatograms correspond to about 5 μgm. acenaphthene (that is, about 60 μgm./gm. of root tip).

An attempt was made to determine the solubility of acenaphthene in water. 10 ml. of saturated solution was evaporated to dryness and any residue dissolved in 0·25 ml. decalin. No trace of acenaphthene was found in this solution. The solubility of acenaphthene is therefore less than 250 μgm./l. These results suggest a considerable uptake of acenaphthene in the root tips, the significance of which will be discussed in a later paper in conjunction with other experiments on the c-mitotic effect.

We wish to thank Mr. A. Adam for his invaluable assistance with the design and construction of the electronic equipment.

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Polypeptide Formation from Asparagine under Hypothetically Primitive Conditions

This is a report of the formation of polyaspartic acid from asparagine[1] in aqueous solution under conditions which satisfy suggested primitive terrestrial conditions[2] better than thermal polycondensation[3,4].

When an aqueous solution of L-asparagine (5–10 per cent) was refluxed for several days, a part of the asparagine hydrolysed to aspartic acid; the other somewhat larger part formed a non-crystalline product[5]. This non-crystalline product was a mixture of aspartyl oligo- and poly-peptides which were separated as either water-insoluble Cu(II) or Pb(II) salts. Lyophilization of the solution of the free peptides, obtained with hydrogen sulphide from the salt, gave a white material. The yield was 62 per cent. When the polymerization of asparagine was carried out at 70–75° instead of 100°, the yield was 18 per cent. Van Slyke amino-nitrogen determination and dinitrophenol end-group analysis indicated average molecular weights between 380 and 530, corresponding to 3·3 and 4·4 units per molecule. Paper chromatogram (butanol–water or phenol–water) demonstrated the presence of a series of aspartic acid peptides, polypeptides and some aspartic acid. β-Aspartylaspartic acid was identified as being one of the oligopeptides present by comparison of the R_F value with that of an authentic sample[6].

Polyaspartic acid of higher molecular weight was obtained by dialysis of the original solution or of the crude polypeptide mixture. The average molecular weight of various preparations ranged from 1,700 to 3,000 (15–26 units) as determined by Van Slyke and dinitrophenol methods. Like α,β-polyaspartic acid[4], this polypeptide was highly soluble in water and gave a positive biuret reaction. The infra-red absorption spectrum was identical with that of polyaspartic acid[4c] (bands: NH stretching near 3μ; CO of carboxyl group 5·85μ; amide I 6·05μ; amide II 6·55μ) confirming the polypeptide structure. It is reasonable to assume that the polymerization of asparagine involves an intermolecular non-enzymatic transamidation reaction.

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BIOCHEMISTRY

Enzymatic Formation of Histamine Ribotide from Histamine Dinucleotide

Alivisatos *et al.*[1,2] reported the formation of histamine dinucleotide, that is, the pyrophosphate of histamine 5′-ribotide and of 5′-adenylic acid, from diphosphopyridine nucleotide (DPN) and histamine, using a soluble, purified preparation of diphosphopyridine nucleotidase[3] (DPNase) from bovine spleen. It has been shown that nicotinamide[4,5] inhibits the release of histamine by antigen from the lung tissue of a sensitized guinea pig. Nicotinamide is a known inhibitor of DPNase and nicotinamide ribosidase[6]. These facts suggest the possibility that part of the histamine released during the antigen–antibody reaction is formed from histamine dinucleotide.

The present report describes some properties of histamine dinucleotide and of histamine ribotide. These compounds were recently isolated as pure preparations in the course of work which was concerned with the biological significance of histamine dinucleotide and related compounds, and with the formation of free histamine from such compounds. The formation of histamine ribotide had hitherto been reported only qualitatively by paper chromatographic and electrophoretic results[2].

$$\text{DPN} + \text{histamine} \xrightarrow[\text{bovine spleen or pig brain}]{\text{DPNase from}} \text{histamine dinucleotide} + \text{nicotinamide} + \text{H}^+ \quad (1)$$

The reaction (1) proceeded easily with DPN and histamine (2-^{14}C) as substrates, and with the use of insoluble, partially purified DPNase[7] from bovine spleen or pig brain. The reaction product, histamine dinucleotide, was found to be present in the fraction eluted from a column of 'Dowex-1' (*X*10, chloride form) by 0·002 *N* hydrochloric acid (*p*H 2·9). The dinucleotide, recrystallized from acetone-water mixture after lyophilization, did not lower the blood pressure of a cat and did not contract the ileum of a guinea pig *in vitro*. The dinucleotide was easily hydrolysed when heated with 2·5 *N* hydrochloric acid in a sealed tube at 150° C. for 5 hr. The free histamine was identified by its action on the cat's blood pressure and the guinea pig's ileum.

$$\text{Histamine dinucleotide} \xrightarrow[\text{diesterase}]{\text{phospho-}} \text{histamine ribotide} + \text{AMP} \quad (2)$$

100 mgm. of histamine (2-^{14}C) dinucleotide (purity, 88 per cent; specific activity, 1,419 c.p.m./μmole), obtained by the reaction (1), was incubated at 37° C. for 5 hr. in the presence of phosphodiesterase[8], purified from snake venom. Chromatography of the reaction mixture through a column of 'Dowex-1' (*X*10, chloride form) gave a radioactive fraction of histamine ribotide, having no absorption at 260 mμ, by elution with 0·001 *N* hydrochloric acid (*p*H 3·1) (Fig. 1). Fig. 1 also shows a substance having absorption at 260 mμ, obtained by elution with 0·004 *N* hydrochloric acid (*p*H 2·4). This substance was identified as 5′-adenylic acid (AMP) by paper chromatography.

The fraction containing histamine ribotide was first lyophilized and recrystallized from acetone–water mixture to 25 mgm. (yield, 58 per cent) of the crystals, m.p. 176–180° C. (decomp.), of this substance. The specific activity of this sample, calculated from the formula of the ribotide as $C_{10}H_{18}O_7N_3P \cdot HCl$ (359·5), gave 1,466 c.p.m./μmole, which agrees with that of the original dinucleotide. The ultra-violet spectrum of the dinucleotide exhibits absorption maxima at around 210 mμ and at 258 mμ, and that of ribotide at around 210 mμ. Both were positive to the ninhydrin and phosphate reactions. The R_F values on a paper chromatogram, using 0·1 *M* acetic acid/ethanol (1 : 1) mixture as a solvent, was 0·45 for the dinucleotide and 0·58 for the ribotide. The observed values for ribose per 1 μmole of the compound were 1·80 (calc., 2·00) for the dinucleotide and 0·96 (calc., 1·00) for the ribotide by periodate consumption, but were 0·86 for the dinucleotide and 0 for the ribotide by the orcinol reaction. The lower values of ribose by the latter method are considered to be due to the stability of the N—C

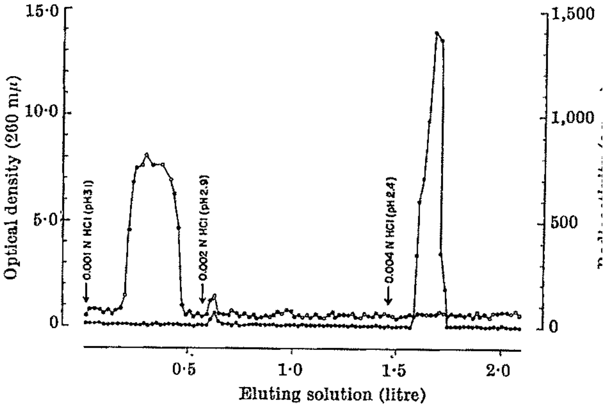

Fig. 1. Separation of histamine ribotide and adenylic acid by 'Dowex-1' (*X*10) in a chloride system. Fractions of 10 ml. were collected, and the radioactivity (○) and the optical density at 260 mμ (●) of each fraction were measured

bond between the imidazole and ribose in both substances[9]. However, this N—C bond is cleaved by decomposition with hydrochloric acid under a high pressure, liberating biologically active histamine in 40–83 per cent yield and inorganic phosphate in a yield close to 100 per cent of the theoretical value. The diazo reaction using sulphanilic acid on a paper chromatogram appears orange coloured in the dinucleotide and yellow in the ribotide. The absorption spectrum of the reaction solution, however, lacks the peak at 530 mμ characteristic of histamine in both substances, and a peak, assumed to be that of an azo compound, is observed at 380 mμ.

Histamine ribotide contracted the ileum of a guinea pig, but this action differed from that of histamine in that the contraction occurred gradually and was not inhibited by an antihistamine ('Neoantergan').

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Inhibition of Chymotrypsin with Di*Iso*propyl Phosphorofluoridate

In a recent article Stein and Laidler[1] described the inhibition of chymotrypsin by di*iso*propyl phosphorofluoridate (DFP). They found that even when the inhibitor-enzyme ratio was as high as 4 : 1 no complete inhibition was observed. This was contrary to the results of Jansen *et al.*[2], who found that when the molar ratio of DFP to enzyme was more than unity, there was practically 100 per cent inhibition.

Stein and Laidler concluded from their results that under their conditions the interaction between DFP and chymotrypsin is reversible. Further evidence of the reversibility was derived from the results of experiments where the enzyme was added to a mixture of DFP and substrate (methyl hydrocinnamate). They found that the greater the substrate : inhibitor ratio the less was the falling-off of the substrate-splitting rate as the reaction proceeded.

The discrepancy between these results and those of Jansen *et al.* was attributed to a small amount of propanol in the reaction medium of the latter.

In a rather comprehensive study on the relationship between structure of organophosphorus compounds and their ability to inhibit esterases and related enzymes[10] I also studied the inhibition of chymotrypsin by DFP.

The inhibition reaction was carried out at 25·0° C. and at *p*H 7·7 either in 0·1 *M* phosphate buffer or in 0·0033 *M* sodium veronal buffer. At intervals, samples were withdrawn and the remaining enzyme activity was measured either by Anson's hæmoglobin digestion method[3] or by measuring the rate of hydrolysis of L-tyrosine ethyl ester at a substrate concentration of 0·025 *M*[4]. In carrying out these activity measurements, the inhibitor-enzyme mixture was diluted about 10 times.

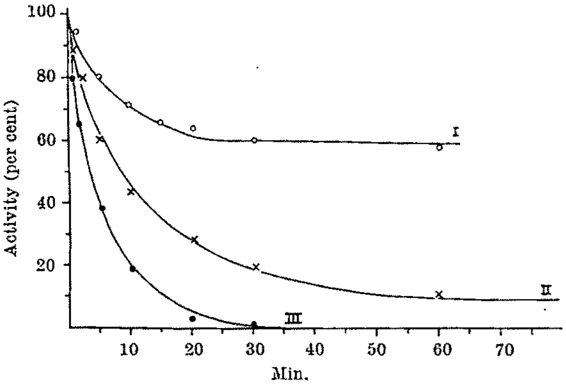

Fig. 1. Inhibition of chymotrypsin by DFP. Concentration of enzyme: $6 \cdot 6 \times 10^{-6}$ *M*. ○, Concentration of DFP, $2 \cdot 76 \times 10^{-6}$ *M*; ×, $6 \cdot 45 \times 10^{-6}$ *M*; ●, $1 \cdot 29 \times 10^{-5}$ *M*. All inhibition reactions carried out at *p*H 7·7 and 25° C.

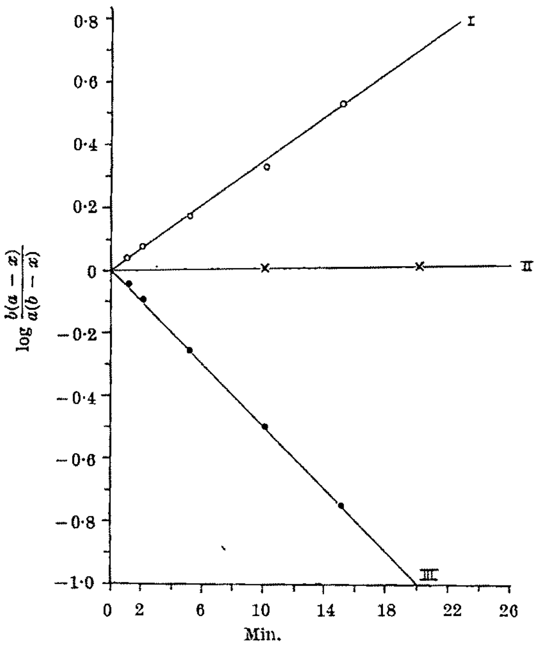

Fig. 2. The rate of inhibition of chymotrypsin by DFP. Concentration of DFP and enzyme the same as in Fig. 1

I was able to demonstrate that under my conditions the inhibition reaction was virtually stopped.

I found that the proteolytic activity is inhibited at the same rate as the esteratic activity, a result which is in agreement with the results of Jansen *et al.*[2] and Hartley and Kilby[5].

Figs. 1 and 2 give the results of three typical runs. From these kinetic data I calculated the bimolecular rate constant for the inhibition reaction. This has been done for the reaction of chymotrypsin with paraoxon by Hartley and Kilby[5]. In their case, however, pseudo first-order kinetics could be used, for a very large excess of paraoxon is needed in order to get proper enzyme inhibition within reasonable time. The authors remarked, however, that DFP inhibits chymotrypsin in such low concentration that the enzyme and inhibitor must be present in about equimolecular amounts.

That this is the case appears from a comparison of the concentrations of DFP used in the experiments of Fig. 1 and the enzyme concentration used, the latter being $6 \cdot 6 \times 10^{-6} M$. In these circumstances the second-order kinetics calculation has to be used. The well-known formula for the rate constant is:

$$k = \frac{1}{t} \times \frac{1}{a-b} \ln \frac{b(a-x)}{a(b-x)} \qquad (1)$$

a and b being the initial concentrations of enzyme and inhibitor respectively and x the concentration reacting in time t. In Fig. 2 $\log \frac{b(a-x)}{a(b-x)}$ is shown in relation to t. Straight lines are obtained; the slopes of these lines give the bimolecular rate constant. It was found that under our conditions:

$$k = 1 \cdot 9 \times 10^4 \text{ l.mol.}^{-1}\text{min.}^{-1}$$

This value corresponds very well with a value published by Jandorf *et al.* ($2 \cdot 0 \times 10^4$) without experimental conditions given, however[6].

The influence of the buffer appeared to be only slight, the value in the veronal buffer being $1 \cdot 7 \times 10^4$ l.mol.$^{-1}$min.$^{-1}$.

The results of Stein and Laidler, given in their Table 2, are obtained at 20·0° C. Unfortunately the *p*H value at which their experiments were carried out is given only as between 7·5 and 8·7. As the influence of the temperature on the inhibition reactions is not very great (I observed apparent activation energy of the order of 10 kcal./mol.) I have assumed that the above-mentioned rate constant is a good approximation of the rate constant under the conditions as given by Stein and Laidler. Using the concentrations of enzyme and DFP as given by Stein and Laidler, I calculated with the above-mentioned rate constant the amount of enzyme inhibition obtained with a reaction time of 10 min., assuming an ordinary bimolecular irreversible reaction and using formula (1).

The results are shown in Table 1, together with the experimentally obtained inhibition values of Stein and Laidler.

When Stein's values are compared with my calculated values striking similarity is seen, especially with the lower DFP concentrations. The fact that with the higher concentrations of DFP the measured inhibition is higher than the calculated values can be explained by the fact, observed by Stein and Laidler, that the addition of substrate (methyl hydrocinnamate) does not stop the inhibition reaction. The results of Stein and Laidler can thus be explained without the assumption of a reversible reaction.

Table 1. RESULTS OF STEIN AND LAIDLER (REF. 1) COMPARED WITH CALCULATED RESULTS FROM MY EXPERIMENTS. $[E]_0 = 3 \cdot 03 \times 10^{-6} M$

DFP × 10^6	DFP/$[E]_0$	Percentage of inhibition found by Stein and Laidler	Percentage of inhibition calculated with second order kinetics
1·18	0·39	17·0	16·0
2·36	0·78	28·6	29·5
3·54	1·17	45·1	40·5
4·72	1·56	48·0	51·5
5·36	1·77	61·8	56·5
8·04	2·65	83·2	72·0
10·72	3·54	87·8	82·5

The results with the mixtures of DFP and substrate are certainly not in contradiction of an irreversible bimolecular second-order inhibition reaction.

Concerning the discrepancy between the results of Stein and Laidler[1] and of Jansen *et al.*[2], I calculated the concentration of enzyme used by the latter authors. Assuming a nitrogen content of 15·83 per cent[7] and a molecular weight of 23,000 [8,9] I found that with 0·060 mgm. protein N/ml., the concentration is $1 \cdot 65 \times 10^{-5} M$. The reaction conditions of Jansen *et al.* are 25·0° C., 0·2 *M* phosphate buffer *p*H 7·7 and 20 min. reaction-time. With an equimolecular amount of DFP, and again assuming the rate constant $1 \cdot 9 \times 10^4$ l.mol.$^{-1}$min.$^{-1}$, I calculated an inhibition of 86·5 per cent, which is certainly much higher than the results of Stein and Laidler. As the concentrations used by Jansen *et al.* are higher and their reaction time longer, the result is not surprising.

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Ammonia Dehydrogenase, Hydroxylamine Dehydrogenase, Hyponitrite Dehydrogenase and Nitrite Dehydrogenase

IT has previously[1,2] been reported that there is an enzymatic cycle of inorganic nitrogen. In the present work, we have demonstrated that the dehydrogenating system in the cyclic processes can be separated from the reducing one, and that each dehydrogenase behaves specifically towards different inhibitors. Moreover, the intermediates in the dehydrogenation have been determined quantitatively.

To fractionate enzymes, an acetone preparation was first made from fresh cockerel liver as described in refs. 1 and 2. The precipitate obtained with 33–55 per cent acetone was dissolved in cold water and mixed with 20–30 parts by volume hydroxyapatite as described by Tiselius, Hyertén and Levin[3]. After 1 hr. at 0° C., the suspension was centrifuged at 6,000 r.p.m. for 5 min. The sediment was washed twice with cooled water and extracted with a mixture of sodium acetate and acetic acid. The extract was dialysed against running water for 3 hr. and used as enzyme solution. Measurement of the action of dehydrogenase or reductase was as described in refs. 1 and 2. For comparing the enzyme activity, the value of $-\log T$ was divided by mgm. of protein nitrogen in the reaction mixture. The results are summarized in Table 1.

Table 1. SEPARATION OF DEHYDROGENASES FROM REDUCTASES

Substrate	0·01–0·1 *M* acetate, *p*H 6·6 Dehydrogenase activity	Reductase activity	2–4 *M* acetate, *p*H 7·5 Dehydrogenase activity	Reductase activity
NH_4Cl	0	—	+1·174	—
NH_2OH	+0·038	−3·808	+0·743	−0·090
$Na_2N_2O_2$	0	−4·115	+1·026	−0·101
$NaNO_2$	0	−4·308	+1·166	−0·103
$NaNO_3$	—	−4·000	—	−0·106

The dehydrogenating system was mostly eluted with 2–4 *M* acetate mixture and the reducing one with 0·01–0·1 *M* acetate. Compared with the enzymatic activity of the recentrifuged supernatant from liver homogenate, the dehydrogenases were purified 50–350-fold by these treatments and the reductases 550–750-fold.

In the inhibition tests for the dehydrogenating enzymes, a dialysed 2–4 *M* acetate extract was mixed with *tris*-buffer and inhibitor, and after 20 min. at 30° C., the substrate was added. Some of the results obtained are given in Table 2.

Table 2. INHIBITOR SPECIFICITY OF DEHYDROGENASES

Inhibitor	Substrate, inhibition (per cent)			
	NH_4Cl	NH_2OH	$Na_2N_2O_2$	$NaNO_2$
Sodium azide, 5×10^{-3} *M*	50	100	0	10
Thiourea, 5×10^{-3} *M*	50	60	20	20
Uric acid, 5×10^{-4} *M*	100	45	85	70
8-Hydroxyquinoline, 5×10^{-4} *M*	100	37	90	0
p-Chloromercuribenzoic acid, 5×10^{-4} *M*	100	0	90	100

It was observed that the dehydrogenation for each substrate is specifically inhibited by certain compounds, indicating that the dehydrogenating system consists of 4 substances.

For estimating the intermediary products in the dehydrogenation process, a larger amount of enzyme and coenzyme, namely, 2 ml. of liver preparation obtained with 33–55 per cent acetone and 8 mgm. of diphosphopyridine nucleotide, were added to the reaction mixture. Ammonia and hydroxylamine were determined by the indophenol method and our modified colorimetry method[4] respectively. It was found that hyponitrite can be estimated by our alkali process[4] for oximes; this was confirmed by using a crystalline salt, $Na_2N_2O_2 \cdot 8H_2O$, prepared according to the description of Jones and Scott[5]. The quantity of nitrite or nitrate was measured by applying Griess's reagent or the 2,4-xylenol method of Buckett, Duffield and Milton[6]. To determine hyponitrite in the reaction mixture containing hydroxylamine, hyponitrite and nitrite, half the mixture was boiled for 5 min. and the other half mixed with 2 volumes of methanol, because boiling for 5 min. decomposes hyponitrite completely but has no influence on the estimation of hydroxylamine and nitrite. After centrifuging, the total amount of hydroxylamine, hyponitrite and nitrite in the mixture treated with methanol as well as that of hydroxylamine and nitrite in the boiled mixture was measured by our alkali process[4]. The results are given in Table 3.

Table 3. DETERMINATION OF DEHYDROGENATION PRODUCTS

Substrate	Amount disappeared (N in μgm.)	Amount produced (N in μgm.)			
		NH_2OH	N_2O_2	NO_2	NO_3
NH_4Cl	32·0	5·1	—	9·5	13·0
NH_2OH	31·8	—	12·6	7·0	8·8
$Na_2N_2O_2$	19·4	—	—	6·5	2·1
$NaNO_2$	20·7	—	—	—	17·5

For the determination of hyponitrite in the hydroxylamine-dehydrogenation, 3×10^{-4} *M* 8-hydroxyquinoline was added to the reaction mixture.

Our experiments have shown clearly that the dehydrogenation and reduction are carried out by different enzyme systems, and our earlier investigation[1] was the first demonstration of such a reducing system in animals. The cyclic oxido-reduction of inorganic nitrogen was also found in yeasts[2]. Klausmeier and Bard[7] reported that di- or triphosphopyridine nucleotide is hydrogenated by *Bacillus subtilis* in the presence of ammonium hydroxide. However, Roussos, Takahashi and Nason[8] proved later that the hydrogenation of pyridine-coenzymes by *B. subtilis* is to be attributed to some factors other than an enzyme specific to ammonium.

The nitrogen cycle formulated by us[1] has been confirmed by these experiments.

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Preparation of Sheep Growth Hormone

EXISTING methods for the preparation of sheep growth hormone by low-temperature ethanol fractionation[1] or salt fractionation, combined with ion exchange chromatography[2], are lengthy and involve a large number of individual steps. The same criticism applies to existing methods for preparation of ox growth hormone[3,4].

During an investigation of the separation of sheep pituitary proteins on a column of diethylaminoethylcellulose (DEAE-cellulose) it was found that under certain conditions all the growth hormone present, in quite simple sheep pituitary gland extracts, passed through the column without adsorption, whereas almost all the other pituitary proteins were adsorbed. Whatman DEAE-cellulose powder (DE60), containing 1·2 milli-equivalents of base per gm. of dry powder, was used in all experiments.

Extracts were prepared by grinding frozen pituitary glands with solid carbon dioxide, and extracting the resultant powder with sodium borate buffer (ionic strength 0·025; *p*H 8·6) for 3 hr. at 4° C. In some experiments these extracts were applied directly to the DEAE-cellulose column. In others the ground glands were extracted with a similar buffer, but to which 0·25 *M* sodium chloride had been added and the extract so obtained dialysed and freeze-dried. The dried material was then dissolved in sodium borate buffer and applied to the column. Both these starting materials contained approximately 0·1 mgm. of growth hormone per mgm. of non-dialysable material, expressed as the equivalent amount of the standard ox growth hormone preparation, *N.I.H. B*2 (kindly supplied by the Endocrinology Study Section, National Institutes of Health, Bethesda). The unadsorbed fraction obtained from the column contained 0·6–0·8 mgm. of growth hormone per mgm. of non-dialysable material. This material in 5 per cent w/v concentration was dialysed against distilled water. The resultant precipitate was equal in potency to the *N.I.H. B*2 growth hormone and yielded the equivalent of 2·5 gm. of growth hormone per kgm. of fresh glands.

Starch-gel electrophoresis did not show the presence of any proteins other than growth hormone (unpublished observations), but the limits of contamination with other pituitary hormones, as determined by bioassay methods, has not yet been investigated.

This method for preparing growth hormone uses only very mild conditions and involves a minimum number of steps. It has been applied successfully to the preparation of growth hormone from quantities of fresh, whole glands, ranging from 7 gm. to 200 gm. and using columns containing 20 gm.–150 gm. of DEAE-cellulose. There appears to be no difficulty in extending the method to either smaller or larger quantities of starting material.

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Acute Effect of Three Antibiotics on Diuresis in the Rat

ONE of the side effects limiting the use of some polymyxins is renal toxicity.

Sodium colistinmethanesulphonate, a new antibiotic with a spectrum of activity very similar to that of the polymyxins, but with much lower systemic toxicity[1], was investigated for its effects upon urinary volume output and was compared in this respect with two polymyxins.

The influence of the administration of each antibiotic upon diuresis was estimated by measuring, after treatment, the urinary output in ml./kgm./hr., 2 and 5 hr. after administration of a water load. This was done in groups of 20 male Wistar rats of approximately 360 gm. which were used twice consecutively in the following manner: first, the normal urinary volume output figures were determined for each group; 2 days later, the same procedure was repeated, each group receiving subcutaneously its respective antibiotic treatment at the same time as the intragastric water load. Each time the rats were starved (food and water) for 18 hr. before intragastric administration of a 50 ml./kgm. tap water load. The animals were then placed in metabolic cages without food and water and their urinary excretion was recorded at 2 and 5 hr., aggregated, and computed as indicated in Table 1.

The results at 2 and 5 hr. indicate that a very marked inhibition of diuresis occurred with all doses of either polymyxin *B* or colistin sulphate up to 1·67 mgm./kgm., whereas doses of sodium colistinmethanesulphonate up to 1·67 mgm./kgm. tended to increase diuresis. However, inhibition of diuresis was observed also with sodium colistinmethanesulphonate at doses above 1·67 mgm./kgm., but it was necessary to administer as much as 16·7 mgm./kgm. in order to obtain a degree of inhibition similar to that noted with 1·67 mgm. of the two other antibiotics. This indicates that, of the three antibiotics tested, sodium colistinmethanesulphonate interferes much less with urinary function, a fact that may explain its higher margin of safety.

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Preparation and Properties of Purified House Dust Allergen

ALTHOUGH house dust allergens have been the subject of many investigations[1], attempts to establish the chemical identity of the specific house dust antigen thus far have met with very little success. This is partly due to the complex nature of crude house dust extracts, which, apart from non-specific toxic substances, contain many other allergens as well (derived from feathers, human and animal dandruff, etc.).

Boatner and Efron[2] were among the first to indicate the general protein nature of active preparations from house dust. Rimington *et al.*[3], in a more detailed study, obtained a purified dust fraction which was found to contain two electrophoretically distinct components of similar chemical composition and skin reactivity. The substance was found to give both protein and carbohydrate reactions. Recently, Vannier and Campbell[4] reported the isolation of a purified dust allergen which they estimated to contain 95 per cent carbohydrate and 5 per cent protein.

Table 1

Control run								Experimental run								
No. of rats		Urinary volume output				Treatment (s.c.)	Dose/kgm. (mgm. base)	No. of rats		Urinary volume output				Per cent change from the controls		No. of rats with macroscopic peripheral vasodilation
		Total (ml.)		ml./kgm./hr.						Total (ml.)		ml./kgm./hr.				
Initial	Final	2 hr.	5 hr.	2 hr.	5 hr.			Initial	Final	2 hr.	5 hr.	2 hu.	5 hr.	2 hr.	5 hr.	
20	20	88	195	5·77	5·14	Polymyxin *B* sulphate	0·20	20	20	52	196	3·51	5·28	−39·2	+ 2·7	0
20	20	113	250	7·55	6·64	,, ,, ,,	0·50	20	20	5·3	29·9	0·35	0·79	−95·4	−88·1	10
20	20	99	247	6·60	6·58	,, ,, ,,	1·00	20	20	3·7	10·7	0·25	0·28	−96·2	−95·7	12
20	20	92	245	6·35	6·78	,, ,, ,,	1·67	20	18	6·4	6·4	0·45	0·18	−92·9	−97·3	20
20	20	96	281	6·33	7·46	Colistin sulphate	1·67	20	20	6·1	31	0·41	0·82	−93·5	−89·0	20
20	20	81	218	5·09	5·49	Sodium colistinmethanesulphonate	0·83	20	20	108	265	6·98	6·83	+ 37·1	+26·3	0
20	20	115	233	8·27	6·68	,, ,, ,,	1·67	20	20	117	285	8·3	8·1	+0·004	+21·3	0
20	20	111	242	7·92	6·92	,, ,, ,,	3·33	20	30	86	216	6·1	6·1	−23·0	−11·8	0
20	20	93	245	6·30	6·63	,, ,, ,,	6·67	20	20	15	21	1·0	0·56	−84·0	−91·6	20
20	20	87	219	6·09	6·14	,, ,, ,,	16·7	20	5	6·4	6·8	0·46	0·19	−92·4	−96·9	20

We wish to report the isolation of a purified house dust allergen with high activity in intradermal skin tests, the substance being apparently devoid of non-specific toxic substances and interfering allergens.

1,700 gm. of pooled house dust (collected during September–October, 1960) were extracted with a phosphate buffer $2 \cdot 8 \times 10^{-2}$ *M*, *p*H 7·0, containing $6 \cdot 5 \times 10^{-2}$ *M* sodium chloride and 0·5 per cent phenol. After two days at room temperature, the thick mud was squeezed and the resulting fluid filtered through a 0·5-cm. thick layer of kieselguhr. From the clear, deep-brown coloured filtrate the allergens were obtained by adsorption on benzoic acid at *p*H 4·00, using Sutherland's method[5]. The precipitated benzoic acid was filtered and dissolved in acetone, in which the allergens are insoluble. After washing once with ice-cold acetone, the crude mixture of allergens was extracted twice with the above-mentioned phosphate buffer, the undissolved residue being discarded.

The clear solution was then dialysed in a semi-permeable 'Cellophane' bag against running tap water, followed by dialysis against 0·01 *M* sodium ethylenediamine tetraacetate solution, *p*H 7·00, and distilled water successively. Further purification could be achieved by ammonium sulphate fractionation, the fraction precipitating between 25 and 100 per cent saturation being collected.

This brown-coloured product was dissolved in water and dialysed. The solution was then adjusted to *p*H 3·1 with hydrochloric acid. The resulting precipitate was separated by centrifugation after one day, dissolved in water and lyophilized. Clinical evidence suggests the identity of this product with the human dandruff allergen (Voorhorst[6]). The supernatant at *p*H 3·1 was treated with 4 volumes of acetone. A flocculent pink precipitate formed which was collected by centrifugation, dissolved in water and lyophilized. The yield was 35 mgm.

The substance thus obtained was very easily soluble in water and in physiological salt solutions. Markedly positive reactions were obtained with 2×10^{-5} mgm. (1 : 5,000,000) in intradermal skin tests with patients showing the typical symptoms of house dust allergy. Reagins against this substance could be demonstrated by means of Prausnitz-Küstner passive transfer tests. Controls were always found to be negative.

Paper chromatography of hydrolysates revealed the presence of 12 amino-acids: glu, asp, arg, lys, ser, val, thr, ala, pro, leu, *iso*leu, and cysteic acid. At least three carbohydrates could be detected: galactose, arabinose and xylose. The substance contains 7 per cent nitrogen (micro-Kjeldahl) and 42 per cent hexose (expressed as equivalent glucose in the orcinol test), which suggests it is a mucoprotein, possibly of plant origin. Preliminary ultracentrifugal analysis gave a sedimentation constant of 2·2, indicating a molecular weight of 22–25,000. Further purification was effected on columns of diethylaminoethyl cellulose.

It should be noted that precipitation of the allergen from saturated ammonium sulphate solutions is not complete; after complete saturation, the supernatant still contains about 7 per cent protein (1·4 per cent nitrogen), derived from unprecipitated allergen, as well as a mixture of polyuronides. A similar phenomenon has been reported in the case of trichophytin[7]. A detailed account of this work will be published elsewhere.

Our thanks are due to Prof. L. H. Jansen for his interest in this work.

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A New Technique of Chromatography and Ionophoresis on Ion-exchange Paper: Application to the Separation of Barbiturate, Salicylate and Acetophenetidin

We have found that, when a mixture containing barbiturate, salicylate and acetophenetidin is subjected to chromatography on Whatman No. *DE*20 cellulosic ion-exchange paper in dilute ammonia solution, a partial resolution of the components is possible. Salicylate is clearly separated from the other two compounds, which move together with only partial resolution (Fig. 1).

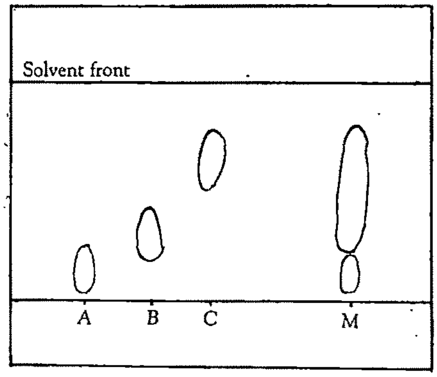

Fig. 1. Ascending chromatography on *DE*20 paper in 0·2 *N* ammonia. Time, 12 min. *A*, salicylate; *B*, phenobarbitone; *C*, acetophenetidin; *M*, mixture of *A*, *B* and *C*

However, if after chromatography the wet paper is subjected to ionophoresis (again in dilute ammonia solution) in a direction at right angles to the chromatographic solvent flow, a complete resolution of all three components of the mixture can be effected in

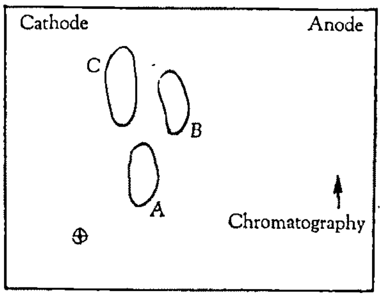

Fig. 2. Ascending chromatography on *DE*20 paper in 0·2 *N* ammonia, followed by ionophoresis for 30 min. in direction at right angles. *A*, *B* and *C*, as in Fig. 1. ⊕, Point of application

only 30 min. (Fig. 2). The chromatography takes only 10–12 min.

By using a micro-electrophoresis tank (Shandon Scientific Co., London), it is possible to carry out the chromatography and the ionophoresis in the same tank with the same solution.

It is interesting to note that when a mixture of salicylate and barbiturate is subjected to ionophoresis on 'ordinary' filter paper, the salicylate moves faster than the barbiturate[1], whereas the reverse is the case with *DE*20 paper. The technique of combining chromatography and ionophoresis on ion-exchange papers may lend itself to rapid separation of mixtures of amino-acids, of mixtures of acids of the Krebs cycle, and of many other mixtures of substances which are either not resolved satisfactorily on paper or which involve conventional chromatography for many hours.

Work is now in progress to develop this technique into a rapid screening test for barbiturates in blood. The technique is also being applied to the separation of alkaloids and other compounds of toxicological interest using different ion-exchange papers. The results of these experiments will be published elsewhere.

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Destruction of Linoleate Hydroperoxide by Soya Extracts

WHEN Balls *et al.* used a thiocyanate assay to estimate oxidation of linoleate by soya lipoxidase, their results suggested that under certain conditions part of the linoleate hydroperoxide produced by the enzyme was afterwards destroyed by crude, but not by purified, enzyme preparations[1]. The reason for this has not been known, although Theorell *et al.* implied that spontaneous decomposition of hydroperoxide might be responsible[2]. Recent work has indicated that there is in crude extracts of soya a factor which destroys β-carotene in the presence of linoleate hydroperoxide[3]. Here, we have examined the possibility that such a factor may also destroy linoleate hydroperoxide enzymically in the absence of β-carotene.

Sumner's thiocyanate assay for peroxide, as modified by Koch *et al.*[4], was used, pure linoleic acid in alcohol solution being added as substrate to *p*H 6·5 phosphate–citrate buffer to give a concentration of 1·36 × 10^{-3} *M* in the reaction mixture. Centrifuged dialysed extracts of 1 gm. defatted soya bean meal in 100 ml. water were used as enzyme source, volumes of 0·1–3 ml. being added to 25 ml. of buffer-substrate.

It was found that with small additions of soya extract (0·1–0·5 ml.) there was proportionality of reaction-rate to added enzyme, but that with larger additions (1–3 ml.) hydroperoxide formation was followed by destruction. Results obtained with 2 ml. soya extract are shown in curve *A* in Fig. 1.

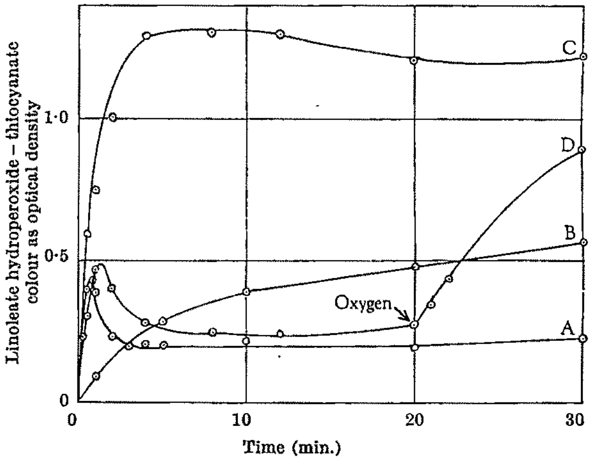

Fig. 1. Formation and destruction of linoleate hydroperoxide. *A*, 2 ml. soya extract ; *B*, 0·3 ml. soya extract; *C*, 2 ml. soya extract and oxygenated buffer; *D*, 2 ml. soya extract and oxygen passed in after 20 min.

Using only 0·3 ml. soya extract, hydroperoxide increase was initially less rapid, but no destruction was observed (curve *B*, Fig. 1) and formation continued steadily up to 80 min. But if a further 2 ml. soya extract were added to the same system at 40 min. from zero time, hydroperoxide destruction, of the same order as shown in curve *A*, resulted. Peroxide destruction did not occur if the 2 ml. soya extract were heated for 3 min. at 80° C. Similar results were obtained when extracts of defatted lentil and of defatted wheat germ (5 gm./100 ml. water) were examined in the same fashion.

These results suggest that the hydroperoxide-destroying factor is enzymic, since it is heat-labile and non-dialysing.

Maier and Tappel[5] have shown that hæmoglobin, acting as a catalyst for the decomposition of linoleate hydroperoxide, is only slightly less active than catalase, and much more active than peroxidase or cytochrome *c*. On the system which we have described, added hæmoglobin solution had to be at least 5 × 10^{-5} *M*, with respect to hæmatin, to destroy as much hydroperoxide as did an equal volume of soya extract. Our soya extracts do not contain enough total hæmatin to be observed spectrophotometrically as pyridine hæmochromogen under conditions where a concentration of 10^{-5} *M* would be obvious, and it may be inferred that if an iron porphyrin compound is responsible for peroxide destruction then its activity in this respect is much higher than those mentioned.

The sudden change from hydroperoxide formation to hydroperoxide destruction may be attributed to exhaustion of available oxygen, since it occurs at a point (Fig. 1) where hydroperoxide, measured as conjugated diene at 232 mμ (assuming an extinction coefficient of 28,000), is 2·3 × 10^{-4} *M*. The calculated molarity of dissolved oxygen in the system is about 3 × 10^{-4} *M*, which suggests that linoleate oxidation ceases because of lack of oxygen, to be succeeded by anaerobic peroxide destruction.

To verify this we repeated the experiment which gave curve *A* in Fig. 1 after first saturating the buffer with oxygen (curve *C*), and alternatively passing in oxygen after the fall in hydroperoxide had occurred (curve *D*). These results confirm the supposition.

It was observed in these experiments that, with small volumes of added soya (0·3 ml.), hydroperoxide formation, calculated from thiocyanate values, was proportional in the early stages to diene formation, but between 2 and 3 times greater. This accords with observations that thiocyanate methods give results between 2 and 3 times the theoretical[6]. We did not establish this proportionality with larger amounts of soya: with these, when destruction of peroxide occurred, there was no corresponding decrease of diene, suggesting that the products of hydroperoxide destruction remain conjugated. A full account of this work will be published elsewhere.

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Reduction of Coenzyme Q by Succinic Acid Dehydrogenase

It has been pointed out to us that our recent communication under this title[1] could be misunderstood as a claim of having first shown the insensitivity to antimycin A of reduction of ubiquinone. The credit of this observation belongs to Drs. A. M. Pumphrey and E. R. Redfearn[2]. The purpose of our communication was to show that this reduction could be obtained with a non-particulate soluble preparation, simpler than those which have mostly been used hitherto.

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PHYSIOLOGY

Functional Interpretation of Cerebellar Histology

The nature of the transformation of patterns of excitation within the cerebellar cortex may be explained by the following argument, based mainly on histological evidence and on some simple physiological assumptions.

For all vertebrates studied the following facts emerge[1-5]: (*a*) The cerebellar cortex is a sheet of grey substance with anisotropic structure. Entirely different histological pictures are obtained in sections cut in frontal and sagittal planes. (*b*) All output fibres arise from one type of neurons (Purkinje cells) the dendritic fields of which are characteristically flattened in the laterolateral direction. (*c*) One set of input fibres (climbing fibres) is connected one-to-one with the set of Purkinje cells. (*d*) Another set of input fibres (mossy fibres) reaches the output (Purkinje) cells via long (about 5–10 mm.), very thin (about 0·2μ), unmyelinated fibres, the so-called parallel fibres, oriented exclusively in the laterolateral direction, each making the same kind of histological contacts with a long row of Purkinje cells (about 130 in the human cerebellum). Fig. 1 shows diagrammatically a fragment of such a laterolateral row with incoming and outgoing fibres.

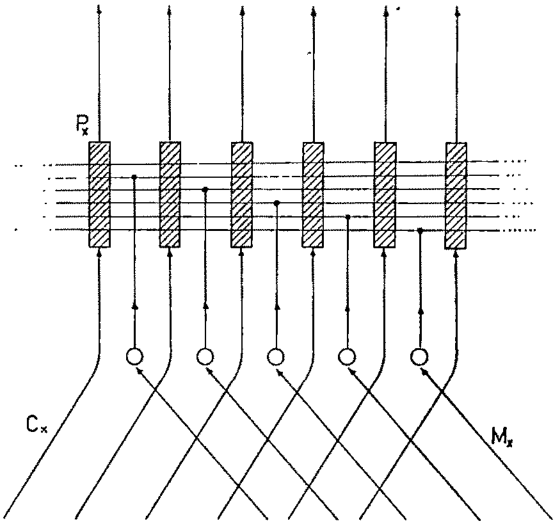

Fig. 1

It has been pointed out[5] that the organization of the cerebellar cortex as it appears in sections cut in the frontal plane becomes understandable if interpreted as a specialization for accurate timing of impulses.

Taking into account the well-known relation between diameter of fibres and velocity of conduction, and interpreting the histological connexion of climbing fibres–Purkinje cells as well as that of parallel fibres–Purkinje cells as an indication of synaptic connexions, this histology allows one to predict widely varying delays interposed between the input in one fibre system (the mossy fibres) and the arrival of activity at different laterolateral distances from the point of input, while, on the contrary, the other input system (the climbing fibres) should influence Purkinje cells after a fixed, short time delay.

Direct physiological support for this interpretation is provided in a paper by Calne[6], who found, in the frog, for some sites of stimulation a fixed delay between stimulus and response in the cerebellar cortex, and for other sites of stimulation delays varying between 20 and 260 msec.

Let $P_x(t)$, $M_x(t)$ and $C_x(t)$ be three sets of functions of time with common indices x. We may interpret them as representing activity in Purkinje cells, mossy fibres and climbing fibres of one laterolateral row, such as those in Fig. 1, with x representing the distance of the elements from an arbitrary origin. For convenience I assume all functions to assume only the values 1 and 0, and consider only integral values of x and t, since these simplifications do not significantly alter our argument. Neglecting the short, fixed synaptic delay between climbing fibres and Purkinje cells, our physiological hypothesis becomes:

$$P_{x_0}(t) = f\left[M_x\left(t - \frac{|x_0 - x|}{v}\right), C_{x_0}(t)\right]$$

where v represents the velocity of conduction in parallel fibres which we suppose to be constant. For a fixed value of C_{x_0}, $P_{x_0}(t) = f\left[M_x\left(t-\frac{|x_0-x|}{v}\right)\right]$, that is, simply a function of a linear combination of time and place of all $M_x(t) = 1$. Explicitly, we may assume the following dependence :

$$P_{x_0}(t) = 1 \text{ if} \left[\sum_x M_x\left(t-\frac{|x_0-x|}{v}\right) + C_{x_0}(t) - \Theta\right] > 0$$

and $P_{x_0}(t)=0$ otherwise, where Θ is a positive integer representing the threshold of Purkinje cells.

This can be used for transforming distances into time intervals and vice versa; in other words, as a clock. Among the many possible modes of operation which suggest themselves I mention only the following examples :

(*a*) Operation as an 'alarm clock'. If $M_{x_0}=1$ only at t_0 and $C_{x_0+\Delta x} = 1$ all the time while all other $C_x=0$, putting $\Theta=2$, there will be a $P_x=1$ only at $t_0 + \frac{\Delta x}{v}$.

(*b*) Operation as a 'stop watch'. $M_{x_0}=1$ only at t_0, all other $M_x=0$. All $C_x=1$ only at the unknown time t. There will be only one $P_x=1$ at t. The distance $|x-x_0|$ will furnish a measure of the unknown time interval $t-t_0$. Again we put $\Theta=2$.

(*c*) Operation as coincidence and time shift detector. Let $M_{x_1}(t)$ and $M_{x_2}(t)$ be two unknown functions. All other $M_x(t)=0$ and all $C_x(t)=0$. We can use $P_x(t)$ with $\Theta=2$ as coincidence detectors between $M_{x_1}(t)$ and $M_{x_2}(t)$. $P_{x_1+x_2/2}$ evaluates $M_{x_1}(t)+M_{x_2}(t)$. Other P_x evaluate the two functions shifted in time one with respect to the other, since :

$$P_x = f\left[M_{x_1}(t) + M_{x_2}\left(t - \frac{x_1 + x_2 - 2x}{v}\right)\right]$$

This embodies the principle of cross-correlation between the two functions, with logical product in place of algebraic product. If we sum each $P_x(t)$ over time, we obtain from one row a direct display of something analogous, in the discrete case, to a cross-correlation function of $M_{x_1}(t)$ and $M_{x_2}(t)$.

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Function of Limb Tremor

THE collaboration of biologists and engineers has led to a hypothesis concerning the biological function of limb tremor in the frequency-range of 6–15 c./sec.

A target spot of light on a cathode ray tube was moved along a horizontal line into different positions at 1-sec. intervals. An operator was given the task of moving a joystick which controlled the movement of a 'follower' spot of light on the cathode ray tube, in the manner required to bring the 'follower spot' into line with the target spot as quickly and accurately as possible. The manually controlled servo-mechanism was a high-gain positional system with no delays in time between control and display movements.

Records of the target and joystick movements were taken on a high-speed pen recorder. Back-lash in the system set an arbitrary lower limit to the amplitude of limb movements which were recorded. Some observations incidental to the main experiment are reported here.

The records of six experimental subjects were analysed for evidence of a relationship between the limb tremor recorded in the range of 6–15 c./sec. and three different states of the subjects' 'controlling' limb : (*a*) at rest ; (*b*) while making the gross rapid movements of primary adjustment ; (*c*) while making the fine movements of secondary adjustment. It was found that 80 per cent of all tremor, of amplitude exceeding the measurement threshold, appeared while making the movements of precise secondary adjustment.

The finding reported agrees with much previous information[1] ; but a new hypothesis has been formulated concerning the function of the type of tremor described.

Mechanical servo-systems have been designed to maintain small oscillations of medium frequency, that is, the servo 'dither' effect because this 'dither' takes up back-lash in the system and minimizes the break-out forces required to overcome frictional resistances when movements are initiated. Peripheral mechanisms for controlling movement of body limbs (for example, muscle spindles, annulo-spiral nerve-endings and associated loops) bear close analogy to such mechanical servo-systems[2], and it is suggested that limb tremor provides the same advantages as 'servo dither'. The fact that tremor was most marked in the phase of the experiment which necessitated the greatest precision of movement lends support to the hypothesis presented.

The functional analogies which have been suggested between limb tremor and servo dither may be applicable to such similar fine tremor of the eyes and of the head in visual and auditory localization.

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Increased Toxicity of Serotonin in Adrenalectomized Animals

ADRENALECTOMIZED rats and mice are more sensitive to anaphylactic shock[1,2], and to the action of histamine[3] and 5-hydroxytryptamine[2,4] which may be involved in the anaphylactic reaction[5], than are intact animals. The toxicity of histamine and of serotonin, as well as anaphylaxis, is increased by the administration of 'pertussis vaccine' and in old and tumour-bearing animals[6]. The present communication reports the toxicity of serotonin in rats and mice in some of these experimental conditions. Sprague–Dawley rats and *ALAL* mice (obtained from the firm ALAL, Milan), of an average weight shown in Table 1, were injected by different routes with 5 - hydroxytryptamine - creatinine - sulphate (kindly supplied by Vister Labs, Casatenovo, Como). Adrenalectomy was performed 72 hr. before injection ;

Table 1. TOXICITY OF SEROTONIN IN VARIOUS EXPERIMENTAL CONDITIONS

Species	No. of animals	Av. body-weight (gm.)	Experimental condition	Route of administration	LD_{50} (mgm./kgm.)	95 per cent confidence limits
Rat	34	204	Intact	s.c.	> 300	—
,,	210	187	Adrenalectomized 72 hr. earlier*	s.c.	14·5	16·4–12·8
,,	41	151	Intact (young)	i.v.	170	204·0–141·6
,,	41	427	Intact (old)	i.v.	46	61·2–34·5
,,	35	177	Adrenalectomized 72 hr. earlier (young)	i.v.	5·8	6·7–5·0
,,	27	397	Adrenalectomized 72 hr. earlier (old)	i.v.	1·2	1·7–0·9
,,	39	269	Walker carcinosarcoma	i.v.	46	61·2–34·5
,,	28	157	Partial hepatectomy 48 hr. earlier	i.v.	155	217·0–111·0
Mouse	60	10	Intact (young)	i.v.	245	303·8–197·5
,,	38	34	Intact (old)	i.v.	148	198·3–110·4
,,	56	19	Intact (adult)	i.v.	223	285·4–174·0
,,	20	19	Intact (adult)	i.p.	> 600	—
,,	20	19	Intact (adult)	s.c.	> 600	—
,,	67	18	Adrenalectomized 72 hr. earlier (adult)	i.v.	0·9	1·4–0·5
,,	87	19	Adrenalectomized 72 hr. earlier (adult)	i.p.	1·9	4·8–0·7
,,	45	18	Adrenalectomized 72 hr. earlier (adult)	s.c.	19	23·4–15·4
,,	45	21	Sarcoma 180 (adult)	i.v.	95	121·6–74·2

* Adrenalectomized animals were kept drinking saline.

partial hepatectomy—according to the method of Higgins and Anderson[7]—was carried out 48 hr. before, and Walker carcinosarcoma was implanted 15 days before.

The mortality, within 24 hr., was observed. The LD_{50} values in mgm./kgm. are expressed as 5-hydroxytryptamine–creatinine-sulphate, and confidence limits were calculated by Litchfield's method[8].

In rats a remarkable increase of serotonin toxicity is observed in older animals as compared with young ones. Adrenalectomy increases the toxicity of serotonin in both young and old animals, when given either by subcutaneous or intravenous routes of administration.

In other experiments, not reported in Table 1, it was observed that animals submitted to adrenalectomy 7 and 14 days before carrying out the test were even more sensitive to serotonin than animals adrenalectomized only 72 hr. before.

Partial hepatectomy has no effect on the toxicity of serotonin, and Walker tumour-bearing animals are relatively more sensitive than normal rats.

In mice, age is not such an important factor in serotonin toxicity as in the rat. Adrenalectomy is more effective in mice than in rats in sensitizing to serotonin toxicity. A factor of 29–36 represents the difference between intact and adrenalectomized rats, whereas in mice, when serotonin is given intravenously, this factor is 239. Mice bearing sarcoma 180 are also more sensitive than normal mice to serotonin.

The reasons for the increased sensitivity to serotonin of adrenalectomized animals are under investigation and will be reported elsewhere.

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Some Properties of Rat Mammary Tissue

IN 1952 Cross and Harris[1] demonstrated that the degree of milk ejection in a lactating rabbit was dependent on the dose of intravenously injected posterior pituitary extract (pituitrin). A minimal effect was seen after injection of 5 mU. of pituitrin and 200 mU. gave a maximal response. On histological evidence, Richardson[2] had suggested that the myoepithelium was responsible for the expression of milk, and Linzell[3] was able to show that oxytocin caused this milk injection by contracting the myoepithelial tissue surrounding the alveoli. He showed further that acetylcholine, histamine and 5-hydroxytryptamine were all capable of causing such contraction when applied directly to lobules of the mammary glands of lactating mice. However, the milk-ejecting response could be made more specific for oxytocin by using atropine and dibenamine, the concentrations needed to block acetylcholine and histamine respectively having no effect on the response to oxytocin. The use of the milk-ejection response in lactating rabbits as an assay method for oxytocin has been described by van Dyke, Adamsons and Engel[4]. These workers could detect no changes in the intramammary pressure to intravenously injected histamine or 5-hydroxytryptamine, and showed that highly purified vasopressin had approximately 17 per cent of the milk-ejecting potency of pure oxytocin, the comparison being on a unit to unit basis. This was in agreement with work on lactating sows by Whittlestone (1955), but Linzell, comparing the two peptides by the amounts needed to produce threshold responses after topical application to mouse mammary lobules, found oxytocin to be even more potent. Highly purified vasopressin had only 8 per cent of the milk-ejecting potency of an equal unitage of pure oxytocin. This discrepancy might be explained as a species difference or by the different routes of administration used, the intravenous route allowing vasopressin to exert its vasoconstrictor action and so possibly enhance falsely the milk-injecting response. Méndez-Bauer, Cabot and Caldeyro Barcia[5] found that rabbit mammary strips in an organ bath gave graded increases in tension to doses of oxytocin and so it seemed worth while to compare vasopressin against oxytocin on rat mammary strips and, in addition, to investigate the usefulness of the rat mammary strip as an assay tissue for oxytocin. Méndez-Bauer, Cabot and Caldeyro Barcia[5] used sections of lactating

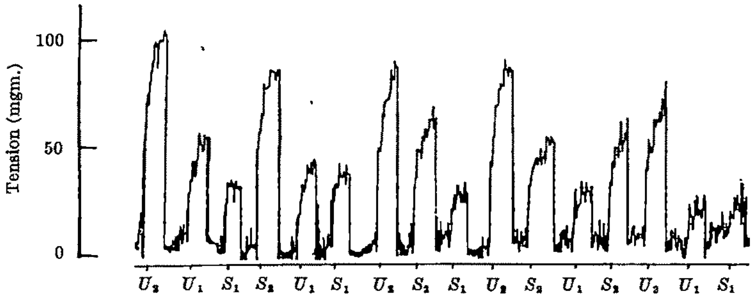

Fig. 1. Graded responses of rat mammary strip to varying doses of oxytocin: S_2, 8 mU.; S_1, 4 mU.; U_2, 10 mU.; U_1, 5 mU.

rabbit mammary glands taken from the nipple up to and including alveoli with associated myoepithelium. In view of the work of Linzell, the rat mammary strips used consisted only of alveoli with myoepithelium and a few small ducts, this tissue being dissected from the lower abdominal mammary glands of a lactating rat anæsthetized with urethane. The Ringer used was that of Edmunds and Cushny[6]; the arginine vasopressin used had been highly purified by the method of Sachs[7]. The oxytocin was supplied by Parke Davis and Co. ('Syntocinon').

To record differences in tension, the mammary strip, suspended in Ringer at a temperature of 37° C. in an organ bath (volume 10 ml.), was attached by a thread to an RCA transducer tube, No. 5734. The transducer acted as a variable resistance in a Wheatstone bridge arrangement. As tension in the muscle increased, the transducer nipple moved slightly, the resistance changed and the current flowing in the circuit was either measured directly on a sensitive micro-ammeter or recorded on paper after amplification. The current flow was directly proportional to the tension exerted on the transducer over the weight range 0–1,000 mgm., and all experimental work was within this tension range.

At the beginning of each experiment the strip was placed under an initial tension of 500 mgm. and the bridge balanced, using an adjustable resistance, so that no current flowed. Doses of oxytocin were then added at approximately 5-min. intervals, the Ringer being changed twice between each addition of oxytocin. Typical responses to four different doses of oxytocin are shown in Fig. 1. To determine the precision of the oxytocin assay, 4 doses, 2 high and 2 low, were chosen. Each dose was given once in any block of four, and one complete assay consisted of 16 responses. A high and low dose was arbitrarily selected as being the solution of 'unknown' concentration and the experimental potency ratio was calculated and compared with the known value. Table 1 shows the results of a series of five such assays.

Table 1. PRECISION OF RAT MAMMARY STRIP ASSAY METHOD FOR OXYTOCIN

R_t	R_c	E	R_1	R_2	Percentage of fiducial limits $P = 0·05$
1·25	1·26	0·8	1·114	1·434	± 12·7
1·25	1·09	12·8	0·910	1·285	± 17·1
1·25	1·22	3·0	1·100	1·355	± 10·5
1·25	1·25	0·2	1·016	1·542	± 21·0
1·25	1·22	3·0	1·052	1·422	± 15·2

E is the percentage difference between R_t, the theoretical potency ratio and R_c, the experimentally calculated potency ratio. R_2 and R_1 are the upper and lower limits of R_t, calculated for $P = 0·05$.

The mean difference between the theoretical and the calculated potency ratios was 3·9 per cent, and the mean fiducial limit ($P = 0·05$) was 15·3 per cent.

Vasopressin was now assayed against oxytocin on mammary strips taken from rats which had been suckled by large litters (greater than 6) for 1–4 weeks. The age of the litter did not appear to affect the relative potency of vasopressin, which was 3·2 ± 0·24 per cent (9) that of an equal unitage of oxytocin.

Thus, mammary tissue *in vitro* has the advantage of being more specific for oxytocin than the *in vivo* preparation. Rat mammary strips provide an accurate and convenient assay method for oxytocin. Each strip can be used for approximately 3 hr., and one rat kept alive under urethane anæsthesia provides many strips over a long period of time. As described here the method is not very sensitive, 2–4 mU. being needed to produce a small response. Using a 2 ml. bath, 0·5–1·0 mU. would, presumably, give the same response, and should an ultra-sensitive assay method be required, a superfused mammary strip could be used. However, it has often been shown that such highly sensitive assays are of low precision.

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Effect of 9α-Fluoro-11β-hydroxy-17-methyltestosterone ('Ultandren') on the Testis of the Immature Rat

'ULTANDREN' (Ciba, Ltd.) is known to be an extremely potent oral androgen, approximately ten times as effective as methyltestosterone[1]. In a preliminary investigation of its effects on the testis of the immature rat comparable doses of 'Ultandren' (0·05 mgm.) and methyltestosterone ((0·5 mgm.) dissolved in 0·5 ml. polyethylene glycol were administered daily by mouth for 10 days to litters of rats aged 28–31 days. To a third group of litter mate controls 0·5 ml. of polyethylene glycol was administered daily.

After killing, one testis was removed and fixed in formol saline. Equatorial transverse sections 8μ thick were cut and stained by the periodic acid–Schiff method, with counterstaining by iron hæmatoxylin. This stain accurately depicts the various stages of the spermatogenic cycle[2].

In each testis the number of tubules showing the acrosome phase of spermiogenesis was expressed as a

Table 1

Treatment	Percentage of tubular cross-sections showing acrosome phase of spermiogenesis	Mean percentage
'Ultandren', 0·05 mgm./day	100, 83, 85, 97, 100, 82	91·1 ± 8·71
Methyltestosterone, 0·5 mgm./day	57, 50, 56	54·3 ± 3·79
Control	67, 58, 94, 72	72·8 ± 15·29

percentage of not less than 60 tubular cross-sections observed in 3 non-adjacent testicular sections. The results are indicated in Table 1.

Comparison of the results in control, 'Ultandren'- and methyltestosterone-treated animals shows that with $n_1=2$, $n_2=10$, $F=13\cdot988$, which is significant at the 0·1 per cent level, thus indicating the heterogeneity of the results of the different treatments.

It is apparent that in 'Ultandren'-treated rats the percentages of tubules showing the acrosome phase of spermiogenesis are higher than in the control animals. This difference is significant ($t=2\cdot34$, $n=8$, $P<0\cdot05>0\cdot025$).

When the methyltestosterone-treated and control animals are compared, the testes of the former group show a lower percentage of tubules in the acrosome phase. However, these differences are not significant at the 5 per cent level ($t=1\cdot95$, $n=5$, $P>0\cdot1<0\cdot2$).

Although the results using methyltestosterone are not significant it may be a depressor of spermatogenesis in the doses used. 'Ultandren', on the other hand, in approximately equally androgenic doses, accelerates the rate at which the acrosome phase of spermiogenesis is achieved in the tubules of the immature testis. This finding is of interest, since previous workers have been unable to accelerate the normal process of maturation of the testis[3-6]. It is proposed to study the matter in greater detail.

I am indebted to Ciba Laboratories, Ltd., for a grant for technical assistance and for supplies of the steroids and solvents used in this experiment.

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HÆMATOLOGY

Sensitivity of Hæmoglobin to Oxidation in Various Conditions

When intact red cells are treated with weak solutions of sodium nitrite in isotonic saline, or hæmolysed red cell suspensions are treated with weak solutions of potassium ferricyanide, the red colour changes to brown as the oxyhæmoglobin is oxidized to methæmoglobin. Infants have long been known to be particularly sensitive to this change[1-3]; but it has only recently been shown[3,4] that this increased sensitivity is also present in children of both sexes up to the age of puberty.

Blood samples have been obtained from pregnant women (100), patients with carcinomata (51), other hospital patients (52) and medical and physiotherapy students (100). 96 per cent of pregnancies of more than 6-weeks duration exhibited increased sensitivity of the hæmoglobin to oxidation. A similar, but less-marked, change occurred in the patients with definite carcinomata. Of the other hospital patients only those with acute coronary thrombosis, hæmolytic blood diseases, multiple fractures or severe rheumatoid arthritis showed any deviation from the normal. No case of increased sensitivity occurred in any of the students.

The sensitivity of the hæmoglobin to oxidation rapidly returned to normal after delivery, and, in the few cases so far examined, following complete extirpation of the malignant tumour. The cause of these changes is as yet unknown, and the phenomenon is at present being investigated. The findings will be reported in full elsewhere.

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Experimental Oxidation of Hæmoglobin: its Relation to Growth-rate in Rats

The sensitivity of oxyhæmoglobin to oxidation with nitrites or ferricyanide is increased in children, pregnant women and patients with carcinomata (refs. 1–4 and preceding communication). The change-over from the increased sensitivity of childhood to the adult reaction occurs at about the age of puberty. To investigate the relationship between this reaction and the hormonal changes of puberty, the phenomenon has been studied in rats, as the growth-rate and pubertal changes can be determined and separated much more readily in small mammals than in man.

Rats' hæmoglobin has approximately the same resistance to oxidation as human hæmoglobin and exhibits the same increased sensitivity in the young and pregnant. The growth-rate of some 60 female Lister hooded Worcester sub-strain rats was determined and related to the oxidation sensitivity of their hæmoglobin. Fig. 1 shows that the change-over from the juvenile type of resistance to oxidation occurs at 40–50 gm., which corresponds closely to the time of most rapid change in the growth-rate (40–

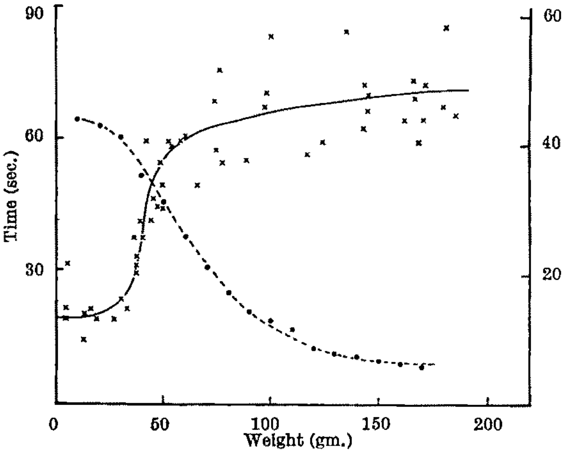

Fig. 1. —×—, Conversion time ; - - ● - - , growth-rate

50 gm.). Puberty, in this strain, as judged by vaginal opening, occurs at 100–110 gm.

These findings strongly suggest that the resistance of hæmoglobin to methæmoglobinization is more closely connected with changes in growth-rate than with any hormonal change associated with puberty.

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Incomplete Antibodies to Chicken Red Cell Antigens

The evidence supporting the existence of incomplete antibodies to chicken red cell antigens is inconclusive[1], and the following experiments were carried out to see if incomplete antibodies, similar to those against the red cell antigens of man, would be demonstrated :

(1) *Heat inactivation.* Sera containing complete agglutinins were diluted 1/4 and then heated for 10 min. at 70° C. The heated sera were then tested against the appropriate cells in saline, by an albumin replacement technique[2] and by a spin antiglobulin technique using rabbit anti-chicken serum. The results obtained are given in Table 1 (scoring as suggested by Dunsford and Bowley[2]).

Table 1

Serum No.	Specificity	Red cells	Saline	Albumin	Antiglobulin test
244 *B*	anti-*B* 32	*B* 32/32	–	–	*V*
296 *A*	anti-*B* 38	*B* 38/38	–	–	+ +
242 *A*	anti-*B* 43	*B* 43/43	–	–	+ +
358 *A*	anti-*B* 39 and *B* 41	*B* 32/39	–	–	+ +
380 *A*	anti-*B* 44	*B* 44/46	–	–	+ +
382 *A*	anti-*B* 45 and *B* 46	*B* 46/47	–	–	*V*
291 *A*	anti-*B* 42	*B* 42/*B* 42	–	–	+ +

(2) *Titration.* Each of the sera of Table 1 was serially diluted, using saline as diluent, and tested against the corresponding cells suspended in saline. The results were read by microscope. The red cells from all the tests giving no agglutination were then well washed four times and tested with anti-chicken serum by the spin antiglobulin technique. The first two positive results of each titration obtained by this method were interpreted as possible enhancement of the reaction of the complete antibody. Any positive results beyond these two were considered due to incomplete antibodies. All seven sera showed this evidence and the results given in Table 2 are typical.

Table 2

Serum 296 *A*	1	5	10	20	40	80	160	320	640	1,280
Saline	*A*	*A*	*A*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
Antiglobulin	Not tested*			*A*	*A*	*A*	*A*	*A*	*N*	*N*

A, Agglutination; *N*, no agglutination.
* Not tested because agglutination was strong.

(3) Sera were obtained from twelve birds which had been inoculated with red cells to produce specific antisera but, judged by saline agglutination methods, they had not been immunized. Serum from each bird was examined against the corresponding cells by saline, albumin and spin antiglobulin techniques. Four of the sera were found to have incomplete antibodies demonstrable by the antiglobulin technique only. These sera were also diluted and heated as in the first experiment and re-tested by the antiglobulin technique. The antibodies were still demonstrable, showing them to be thermostable.

These experiments leave no doubt as to the existence of incomplete antibodies to chicken red cells. Fuller details of the methods used, specificity of the antibodies, etc., will be published elsewhere later.

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Circulating Platelets in Mice subjected to Simultaneous X-ray and Ultra-violet Irradiation

The circulating platelet count of mice increases significantly after ultra-violet irradiation. Five to seven days after a single whole-body ultra-violet irradiation the increase is about 100 per cent. Parabiosis experiments, as well as the positive effect of isologous serum transfer, suggested that humoral factor(s) are responsible for the thrombocytopoietic effect and the agent seemed to be a protein-like macromolecule[1] as already described for human serum[2]. In the present experiments we investigated the simultaneous action of whole-body X-ray irradiation, that is, a procedure causing severe thrombocytopenia, and ultra-violet radiation, that is, a procedure causing marked thrombocytosis.

Mice weighing 20 gm. were subjected to ultra-violet irradiation using a Hanau quartz-lamp (no filter, 210 V., 5·2 amp., 50 cm. distance, 30 min.). The dose of whole-body X-ray irradiation was 300 r. (0·5 copper, 180 kV., 10 m.amp., 50 cm. distance). Platelets were counted by a direct phase-contrast microscope method as described by Feissly and Lüdin[3]. 0·2 ml. sera from ultra-violet irradiated mice was injected into the tail vein of recipients.

In the first experiments sixty mice were subjected to ultra-violet irradiation and five animals were killed at each interval marked on Fig. 1. The serum of these animals was given intravenously to 5–10 recipient mice in each group. Platelets were enumerated

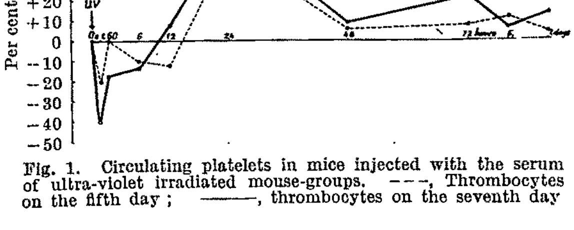

Fig. 1. Circulating platelets in mice injected with the serum of ultra-violet irradiated mouse-groups. – – –, Thrombocytes on the fifth day ; ———, thrombocytes on the seventh day

before and 5 and 7 days after serum injections. As shown, the most pronounced thrombocytosis (50–60 per cent) resulted with sera taken 24 hr. after irradiation. Although the platelet count of irradiated animals did not diminish, early serum samples, up to 6 hr. after irradiation, tended to diminish the platelet count of recipient mice.

As Fig. 2 demonstrates, about 60 per cent diminution of circulating platelets followed the 300 r. whole-body X-ray irradiation of mice 10 days later. However, if ultra-violet irradiation was given 24 hr. before this X-ray dose, (*a*) the platelet count did not diminish at all, and an average 21 per cent increase occurred on the tenth day. Furthermore, as demonstrated by (*b*), even ultra-violet irradiation administered 24 hr. after X-rays significantly reduced platelet count diminution.

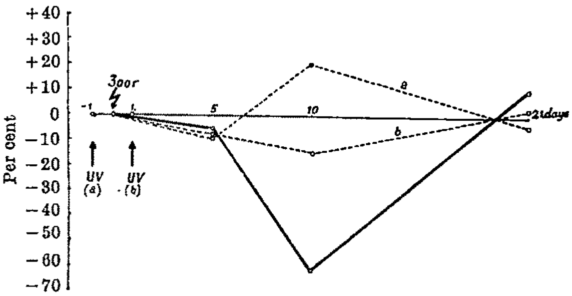

Fig. 2. Circulating platelets in mice subjected to simultaneous X-ray and ultra-violet irradiation. The solid line represents the platelet count of X-ray irradiated animals

In Fig. 3 we see that sera taken from mice treated with ultra-violet irradiation 24 hr. before prevent thrombocytopenia when given 24 hr. after the above X-ray dose (*a*), but cannot prevent thrombocytopenia 5 days after the X-ray injury (*b*).

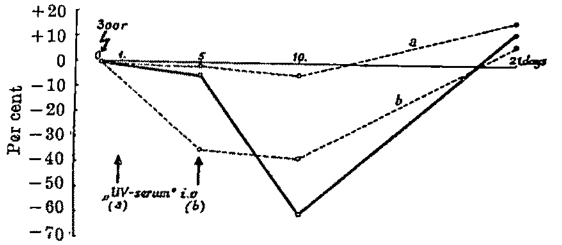

Fig. 3. Circulating platelets in X-ray irradiated mice injected with sera from ultra-violet irradiated mouse-groups. The solid line represents the platelet count of X-ray irradiated animals

X-ray induced thrombocytopenia results from the injury of the megakaryocyte system. It is of some interest, therefore, that the agents which are responsible for the thrombocytosis of whole-body ultra-violet irradiation are effectively present even in mice treated with 300 r. Perhaps these agents do not originate from the bone marrow. On the other hand, the failure of an otherwise effective ultra-violet-light activated serum 5 days after total-body irradiation suggests that the target organ of this thrombocytopoietic factor is the bone marrow.

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RADIOBIOLOGY

Incorporation of Thymidine into Deoxyribonucleic Acid by Extracts from Bacteria exposed to Ultra-violet Light

Ultra-violet light induces a lag in the synthesis of deoxyribonucleic (DNA) acid in *Escherichia coli*[1]. After a dose-dependent incubation interval, the irradiated bacteria will resume synthesis at almost the same rate as prior to exposure. Recently, various investigators[2–4] have shown that blockage of ribonucleic acid (RNA) or protein synthesis prevents the recovery of DNA synthesis in bacteria irradiated with ultra-violet light. The capacity of the bacteria to synthesize DNA after exposure is regained with the initial doubling of RNA in the culture[5].

In this investigation, by measuring ^{3}H-thymidine incorporation into an alkaline-stable, DNAase-sensitive, cold acid-insoluble fraction[6], it has been determined that following exposure to doses of ultra-violet light which inhibit net DNA synthesis *in vivo*, cell extracts showed unimpaired thymidine phosphorylase and DNA polymerase activity. In addition, an increase in activity was noted in the extracts from exposed cells that had been re-incubated in a growth medium. In the system used here ^{3}H-thymidine incorporation into DNA depends on the presence of the other three deoxyriboses in the triphosphate form[6]. This strongly suggests that we are measuring DNA replication rather than thymidine replacement in the pre-formed DNA. Kornberg *et al.*[7,8] and Lehman *et al.*[9] have shown that the polymerase present in a crude enzyme preparation from another strain of *E. coli* is capable of net DNA synthesis.

Cultures of *E. coli* strain *B/r* in the exponential phase of growth were exposed to ultra-violet light from a Hanovia model 30,600 high-pressure mercury vapour arc lamp. The techniques used for radiation exposure, determination of numbers of colony-forming organisms and the measurement of RNA, DNA and protein have been described[10]. The coli suspension at a depth of 1·5 mm. was exposed to 980 ergs per mm.^{2} ultra-violet light while being stirred vigorously. The cells were broken in a French pressure cell to yield the extracts tested[6]. By centrifugation at 10,000*g* for 20 min., a pellet of intact cells and cellular debris was formed. The resultant extracts contained less than 10^3 colony-forming bacteria per mgm. protein.

This dose of ultra-violet light does not decrease the activity of the thymidine-incorporating enzymes found in the extracts from the exposed cells (Table 1). This exposure is effective in delaying *in vivo* synthesis of DNA for a period of 75 min. Furthermore, during this period, in which synthesis of DNA is blocked, a considerable increase in RNA, protein and the thymidine-incorporating enzymes is observed. Additional experiments with higher doses of ultra-violet light have shown that exposures sufficient to reduce survival as low as 0·1 per cent do not diminish the capacity of extracts from such cells to incorporate thymidine into DNA.

If the enzymatic activity present in the extracts reflects the enzyme content of the intact cells, then these results indicate that the blockage of synthesis of DNA by ultra-violet cannot be ascribed to an effect on the polymerase or the enzymes responsible for phosphorylation of thymidine. These results are comparable to those of Billen, who determined that

Table 1. EFFECT OF ULTRA-VIOLET LIGHT ON SURVIVAL AND SYNTHESIS OF NUCLEIC ACIDS AND PROTEIN BY *Escherichia coli* STRAIN B/R AND ON THE ^{3}H-THYMIDINE-INCORPORATING CAPACITY OF BACTERIAL EXTRACTS

Ultra-violet exposure 980 ergs/mm.^{2}	Incubation time (min.)	Intact bacteria				Extracts	
		RNA μgm./ml.	DNA μgm./ml.	Protein μgm./ml.	Colony-forming bacteria/ml.	^{3}H-thymidine incorporation*	Relative enzymatic activity†
–	0	25·2	5·4	82·9	$2\cdot5\times10^8$	99	400
+	0	23·2	5·0	80·8	$4\cdot6\times10^7$	87	440
+	30	28·0	5·2	107·0	$6\cdot0\times10^7$	134	550
+	60	32·6	5·2	125·8	$4\cdot7\times10^7$	181	430

* μμmoles of ^{3}H-thymidine incorporated in 20 min. The extract used per reaction vessel is equivalent to 4 ml. of the bacterial suspension

† μμmoles of ^{3}H-thymidine incorporation into DNA per mgm. of extract protein.

extracts from cells lacking a component of the DNA synthesizing system because of amino-acid starvation[11] possessed enzymatic activity for DNA synthesis *in vitro* equivalent to cells fully capable of DNA synthesis[6]. It seems clear that the blockage of DNA synthesis, either by ultra-violet light or amino-acid starvation, cannot be ascribed to an effect on DNA polymerase *per se*. While the possibility exists that the extraction procedure may remove the enzymes from some inhibitory effect of the ultra-violet light, this possibility seems less likely in the case of the enzymes synthesized after exposure to ultra-violet light. Thus, at 60-min. incubation, the enzymatic activity in the extract has doubled while DNA synthesis *in vivo* remains blocked for an additional 15 min.

Though these investigations have not measured the activities of the kinases of the nucleosides other than thymidine, it seems unlikely that the blockage of DNA synthesis by ultra-violet light can be ascribed to an inhibition of synthesis of any of the deoxyribonucleotides, since Kanizir and Errera[12] found extensive accumulation of deoxyribonucleotides *in vivo* following exposure to ultra-violet light. Rather, it would appear that we must ascribe the ultra-violet light block to an effect on some other component of the *in vivo* DNA-synthesizing system which is restorable through RNA and protein synthesis following exposure. In view of the results of Doudney and Haas[5], which relate ultra-violet-induced mutation to the restoration of this component of the DNA-synthesizing system, it may be predicted that the component plays an intimate part in the transfer of genetic specificity from parental to daughter DNA.

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Nitrogen Protection of Fecundity and Fertility in Female *Habrobracon* treated with X-rays

BRACONID anoxia experiments were performed as a logical sequel to investigations with ingested chemical protectors[1]. In both cases, damage was assessed by scoring the eggs produced per unit of time and the larvæ emerging therefrom. Eggs are dispensed in exact order from the single developmental series identical for each of the four ovarioles. This feature makes it possible to correlate quantitative modifications in the number of eggs, and their hatchability, with the cellular state at the time of irradiation.

Cysteine or glutathione fed to female *Habrobracon* prior to irradiation protected a significant fraction of the cells which afterwards gave rise to the eggs laid toward the end of the first week and at the beginning of the second[1]. At the time of exposure the cells involved were mitotically active and engaged in transition from oogonia to differentiated trophocyte–oocyte units. According to a widely accepted hypothesis, the sulphydryl types of protective compounds operate by reducing the availability of oxygen in irradiated cells. The present experiments were carried out to determine whether anoxia by gas replacement would protect only the mitotically active cells or whether protection could be extended to all the cell types present in the wasp ovariole.

The virgin females used were isolated from stock No. 33 (Whiting) of *Habrobracon juglandis* = *Bracon hebetor*. Groups of 15 were irradiated in the small plastic chambers developed during the examination of gas modification of radiosensitivity of developing *Habrobracon*[2]. In gas replacement experiments nitrogen was passed through the chamber for two minutes before sealing. Including two minutes of such flushing, the wasps were in nitrogen for a total of 15 min. X-rays were delivered by the Woods Hole Marine Biological Laboratory generator, which operates at 28 m.amp. and 200-kV. peak with filtration equivalent to 0·2 mm. of copper.

Exposures in both air and nitrogen were made at 2,250 r. and 4,500 r. A higher series of doses ranging from 9,000 r. to 13,600 r. were delivered in nitrogen. An unirradiated group given nitrogen for 15 min. demonstrated that such treatment did not decrease egg production.

A nitrogen atmosphere afforded nearly complete protection from the 2,500 r. dose. Except on days 3, 4, and 5 egg production was not significantly lower than controls. In fact, on a majority of days control values were exceeded. Fig. 1 presents egg production after higher doses selected for contrast and comparison. 4,500 r. delivered in air is near the threshold for permanent infecundity[3]; but if delivered in nitrogen is found to be much less effective. Indeed, during the latter part of life, egg production compares well with

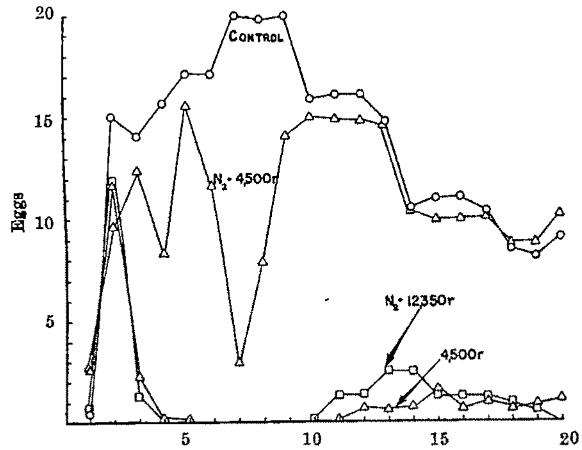

Fig. 1

control values. The eggs deposited during this period are derived from cells which were oogonia at the time of treatment.

The nitrogen series provided a family of curves demonstrating that low egg production equivalent to that following 4,500 r. in air is attained between 12,350 and 13,600 r. Records for the former are shown in Fig. 1. The higher dose resulted in only scattered eggs on the 12th to 14th days. For the sake of clarity the family of curves obtained in the nitrogen series is not shown in the graph. In a regular expected order they would fill in the space between the 4,500 r. and the 12,350 r. curves.

A summary of hatchability for the eggs of Fig. 1 is shown by Table 1. The basis of comparison reflects cytological conditions of the developing units at the time of irradiation. Protection afforded by the replacement of air by nitrogen is apparent not only in a significantly higher hatchability after 4,500 r. but also in hatchability which approaches control values after the eleventh day. Standard errors range from 1 to 3 per cent. Hatchability records for the 12,350 r. nitrogen experiment are included as a demonstration that larvæ are obtained from eggs laid during both the decline phase and recovery phase of egg production. No exactly comparable data are available.

These results demonstrate that the protective effect of gas replacement anoxia is afforded to all types of cells in the polytrophic ovariole. They comprise differentiated, transitional and primitive cells. The progress of meiotic prophase, mitosis, and interphase characterizes the respective periods, with mitotic activity explaining the radiosensitive 6–10 day period. Limitation of protection by SH compounds to the sensitive period might indicate merely a selective concentration of the chemical substances and localized reduction of oxygen availability. Alternatively, the present results would raise the question of whether reduced oxygen is the explanation of sulphydryl protection. This alternative is an interesting possibility in view of the recent demonstration that the mode of action of cysteine includes one reaction that can occur independently of oxygen[4].

Table 1. PERCENTAGE OF HATCHABILITY FOR EGGS OF FIG. 1. 5-DAY PERIODS REFER TO THE CYTOLOGICAL CONDITION OF THE CELLS AT THE TIME OF IRRADIATION FROM WHICH EGGS MAY AFTERWARDS BE OBTAINED DURING THE PERIODS INDICATED

X-ray	Gas	Days after irradiation			
		1–5	6–10	11–15	16–20
None	Air	93·2	95·1	91·8	85·6
4,500 r.	N_2	79·9	74·6	90·7	80·3
	Air	65·2		14·4	52·7
12,350 r.	N_2	51·3		40·2	0
Cytological stage when irradiated		Oocytes	Transitional cells	Oogonia	Oogonia (senility)

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Reversal of γ-Ray-induced Dormancy of Potato Tubers by Gibberellic Acid

POTATOES (var. Up-to-date), which were free from sprouting, were packed in polyvinyl chloride bags and irradiated with 8,000 and 12,000 rads of γ-rays during a 'shut-down' period of the swimming-pool-type reactor *Apsara*. The dose-rate during the period of irradiation was 200 rads/min. Based on a sample of 81 potatoes in each case, the numbers sprouting, 35 days after irradiation, were 81 in control, 9 with a dose of 8,000 rads and none with a dose of 12,000 rads.

γ-Ray-treated potatoes were dipped for 5 min. in 250 and 500 p.p.m. solutions of sodium salt of gibberellic acid, 35 days after irradiation. In the case of potatoes irradiated with 8,000 rads, only those free from sprouting were chosen for treatment with gibberellic acid. The control potatoes were dipped in distilled water for 5 min. The results are recorded in Table 1. In the potatoes treated with 8,000 rads of γ-rays, 20 out of 20 potatoes sprouted in 10 days with 500 p.p.m. and in 15 days with 250 p.p.m. of gibberellic acid. Similarly, in the case of potatoes that had received a dose of 12,000 rads of γ-rays, 20 out of 20 potatoes sprouted in 15 days with 500 p.p.m. and in 20 days with 250 p.p.m. of gibberellic acid.

As a result of irradiation with 8,000 and 12,000 rads of γ-rays, the buds on the potatoes degenerated. Similar degeneration of potato buds as a result of γ-irradiation has been observed by other workers[1,2] also. In the present experiment, new buds arose

Table 1. EFFECT OF GIBBERELLIC ACID ON THE REVERSAL OF γ-RAY-INDUCED DORMANCY OF POTATO TUBERS STORED AT 23–32° C. (RELATIVE HUMIDITY, 75–95 PER CENT)

Treatments (γ-radiation in rads : gibberellic acid in p.p.m.)	No. of tubers	No. sprouting after, days			
		5	10	15	20
(1) 8,000 : 0·0	20	Nil	Nil	Nil	Nil
(2) 8,000 : 250	20	7	16	20	20
(3) 8,000 : 500	20	13	20	20	20
(4) 12,000 : 0·0	20	Nil	Nil	Nil	Nil
(5) 12,000 : 250	20	5	12	17	20
(6) 12,000 : 500	20	8	14	20	20

adjacent to those degenerated after treatment with gibberellic acid.

It appears that γ-irradiation impairs a possible endogenous gibberellin-synthesizing system in the potato and that the effects of this damage can be counterbalanced by exogenous gibberellic acid.

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Incorporation of Phosphoryl-choline labelled with Phosphorus-32 into the Liver Phospholipids of Rats treated with Growth Hormone

THE rate of incorporation of acetate labelled with carbon-14 into the liver phospholipids of rats treated with growth hormone is reduced to about 50 per cent of that found in normal animals[1]. On the other hand the rate of incorporation of orthophosphate labelled with phosphorus-32 in similarly treated animals is increased by about 30 per cent[2]. It thus appeared that growth hormone might affect the rates of synthesis of the fatty acid skeleton and the phosphoryl-base moiety separately.

Table 1. EFFECT OF PITUITARY GROWTH HORMONE ON THE INCORPORATION OF ORTHOPHOSPHATE-^{32}P, GLYCINE-2-^{14}C AND ACETATE-1-^{14}C INTO PHOSPHOLIPIDS OF RAT LIVER SLICES

Precursor	Specific activity (counts/100 sec./mgm.)			Total incorporation (counts/100 sec./flask)		
	Hormone-treated	Control	$\frac{\text{Treated} \times 100}{\text{Control}}$	Hormone-treated	Control	$\frac{\text{Treated} \times 100}{\text{Control}}$
Orthophosphate-^{32}P	1,024	1,042	98	8,735	6,731	130
Glycine-2-^{14}C	969	977	99	8,266	6,311	131
Acetate-1-^{14}C	139	805	17	1,186	5,200	23

Adult female rats were injected with 1 mgm. pituitary growth hormone 6 hr. before killing. The rats were killed and the livers rapidly excised and slices cut on a Stadie–Riggs microtome[3]. 400 mgm. slices were incubated for 3 hr. in 5 ml. Krebs–Ringer bicarbonate (gassed with 95 per cent oxygen–5 per cent carbon dioxide) in the presence of either 0·5 μc. $H_3{}^{32}PO_4$, glycine-2-^{14}C or sodium acetate-1-^{14}C. At the end of the incubation the slices were collected and homogenized in 5 ml. 5 per cent trichloroacetic acid. The phospholipids were separated, plated and counted as described previously[1]. The results of such an experiment are given in Table 1.

It will be seen that, on the basis of specific activity, there is no change in the rate of incorporation of either orthophosphate or glycine, but acetate incorporation is greatly reduced. However, in view of the massive mobilization of lipids to the liver[4], the values for the specific activities are not as meaningful as the total counts incorporated, also given in Table 1. From these it will be seen that there is a reduced rate of incorporation of acetate, but an increased incorporation of phosphate and glycine which fully confirms the previous results. Because there is a strong possibility that the phospholipins as extracted above might be more or less contaminated with phosphatidic acids, the phospholipids obtained were further purified by adsorption chromatography by Kennedy's method[5]. The results obtained with this purified material were the same as those already described.

Since the glycine is only incorporated into the base of phospholipids[6], it would thus appear that both the phosphate and the base are incorporated more rapidly in the hormone-treated animals than in the controls. Since this could be due either to a more rapid rate of formation of phosphoryl-base or, alternatively, to a more rapid incorporation of the phosphorylated base into phospholipin, we have investigated the incorporation of phosphoryl-choline into the liver phospholipids of normal and growth hormone-treated animals.

Phosphoryl-choline labelled with phosphorus-32 was prepared according to Riley's method[7], and calcium ion was removed by use of an exchange resin '*IR*–120' (K^+ cycle). The rats were injected as above and the livers removed. A 'particle fraction', prepared according to Kennedy[8], was used for the incorporation experiments. The incubation period was 1 hr. The phospholipids were extracted and purified according to Kennedy[8] and counted as before. The results are given in Table 2.

In each of the three experiments the phosphoryl-choline was incorporated into the phospholipids of the injected animals at a rate about one-third greater than in the control animals, that is, at about the same rate as the incorporation of phosphorus and glycine.

To account for these results two explanations may be advanced: (1) that there exists a smaller pool of phosphoryl-choline in the livers of the injected animals; (2) that there is an increased rate of phospholipin synthesis in the livers of the hormone-treated animals. In this second case the very much lower rate of incorporation of acetate in the injected animals may be accounted for in terms of the inhibition of some step between acetate and diglyceride, or alternatively to a dilution of the diglyceride pool in the livers of growth hormone-treated animals. It has already been suggested that the incorporation of

Table 2. EFFECT OF PITUITARY GROWTH HORMONE ON THE INCORPORATION OF ^{32}P-CHOLINE INTO THE PHOSPHOLIPIDS OF RAT LIVER 'PARTICLES'

Exp.	^{32}P-Choline added (μmoles/flask)	^{32}P-Choline incorporated (mμmoles/flask)		
		Growth hormone-treated	Control	$\frac{\text{Treated} \times 100}{\text{Control}}$
I	4	6·3	4·6	137
II	18	12·3	9·5	129
III	20	21·6	14·1	153

Each flask contained initially 100 μmoles of phosphate buffer, *p*H 7·4, 10 μmoles ATP, 1·5 μmoles CTP, 20 μmoles $MgCl_2$, ^{32}P-Choline (33,700 c.p.m. per micromole) as indicated and 'particles' derived from 0·4 gm. liver. 1·5 μmoles of ATP and 0·03 μmoles of CTP were added every 12 min. during the course of the experiment. The flasks were incubated at 37° C. for 1 hr.

acetate into fatty acids is greatly reduced in growth hormone-treated rats[1] and experiments are now in progress to establish whether there is another block between fatty acids and diglycerides and whether there is a dilution of the diglyceride pool.

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PATHOLOGY

The Liver in Infective Hepatitis

THE changes in the liver during the acute stage of infective hepatitis consist of marked alterations to the liver cells, especially in the central regions of the lobule, and an infiltration of the portal tracts and sinusoids with inflammatory cells. Except in fatal cases, necrosis of liver cells is infrequent[1-4]. The disease is thought to be a virus infection of the hepatic parenchyma; but the causative organism or organisms have so far eluded laboratory isolation[5].

This communication reports the results of work on 26 needle biopsies which have been carried out on the livers of African soldiers suffering from infective hepatitis. Biopsies were performed between 5 and 73 days after the onset of disease. For comparison, liver biopsies have also been performed on healthy human volunteers. Liver specimens have been studied by a variety of cytological and cytochemical methods and by electron microscopy after conventional osmium fixation and methacrylate embedding.

Histological examination of the infective hepatitis livers showed the typical parenchymal and stromal changes of this disease. Although the sequence of liver cell alterations could not always be accurately determined, the earliest change, as shown by light microscopy, appears to be enlargement of the nucleolus and a concurrent increase in ribonucleoprotein. This increase in ribonucleoprotein is progressive, and later results in marked enlargement and distortion of the nucleolus. The nucleus also becomes swollen; but an increase in nucleoprotein has not been detected in the nucleoplasm. While these changes are taking place, the cytoplasm shows a progressive increase in ribonucleoprotein which commences in the region of the nucleus but later fills most or all the cell. Cytochemical tests indicate that this newly formed ribonucleoprotein contains the amino-acids, arginine, typtophan and tyrosine, and small amounts of cysteine and cystine. It does not contain deoxyribonucleic acid.

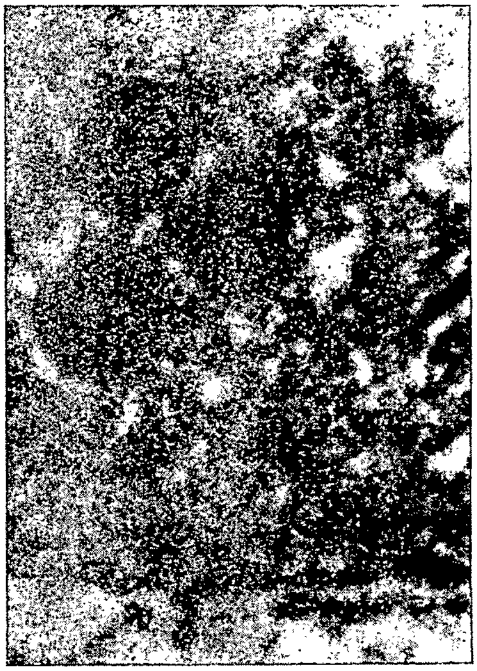

Fig. 1. Periphery of liver cell showing numerous roughly spherical bodies in the cytoplasmic matrix. (× 23,700)

In some cells the mitochondria show variable degrees of swelling; in others they appear increased in number. The Golgi complex is clearly seen in healthy biopsies, but because it is not demonstrable in infective hepatitis livers it is thought to have undergone degeneration.

Electron microscope studies on 14 infective hepatitis livers reveal marked cell changes in all cases. In some instances the nucleoplasm exhibits an increased amount of granular material and the nucleoli are often distorted. Within the cytoplasm the endoplasmic reticulum is usually dilated, and the mitochondria are often grossly swollen. In some cells, however, the mitochondria are increased in numbers, fresh forms arising from existing mitochondria. Not infrequently the microbodies are increased also. The cytoplasmic matrix of some liver cells contains numerous granules which measure approximately 21·5 mμ in diameter and, therefore, are larger than the ribonucleoprotein-containing granules of healthy cells. Often 6–12 of these granules appear to be aggregating to form irregularly spherical bodies measuring 76–103 mμ in diameter (Fig. 1). Such spherical bodies were seen in 7 of the 14 infective hepatitis livers but in none of 6 healthy livers. In one case they were present 29 days after the onset of disease. The bodies are always confined to the cytoplasmic matrix, but occasionally they appear to be passing from the liver cells into the sinusoids and are present in the blood serum. Spherical bodies, varying between 30 and 70 mμ, have already been visualized in the cytoplasm of liver cells in infective hepatitis[6,7].

Although it has not been possible to determine accurately the progression of liver cell changes in infective hepatitis, it is thought that it parallels the sequence of alterations which occur in the liver cells of rhesus monkeys infected with yellow fever[8]. In this latter condition there is an increased synthesis of ribonucleoprotein in the nucleolus. The newly formed ribonucleoprotein passes in a finely granular state through the nucleoplasm to the cytoplasmic matrix. In the matrix the granules aggregate to form small rosettes which later contract to give rise to spherical virus particles. Although further on infective hepatitis is required before the true nature of the spherical bodies is elucidated, it is considered on the basis of present evidence that they may possibly represent the causative virus.

More detailed reports on the liver in infective hepatitis are to be published later.

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Precipitation Reaction between Serum and Lysed Erythrocytes

Human serum reacts with hæmolysates of human erythrocytes to form a precipitate; this occurs even with the isologous hæmolysate. When the hæmolysate is added to serum in tubes at 37° C., precipitation continues for several days. In agar-gel, using a double diffusion technique, a well-marked precipitate band is formed (Fig. 1). Peetoom *et al.*[1] described a precipitate of this type in agar-gel, but only minimal precipitation in fluid media. In spite of antigenic differences between adult and fœtal hæmoglobins[2], serum and hæmolysate from adult and umbilical cord blood form precipitates in agar-gel, which are qualitatively the same. Cross-precipitation also occurs between human and some animal sera and hæmolysates. As the reaction is of general occurrence, it causes false positive results in tests for auto-antibodies when the tissue extract used as antigen contains sufficient lysed erythrocytes.

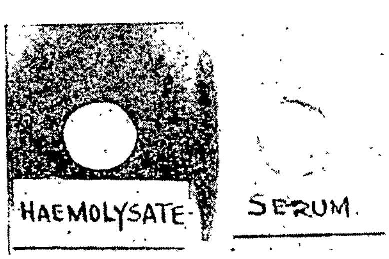

Fig. 1. Precipitate band formed by double diffusion of serum and hæmolysate in agar-gel

The precipitate forms with serum and hæmolysate from blood of the same or different *ABO* blood-groups. After absorption of the α- and β-antibodies with intact erythrocytes, serum continues to form the precipitate. The cell envelope is not involved, as precipitation occurs with Seitz-filtered hæmolysates. Thus, the reaction is independent of the *ABO* groups.

Although haptoglobins were not detected by Tuttle[3] in cord blood, serum from cord blood gives a precipitate with adult and cord hæmolysates. Also adult serum, after absorption for 3 days at 37° C. with hæmolysate containing 8–12 mgm. hæmoglobin per ml., loses its ability to precipitate with hæmolysate in agar-gel; but Allison *et al.*[4] found that the haptoglobins in 1 ml. of serum could react with only 1·35 mgm. of hæmoglobin. Therefore, serum factors other than haptoglobins must be involved.

The reaction titres of sera from normal adult and cord blood, tested in agar-gel, are similar; but some sera from cases of hæmolytic disease of the new-born and of acute hæmolytic anæmia have reduced titres. Thus, this precipitation reaction may be part of the physiological mechanism for the removal of products of erythrocyte lysis.

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Antigenicity of Deoxyribonucleic Acids from Mouse Liver and from the Ehrlich Ascites Tumour

In recent years, a number of reports have appeared concerning the antigenicity of mammalian and bacterial deoxyribonucleic acid. Evidence has been presented indicating that complement-fixing[1] and precipitating[2] antibodies may be produced by the injection of such preparations into experimental animals. In addition, serum from patients with systemic lupus erythematosus has been shown to react immunologically with highly purified deoxyribonucleic acid from a variety of sources[3]. The present communication concerns the production of antisera by the immunization of rabbits with deoxyribonucleic acid preparations isolated from mouse liver and from cells of the Ehrlich ascites tumour. The results obtained indicate that anti-tumour cell deoxyribonucleic acid serum has an inhibitory effect on the growth of the tumour *in vivo*.

Mouse liver and Ehrlich tumour cell deoxyribonucleic acids were isolated by the detergent method of Kay, Simmons and Dounce[4]. The preparations had nitrogen/phosphorus ratios of 1·65–1·68. In the analytical ultracentrifuge (Spinco, model *E*) equipped with an ultra-violet optical system, solutions containing 25–30γ DNA/ml. in 0·2 *M* potassium chloride – 0·01 *M* phosphate buffer, *p*H 7·0, were found to contain components having sedimentation velocities ranging from 20 to 40 Svedberg units, with mean sedimentation velocities of 24–25 *S*. Contamination with polypeptide material was minimal. One-dimensional paper chromatography of hydrochloric acid hydrolysates of the deoxyribonucleic acid (using methyl ethyl ketone/glacial acetic acid/water, 2:1:1, as solvent) revealed the presence of amino-acids with the R_F values of leucine (0·04–0·08 per cent), methionine (0·02–0·04 per cent), glutamic acid (0·008–0·016 per cent), arginine (0·01–0·02 per cent) and lysine (0·02–0·06 per cent). Polysaccharides containing hexosamine could not be detected by the Rimington[5] modification of the method of Elson and Morgan[6]; that is, if such substances were present they represented less than 0·01 per cent of the nucleic acid preparations.

Antisera were produced in rabbits (New Zealand whites) either by the intravenous injection of solutions of the deoxyribonucleic acids in physiological saline or by the intramuscular injection of the nucleic acids suspended in Freund's adjuvant. In the first case (pool 1), each rabbit received 4·0 ml. of deoxyribonucleic acid solution (containing 3 mgm./ml.) twice weekly for a total of eight injections. In the second case (pool 2), each rabbit was injected twice-weekly for a total of eight injections with 4 ml. of antigen suspension containing 2 mgm. deoxyribonucleic acid per ml. Rabbits were bled by cardiac puncture two weeks after the final injection. In each case, the sera from three immunized rabbits were pooled before testing. Normal rabbit serum was obtained from the same rabbits prior to immunization.

The two methods of immunization yielded antisera of much the same potency. In complement fixation tests, performed by a slight modification of the Kolmer–Boerner technique[7] employing 250γ antigen per tube, complete fixation was observed with serum dilutions of 1 : 128 to 1 : 256. Illustrative results are presented in Table 1. Although the antisera appeared to react somewhat more extensively with the homologous than with the heterologous antigen (with the exception of the reaction between anti-mouse liver DNA serum and DNA isolated from the *AH*130 rat ascites hepatoma) the results in Table 1 do not permit one to make a case for their specificity.

Table 1. COMPLEMENT-FIXATION TESTS WITH SERA FROM RABBITS IMMUNIZED WITH DEOXYRIBONUCLEIC ACID ISOLATED FROM MOUSE LIVER AND FROM THE EHRLICH ASCITES TUMOUR

Serum	Antigen*	Complement-fixation titres†
Anti-Ehrlich DNA, pool 1	Ehrlich DNA	128
	Mouse liver DNA	64
Anti-liver DNA, pool 1	Ehrlich DNA	64
	Mouse liver DNA	128
Anti-Ehrlich DNA, pool 2	Ehrlich DNA	256
	Mouse liver DNA	128
Anti-liver DNA, pool 2	Mouse liver DNA	128
	*AH*130 rat hepatoma DNA	128
	6*C*3*HED* lymphosarcoma DNA	32
	DBA lymphoma DNA	64

* Concentration employed in complement-fixation test = 250γ/tube.

† Expressed as reciprocal of serum dilution showing complete fixation of complement.

Investigations of the effects of the antisera on the growth of the Ehrlich ascites tumour in mice do suggest, however, that the antisera have some measure of specificity. The antisera were tested in the following manner for their ability to inhibit tumour growth. The cell concentration in a pool of ascitic fluid was adjusted to the desired value (40 or 80 × 10^6/ml.) by the addition of Hanks's balanced salt solution. Aliquots of this cell suspension were mixed either with equal volumes of the antisera or with normal rabbit serum. Mice were injected intraperitoneally with 0·25 ml. (2·5 or 5·0 × 10^6 cells) of the resulting cell suspensions. In each experiment, mice injected with cell antiserum mixtures were divided into two groups. One group received no further treatment; mice in the second were injected with antiserum subsequent to tumour implantation according to the schedule indicated in Table 2.

Table 2. EFFECT OF ANTISERA PRODUCED IN RABBITS AGAINST EHRLICH ASCITES TUMOUR AND MOUSE LIVER DEOXYRIBONUCLEIC ACIDS ON THE GROWTH OF THE EHRLICH TUMOUR IN MICE

Antiserum	No. of cells implanted	Survivors	Mean survival time (days)	*P*§
Normal rabbit serum	5 × 10^6	0/10	16·4	
Anti-liver DNA (pool 1)*	,,	0/10	16·0	0·7
,, †	,,	0/10	17·3	< 0·6, > 0·5
Anti-Ehrlich DNA (pool 1)*	,,	2/10	24·5	< 0·001
,, †	,,	5/10	24·2	< 0·01, > 0·001
Normal rabbit serum	5 × 10^6	0/10	15·4	
Anti-liver DNA (pool 1)*	,,	0/10	15·8	< 0·9, > 0·8
,, †	,,	0/10	16·2	< 0·7, > 0·6
Anti-Ehrlich DNA (pool 1)*	,,	0/10	19·0	< 0·05, > 0·02
,, †	,,	2/10	27·5	< 0·001
Normal rabbit serum	2·5 × 10^6	0/10	15·9	
Anti-liver DNA (pool 2)*	,,	0/10	20·6	< 0·02, > 0·01
,, ‡	,,	0/10	27·6	< 0·001
Anti-Ehrlich DNA (pool 2)*	,,	0/10	25·8	< 0·001
,, ‡	,,	7/10	31·7	< 0·01, > 0·001

* No post-tumour implantation therapy.

† 0·25 ml. serum injected intraperitoneally 24 and 72 hr. post-tumour implantation.

‡ 0·50 ml. serum injected intraperitoneally 24 hr. post-tumour implantation.

§ Probability of mean differing from that of controls by chance alone calculated by the *t* test.

The results of these experiments are summarized in Table 2. Antiserum against mouse liver DNA was without significant effect on tumour growth. Of sixty mice injected with Ehrlich cells suspended in this anti-serum, sixty developed and died of the ascites tumour; and in only one of three experiments was there a significant increase in mean survival-time compared with the control group. In contrast, mice which died with ascites following injection with cells plus anti-Ehrlich DNA serum showed in every case a significant increase in mean survival-time compared with the control group. More significant was the fact that, of thirty mice in this category which received some post-tumour implantation therapy, fourteen survived without evidence of tumour development, either in ascites or solid form, during the observation period of sixty days.

These results provide evidence that highly polymerized DNA is antigenic in the rabbit when administered by the intravenous or intramuscular route. They furthermore show that antisera produced in this way possess a degree of specificity—a specificity not apparent in complement-fixation tests, but quite evident in bio-assays based on the effects of antisera on tumour growth.

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Longevity in Mice with a genetically determined Muscular Dystrophy

The recent communications by Dowben[1] and Tubis *et al.*[2] regarding possible therapeutic procedures for mice with a genetically determined form of myopathy[3] have prompted this preliminary communication commenting on some problems relating to longevity in animals with this defect.

The value of such therapeutic work is three-fold. First, by prolonging the life-span of these animals it becomes possible to obtain critical details on the mechanism of the disease as it progresses. Secondly, effective therapeutic agents may give valuable leads regarding the primary metabolic nature of the defect. Thirdly; agents which prolong the life of animals with muscular dystrophy or alleviate the disease process would seem to be logical agents to employ in a clinical testing programme involving human beings with muscular dystrophy.

The last point is important because at present the rationale for employing certain therapeutic measures in patients suffering from various forms of muscular dystrophy is based largely on the results of tests in which experimental animals have been used[4]. If the results of these tests are accepted uncritically or their limitations not fully realized, it is likely that large amounts of time and money will be expended on clinical trials which unfortunately do not have the slightest probability of success.

Approximately two years ago we designed and initiated an experiment to test the efficacy of several agents in extending the life-span, and arresting the disease process, in mice suffering from a genetically determined muscular dystrophy. The agents used were felt to have potential value, by virtue of previous work either in our laboratories or in those of other investigators. It was our impression that in some reported work not enough attention had been paid to simple problems of animal management that might significantly influence the outcome of the experiments. Therefore, the method of handling the control animals in our experiment was determined by two observations made in routine mouse-colony maintenance. First, the transition period (between 2 and 2½ weeks of age), during which a suckling mouse changes to a diet of solid food, can be facilitated by the temporary use of a powdered form of the food pellet regularly used in the colony. Secondly, mice with muscular dystrophy often experience considerable difficulty in managing pelleted food and water when they are presented in the usual way through small glass tubes attached to inverted bottles. Regardless of whether these difficulties result from a generalized muscular weakness or from a particular involvement of the muscles of mastication, it was apparent that we had to entertain the hypothesis that these animals succumb to inanition resulting from their inability to consume sufficient pelleted feed or to a self-imposed inanition arising from an inadequate intake of water. It was decided, therefore, to maintain each of the animals in separate pens supplying them each day with fresh powdered food (U.B.C. mouse ration No. 6) and fresh water in small open vessels placed directly on the floor of the pen. The therapeutic substances being tested were added in appropriate levels to the powdered food.

While a number of the mice in various 'treatment' groups have lived for periods exceeding nine months, several of the untreated dystrophic animals in the control series have lived equally as long. One animal in the control group died at the age of eleven months and another still alive promises to exceed this record. So far as we are aware these are the oldest dystrophic mice yet reported.

Although the experiment is not yet complete, we believe that the results so far are significant in two principal respects: First, any claims regarding the ability of a particular agent to prolong the life-span of mice suffering from this form of muscular dystrophy must be examined with extreme caution and viewed against the background of adequately nourished control dystrophic survivors. Secondly, the progressive pathological changes previously described in this disease in the house-mouse should be re-examined in order to differentiate between those attributable to concomitant dietary deficiencies and those truly resulting from primary muscle disease.

Three additional points should be emphasized: First, not all the animals in this experiment have lived to the ages mentioned above. Secondly, there is no evidence that the disease process of the muscle is modified in any way since the affected animals still die prematurely and post-mortem examination demonstrates that muscle wasting is still extreme. Thirdly, our management procedure is begun some time after the first signs of the disease are observed[3].

On the basis of these three points we do not believe that the 'therapy' used in our experiments has any effect on the fundamental metabolic error underlying the disease, and we must stress emphatically that we are making no claims whatever about the life-prolonging ability of our diet. It seems more reasonable to believe that this ability, and perhaps that of the diet reported by Coleman[5], results from the provision of more of the essential nutrients per unit mass of feed and hence assures what is essentially an adequate intake of protein, energy and micronutrients.

A full report on this experiment together with pathological findings arising from it will be presented at a later date.

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Agglutination of Sensitized Alligator Erythrocytes by Rheumatoid Factor(s)

Recently, it was found that alligator erythrocytes can be effectively used as carriers for reactant globulin in tests for rheumatoid factor(s)[1–3]. The cells are remarkable because most human sera do not contain heterophile antibodies to them, and the cells show extraordinary durability under osmotic and mechanical stress[1,2].

The present investigation was carried out to determine the effects of euglobulins from healthy individuals and from patients with rheumatoid arthritis and other diseases, in agglutination of sensitized alligator erythrocytes by rheumatoid factor(s). Globulin fractions of ovalbumin (rabbit) antiserum were also tested.

Serum euglobulin fraction[4] or other inhibitors to be tested, in 0·2-ml. serial dilutions, were mixed with 0·2 ml. volumes of positive 2 plus agglutinating serum of a patient with rheumatoid arthritis, in 10 × 75 mm. test-tubes. The mixtures were incubated at 37° C. for 30 min. before testing with sediments of 0·1 ml. of 2 per cent saline suspension of sensitized alligator cells. The agglutination of sensitized alligator erythrocytes by rheumatoid arthritis serum was not readily inhibited by the euglobulin fractions of normal sera, unless the sera were native (not inactivated at 56° C. for 30 min.) and only if sera were diluted no more than 1 : 4. In contrast, euglobulins of subjects with rheumatoid arthritis gave even stronger agglutination than the control, suggestive of an enhancement, or summation effect. Results of a typical experiment are shown in Table 1. Some degree of depression of agglutination occurred with serum euglobulins tested of 3 patients with gout, 1 with lupus erythematosus, 1 with osteoarthritis, and 1 with rheumatoid spondylitis. No inhibition or depression of agglutination was observed with the euglobulins tested of 11 rheumatoid arthritis patients. Random readings of coded dilution tubes have indicated that one can reliably distinguish macroscopically between 2 plus and 1 plus degrees of agglutination of alligator cells. The nature of the depression, or summation of agglutination of sensitized alligator erythrocytes by euglobulin fractions, is under study. Well-defined inhibition of sensitized sheep cells and coated latex particles by euglobulins of sera of normal subjects has been reported by other authors[4-7]. The degree of inhibition of sensitized alligator cells was not so remarkable as reported by workers with red cells of other species or with latex particles.

Table 1. EUGLOBULIN INHIBITION OF ALLIGATOR ERYTHROCYTE AGGLUTINATION

Subjects	Sensitized alligator erythrocyte sediment								
	Dilutions of patient euglobulin								
	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	Saline control
	Fixed amount of positive-reacting rheumatoid arthritis serum								
Normal (*D*)	0	0	1	1	1	1	1	1	1
Normal (*C*)	0	1	1	1	1	1	1	1	1
Rheumatoid arthritis (*T*)	2	2	2	2	2	1	1	1	1
Rheumatoid arthritis (*S*)	2	2	2	2	2	1	1	1	1

Agglutination of sensitized alligator cells by 1 volume of rheumatoid arthritis serum was readily inhibited by addition of 1 vol. of 5 per cent human, rabbit, or bovine γ-globulins. However, 5 per cent bovine albumin ('Ortho') or human albumin ('Cutter') added to the saline buffer diluent enhanced the degree of agglutination. The mechanism of the enhancement is being investigated.

Inhibition of the agglutination of sensitized alligator erythrocytes was observed by γ-globulins obtained from ovalbumin rabbit antiserum and by γ-globulin fragments, prepared by Porter's method[8]. Chromatographic fractions (Porter fractions I, II and III) of the rabbit antibody γ-globulins were also tested. Fraction III, at 1·75 mgm./ml., inhibited rheumatoid factor agglutination of sensitized alligator cells. Fraction I, which is reactive with the ovalbumin antigen, did not inhibit rheumatoid factor agglutination at a maximal concentration tested of 4·15 mgm./ml.; neither did fraction II, which is also reactive with ovalbumin antigen, at a concentration of 1·90 mgm./ml. Limited amounts of the globulin fragments were available. Ultracentrifugal examination showed no visible contamination by undegraded whole globulin. The observations indicate that not all globulin fragments are active or equally active as inhibitors of rheumatoid factor hæmagglutination and suggest that there are one or more specific combining sites on the rabbit globulin which interact with the rheumatoid factor(s). In the light of the recent report of Edelman *et al.*[9] of antigenic specificity of 2 human γ-globulin fragments (*F* and *S*) obtained after papain treatment, it might be important to investigate their capacity to combine with rheumatoid factors. The physico-chemical characteristics of globulin fragments which determine inhibitory or lack of inhibitory capacity require further study.

Alligator erythrocytes are found to lend themselves to detection of rheumatoid factor(s) by direct or inhibition hæmagglutination procedures.

We are indebted to the Buffalo Zoo for alligator cells and to Mrs. L. Lipman, Mr. K. Crampton and Mr. Wm. Leumer for technical assistance.

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BIOLOGY

Effect of *Iso*nicotinyl Hydrazide on the Path of Carbon in Photosynthesis

IN a study of the pathways of incorporation of carbon-14 from labelled carbon dioxide into the amino-acids glycine and serine during photosynthesis the compound *iso*nicotinyl hydrazide (isoniazid) was used. This compound has been shown to be an inhibitor of transaminases *in vitro* when used at high concentrations (about 10^{-2} *M*)[1,2].

Cells of *Chlorella pyrenoidosa* (Emerson strain) were grown for four days at 25° C. in illuminated culture tanks. 1,200 µl. wet packed volume of cells were suspended in 105 ml. of 10^{-4} *M* potassium dihydrogen phosphate at *p*H 4·5 and the suspension divided into three samples. Isoniazid was added to two of the samples to give concentrations of 10^{-2} *M* and 10^{-3} *M*. The third sample was used as a control. The samples were aerated in the dark for 1 hr. and were then illuminated (light intensity approximately 10,000 lux). Air containing 0·1 per cent carbon dioxide was passed through the suspensions. After 15 min. photosynthesis, 30 µc. of sodium carbonate-^{14}C were added to the suspensions. After a further 10 min. photosynthesis the cells were killed by boiling ethanol. The distribution of carbon-14 among the ethanol-soluble compounds was examined by the procedures of Benson *et al.*[3].

The total fixation of carbon-14 and the distribution of carbon-14 between the ethanol-soluble and ethanol-insoluble fractions is shown in Table 1.

Table 1. CARBON-14 FIXED BY *Chlorella* DURING PHOTOSYNTHESIS (EXPRESSED AS COUNTS PER MIN. PER ML. OF CELLS × 10^{-3}). THE LIMITS OF VARIATION ARE ABOUT ± 5 PER CENT)

	Control	Isoniazid (10^{-3} *M*)	Isoniazid (10^{-2} *M*)
Total fixation	5,340	5,960	5,630
Ethanol soluble fraction	2,978	3,630	3,460
Ethanol insoluble fraction	2,395	2,339	2,157

Isoniazid had only a small effect on the total fixation of carbon-14 and on the distribution between the ethanol-soluble and ethanol-insoluble fractions. There is a slight stimulation of total uptake and carbon-14 content of the ethanol-soluble fraction which is probably significant.

The effect of isoniazid on the distribution of carbon-14 among the compounds of the ethanol-soluble fraction is shown in the histogram of Fig. 1.

The incorporation of carbon-14 into sugar phosphates, phosphoglyceric acid, phosphoenol pyruvic acid, aspartic acid, malic acid, fumaric acid and alanine is considerably reduced by treatment with isoniazid. Incorporation into sucrose is stimulated at the lower concentration and slightly inhibited at the higher concentration. Incorporation into the two-carbon compounds, glycollic acid and glycine, is stimulated about 5- to 8-fold by treatment with 10^{-2} *M* isoniazid and these two compounds account for more than 80 per cent of the total ethanol-soluble carbon-14. Incorporation into serine, which previous evidence suggests is derived from glycine during photosynthesis[4], is inhibited to the same extent as that into sugar phosphates, phosphoglyceric acid, etc. It has been shown by Dac and Wriston[5] that isoniazid inhibits the condensation of formaldehyde and glycine during the formation of serine from sarcosine. Therefore the above results are not necessarily incompatible with the view that serine is derived from glycine during photosynthesis.

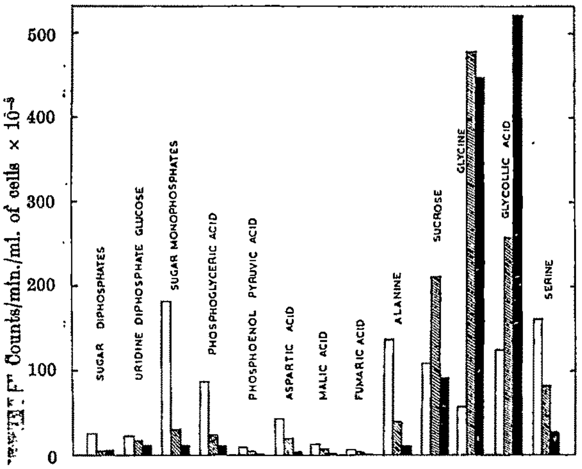

Fig. 1. The effects of treatment of *Chlorella pyrenoidosa* with *iso*nicotinyl hydrazide on the incorporation of carbon-14 into ethanol-soluble compounds during photosynthesis in $^{14}CO_2$. Counts were made directly on-paper chromatograms and have not been corrected for self-absorption. White, control; hatched, 10^{-3} *M* isoniazid; black, 10^{-2} *M* isoniazid

Thus in its effect on the photosynthetic carbon cycle, isoniazid differs from azaserine. The latter substance has been shown to inhibit the transaminases fairly specifically and does not inhibit incorporation of carbon-14 into sugar phosphates and phosphoglyceric acid[6]. The effects of isoniazid on *Chlorella* are similar to those obtained by treatment of *Scenedesmus* with low concentrations of ethanol[7]. Incorporation of carbon-14 into phosphoglyceric acid and sugar phosphates during photosynthesis was inhibited by ethanol treatment while incorporation into glycollic acid and glycine was unaffected or even enhanced. Ethanol, however, inhibited total fixation of carbon-14 quite markedly while in our experiments isoniazid did not. The above results do not indicate specific inhibition of transaminases by isoniazid but suggest a different site of action.

This work is part of a research programme primarily concerned with carbon metabolism in photosynthetic organisms sponsored by the Office of Scientific Research of the Air Research and Development Command, U.S. Air Force through its European Office, under Contract No. *AF* 61(052)-245. The implications of these results will be discussed in a fuller account of this work to be published elsewhere.

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Taxonomic Significance of Surface Lipids of Plants

WHILE investigating the composition of surface lipids of plants, we have observed that the chemical composition of the lipid appears to be characteristic of the species of plant. The lipids were extracted from leaves by immersing them for 10 sec. in ether. According to Martin *et al.*[1], and in harmony with our own findings, this treatment does not extract lipids from within the leaf. After removal of the solvent, the residues were chromatographed on thin films of silica supported on glass plates. Three solvents were used for the elution: benzene, benzene containing 5 per cent methanol, and benzene containing 10 per cent methanol. The positions of the components were revealed by spraying separate plates with two reagents: chloroform saturated with

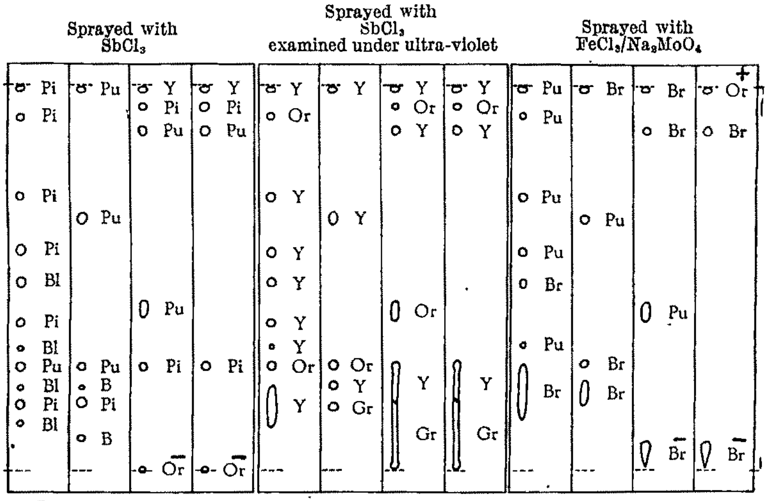
Sprayed with SbCl₃
Sprayed with SbCl₃ examined under ultra-violet
Sprayed with FeCl₃/Na₂MoO₄
R I O G R I O G R I O G

Fig. 1. Drawing of two plates eluted with benzene containing 10 per cent methanol. *R, Rhododendron ponticum* ; *I, Ilex aquifolium* ; *O, Festuca ovina* ; *G, Festuca rubra genuina S*59 : *B*, black ; *Bl*, blue ; *Br*, brown ; *Gr*, green ; *Or*, orange ; *Pi*, pink ; *Pu*, purple ; *Y*, yellow ; +, intense ; –, pale

antimony trichloride, and saturated aqueous ferric chloride followed immediately by 0·1 molar sodium molybdate. Both series of plates were heated to 200° C. for 10 min. for full development of the colour. If the plates which were to be sprayed with antimony trichloride were first heated to 200° C. for 10 min. and sprayed while hot, the components showed a large range of colours. Subsequent examination in ultra-violet light showed a less-extensive range of fluorescent colours. Specimen chromatograms in one solvent are shown in Fig. 1.

Although the relative positions occupied by the components are always the same, their R_F values are sufficiently variable to render them of little use in comparing one chromatogram with another. For simplicity, we shall refer to the complete data from six separate chromatograms for each lipid, having regard to the colours of the spots obtained with each spray reagent, and their relative positions when eluted with different solvents, as its pattern. Characteristic patterns were given by each of the surface lipids from 10 species of monocotyledons (*Festuca elatior pratensis S*56 ; *F. ovina* ; *F. rubra genuina S*59 ; *Dactylis glomerata S*143 and *S*26 ; *Phleum pratense S*50 and *S*51 ; *Agrostis tenuis* ; *Alopecurus pratensis* ; *Lolium italicum* ; *L. perenne* ; *Holcus mollis*) and 14 species of dicotyledons (*Rhododendron ponticum* ; *Ilex aquifolium* ; *Fraxinus excelsior* ; *Aesculus hippocastanum* ; *Malus* spp. edible form ; *Rosa* hybrid *H.T.* garden variety ; *Brassica oleracea* sub-sp. *gemmifera* ; *B. oleracea capitata*, red and winningstadt ; *Chrysanthemum* garden variety ; *Antirrhinum majus* cultivated ; *Petunia* spp. ; *Beta vulgaris* (sugar beet type *E*) ; *Trifolium repens S*100).

Before the taxonomic value of the patterns could be considered seriously, three further variables had to be examined : first, it must be established that foliage from different parts of the plant gives the same pattern. For brussels sprout this is certainly true. Lipids from the main leaves and from the outer and inner leaves of the bud all had the same pattern, although the relative concentrations of the components were different, and so too were the weights of lipid per unit area on the inner and outer leaves of the bud.

Secondly, we found that the lipids on the leaf are relatively stable; precisely the same patterns were obtained from freshly cut leaves as from leaves which had been stored for thirteen days. However, once the lipids had been extracted from the leaf, auto-oxidation was detectable by a change in the pattern after the same period.

Thirdly, it was established that the pattern did not change as the plant aged. Waxes from leaves of various ages from *Rhododendron ponticum* and from *Ilex aquifolium* had patterns that were independent of age. Two groups of the 12 grasses were examined; one group had been grown from seed one year before the second group. Once again the patterns were independent of time.

Bate-Smith *et al.*[2] have suggested that the occurrence of tannins in plants may be a useful taxonomic character. Our observations show that a brief qualitative analysis of the surface lipids of plants provides more useful data, even if the identities of the components have not been established.

The technique may be capable of extension to Arthropoda.

We are indebted to Prof. J. B. Speakman, to Miss D. M. Turner for advice and supplies of material, and to the Agricultural Research Council for financial assistance.

Note added in proof. The observations have now been extended to a total of 63 species.

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Botryodiplodia theobromae Pat. and *Nigrospora* sp. in the Air of a Jamaican Banana Plantation

Cammack[1] has commented on the lack of detailed information about the composition of the air spora in tropical climates. On the other hand, Hirst[2,3] and others have contributed much to our knowledge of fluctuations in the air spora in a temperate climate. Seasonal changes in three common constituents (*Puccinia polysora*, *Nigrospora sphaerica*, *Cladosporium* spp.) of the air spora of Southern Nigeria have been described by Cammack[1]. The observations described here were made during an investigation[4-6] chiefly designed to provide information about dispersal of *Deightoniella torulosa*, the cause of fruit-spot ('speckle') of Jamaican bananas.

Botryodiplodia theobromae Pat. is a widespread saprophyte and wound-parasite on a considerable range of hosts in the tropics. It is an important parasite of bananas in storage, causing several forms of fruit-rot[7,8]. The pycnospores are elliptical, at first unicellular and hyaline, becoming brown and 1-septate, sometimes with longitudinal striations: dimensions are 20–30 × 10–18μ. There is little risk

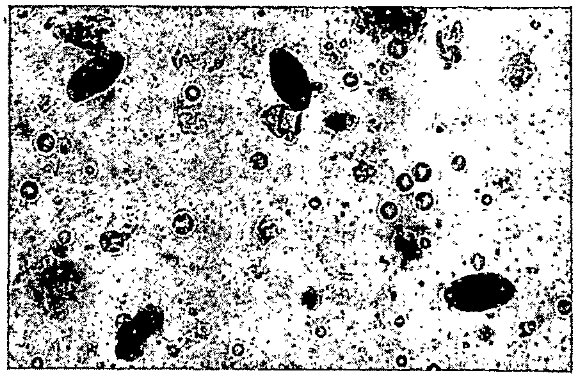

Fig. 1. Relatively large dark-coloured pycnospores of *Botryodiplodia theobromae* from air sampled in a Jamaican banana plantation. (× *c.* 300)

of confusing these spores with those of any other species (Fig. 1). The fungus produces pycnidia on dead or dying banana leaf 'trash', bracts and decaying fruit in the plantation.

A species of *Nigrospora* variously named *N. sphaerica* (Sacc.) Mason[9] or *N. musae* McLennan and Hoette[10] is responsible for the serious 'squirter' disease of banana fruits in Queensland. It is a common saprophyte on banana debris inside the plantation. *N. oryzae* (Berk. and Br.) Petch is also saprophytic on decaying banana tissue. These fungi may be synonymous, and, until further work has been carried out in Jamaica, it is proposed to refer to the fungus discussed here as *Nigrospora* sp. The conidia, which are borne terminally on simple conidiophores, are sub-globose or globose, black and measuring 15 × 18μ or 13–18μ in diameter (Fig. 2).

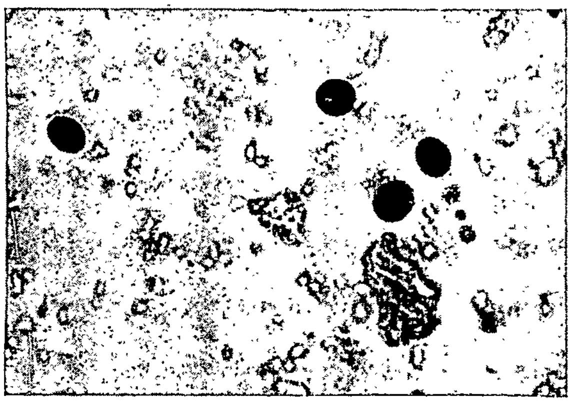

Fig. 2. Sub-globose or globose, black conidia of *Nigrospora* sp. trapped in a banana plantation. (× *c.* 300)

A Hirst spore trap (automatic volumetric) was set up inside a plantation of Lacatan bananas situated on the plains of St. Catherine, Jamaica. The trap orifice was three metres above ground-level. Slides were prepared and mounted in the manner described by Hirst[3], and were changed daily at 09.00 hr. E.S.T. On each slide, traverses 40μ wide and at right angles to the direction of slide movement were scanned at positions representing 2-hourly intervals. The number of spores counted on each cross-traverse was then converted into an estimated number per cubic metre of air. Since no corrections for variation in trapping efficiency were made, all concentrations recorded were under-estimates of the true ones.

The diurnal periodicity curve for *Nigrospora* sp. (Fig. 3) was derived from the geometric means for spore concentration at each even hour, plotted according to Hirst[3]. Also shown are the 2-hourly means for temperature and relative humidity. Each mean was based on counts over sixty-three days (July 19–September 20). The fungus exhibited a well-defined periodicity with peak concentrations occurring near 08.00 hr. each day. After this there was a progressive decrease to a characteristically low night level. This periodicity is similar to that for *D. torulosa*[4], *N. sphaerica*[1], *Phytophthora infestans*[3] and *Polythrincium trifolii*[3,11]. Hirst[3] has suggested that such regularity may be due to the operation of a definite discharge mechanism or to the occurrence each day of environmental conditions favouring spore liberation. Violent spore liberation in *D. torulosa* occurs under conditions of rapidly decreasing vapour pressure[4], usually between 08.00 hr. and 10.00 hr. Webster[12] has described active discharge accompanying drying in *N. sphaerica*, thus helping to explain the periodicity of the fungus in Nigeria. The possibility that a similar process occurs in *Nigrospora* sp. is being investigated.

Variation in absolute numbers of spores of *Nigrospora* sp. appears to be related to rainfall and humidity; highest counts were obtained during predominantly dry weather. The highest 2-hourly estimate recorded so far is 20,000 spores per cubic metre of air at 08.00 hr. There is considerable variation in spore

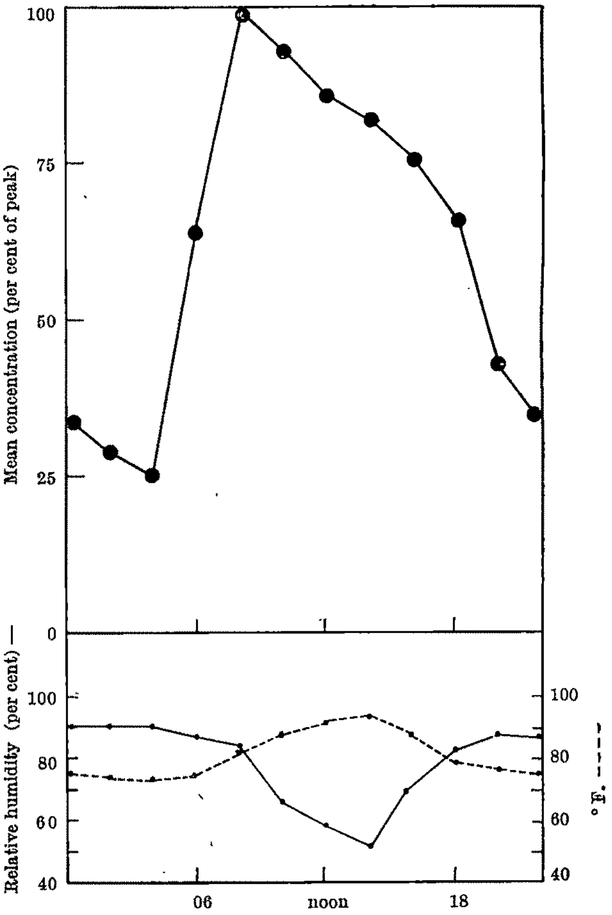

Fig. 3. Mean diurnal periodicity curve for *Nigrospora* sp. in the air of a banana plantation

content between different plantations. Although this will be discussed more fully elsewhere, it seems clear that *Nigrospora* sp. is an extremely common component of the air spora of Jamaican banana plantations.

Because of the small numbers of *B. theobromae* spores trapped, it was not possible to distinguish any clearly defined periodicity. It was, however, unusual to trap spores during the period 20.00–06.00 hr. The highest 2-hourly estimate recorded was 858 spores per cubic metre, when a clump of spores was trapped. The average daily mean concentration throughout the sampling period was only 8 spores per cubic metre. Such meagre counts could not be correlated with fluctuations in weather conditions. It was concluded that this fungus is a relatively infrequent member of the plantation air spora.

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Biotin Requirement and Carbon and Sulphur Sources in *Aspergillus* and *Neurospora*

Conidia of the mould *Aspergillus nidulans* were unexpectedly found to require a small amount of biotin (or larger amounts of desthiobiotin) for initial growth, when plated on to a minimal medium of Czapek–Dox, at a density of no more than 10^3 per dish. This fact seems to be in contradiction to what was previously considered to be their basic requirement. Biotin must be added to the minimal medium, for a total germination of wild-type conidia, to the extent of 10^{-6} gm./ml. This corresponds to about 1/100–1/10 of the amount required by the biotinless mutant *bi*-1.

Sometimes a few wild type conidia germinate in the absence of biotin to give rise to normal colonies, the conidia of which, however, have not acquired biotin independence.

When the carbon source (usually glucose 4 per cent) is replaced with an equal concentration (w/v) of fructose, ribose, glycerol or intermediates of the Embden–Meyerhof, of tricarboxylic, or pentose cycle (pyruvate, acetate, succinate, citrate, gluconate) good growth takes place in the absence of biotin. If the glucose is replaced with an equal concentration of sucrose, maltose or dextrin, the basic requirement for biotin is retained. Under our conditions glucose-6-phosphate or glucose-1-phosphate are not utilized as carbon sources (even with added biotin).

Mixtures of glucose and fructose support growth in the absence of biotin, if the amount of fructose is equal or superior to the amount of glucose, whatever the total sugar concentration in a large range (0·2–8 per cent).

A role of biotin in glycolysis, namely as a coenzyme in the hexokinase or in glucose-6-phosphate dehydrogenase systems, was proposed by Moat and Liechstein and Strauss and Moat[2] in biochemical work with *Saccharomyces*. However, this finding was not confirmed by Siepmann and MacDonald[3]. Our results with the carbon sources are compatible with a role of biotin in some initial steps of glucose metabolism.

A variety of nutritional mutants (*ribo*-1, *an*-1, *pro*-1, *paba*-1, *ad*-20, *nic*-2, *lys*-5, *orn*-4) retained the basic biotin requirement of the wild-type, except the parathiotrophic mutant *s*-12, which grew, although with a delay of 24–48 hr., in the absence of biotin. (For description of mutants see Käfer[4].) As this mutant requires reduced sulphur as the only addition in a minimal medium, we looked for a possible effect of the replacement of the source of sulphur on the biotin requirement.

Replacement of 4 m*M* sulphate (which is the only source of sulphur in the Czapek–Dox minimal medium) by an equimolecular concentration of reduced sulphur (sulphite, thiosulphate, sulphide, cystein) or cysteic acid, allows wild-type conidia to give rise to colonies in the absence of biotin, though growth is delayed 24–48 hr. Addition of biotin ($2\cdot5 \times 10^{-9}$ m*M*) restores the growth to a normal rate.

Methionine, which is utilized in the presence of biotin as a reduced sulphur source by the parathiotrophic mutant, does not support growth in the absence of biotin. Moreover, the methionineless mutant *meth*-1 retains the biotin requirement of the wild-type.

A wild-type strain of *Neurospora crassa*, commonly regarded as a biotin-requiring mould, is able to grow in the absence of biotin if glucose is replaced by fructose, or sulphate by sulphide, on the same Czapek–Dox minimal medium. In the latter case, growth is improved by the addition of biotin.

Many species of moulds are reported to need biotin[5]. It would be of interest to elucidate the mechanism of this requirement, which appears to be similar in *Aspergillus* and *Neurospora*.

This work was aided by a grant to one of us (P. S.) from the Comitato Nazionale per l'Energia Nucleare.

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Transfer of Ova to the Bursa Ovarii and the 'Suspended Loop' of Tube in the Rabbit

In mammalian species successful ova transfer is possible when the ova are replaced inside the Fallopian tubes or uteri of laparotomized synchronous recipients. In species with confined bursa ovarii, like the mouse, the ova can be transferred into the fat pad adjacent to the bursa ovarii, through the capsular membrane and into the bursa proper[1]. Intravaginal ova transfer was not successful in cattle[2], owing

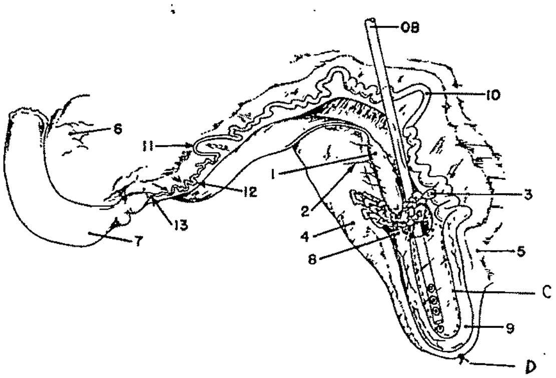

Fig. 1. Diagram of experimental treatments; removal of membrane of bursa ovarii (*C*), induced adhesions in suspended loop (*D*) and ova transfer to ovarian bursa (*OB*). 1, ovary; 2, ovarian artery and vein; 3, appendix vesiculosa; 4, mesosalpinx; 5, suspended mesosalpinx; 6, broad ligament; 7, uterus; 8, infundibulum; 9, suspended loop (ampulla); 10, embedded ampulla; 11, major loops (isthmus); 12, minor loops (isthmus); 13, uterotubal junction (intramural sphincter)

to increased sensitivity to bacterial infection and pyometra during the luteal phase[3]. A few cases of intravaginal ova transfer in the rabbit were successful when antibiotics were used[4]. Intraperitoneal insemination has been successful in several species such as the rabbit[5], guinea pig[6,7], cattle[8], pigeon and hen[9]. Intraperitoneal ova transfer has not been possible yet.

The tubo-ovarian anatomical relationships are of some importance for the reception of ova. The ampulla of the tube is not held in position, but it forms a loop *suspended* in the pelvic cavity (Fig. 1). This loop is attached to a thin fold of the peritoneum which is connected to the mesosalpinx. This thin fold, together with the fatty pad attached to the suspended loop, makes the ovarian bursa. The transit of the ovum from the ruptured follicle to the uterotubal junction is ensured by two series of complex processes; mechanisms for 'reception' or 'adjustment' and mechanisms for tubal 'transportation'.

This work was undertaken to evaluate the physiological significance of suspended loop in ova reception and the possibility of transfer of ova in the ovarian bursa of the rabbit.

Exp. 1: Seven adult does were laparotomized on both sides and the Fallopian tubes exposed. On one side, the membrane attached to the suspended loop of the ampulla (which is part of the ovarian bursa) was removed by fine scissors (Fig. 1). The does were allowed 3 days to recover from the operation, then mated (with or without superovulation) to fertile bucks and killed 40, 50 and 98 hr. *post coitum.* The ampulla, isthmus and uterus were dissected and flushed separately with saline. The ova were examined microscopically. The diameter of blastocysts was measured.

Exp. 2: Twenty donors were superovulated, mated to fertile bucks, killed 16–40 hr. *post coitum,* and the ova were flushed out. The healthy ova were transferred to twenty synchronous pseudopregnant recipients. In one side of the recipient, the ova were transferred to the bursa ovarii (Fig. 1). In the other side, the ova were transferred at different parts of the tube. The recipients were either killed 2, 3 or 4 days *post coitum,* or laparotomized 8 days *post coitum.* When killed, the reproductive tract was dissected and the ampulla, isthmus and uterus flushed separately. The flushings of each portion were examined microscopically. Pregnant recipients were allowed to proceed to term.

Exp. 1: When the membrane of the bursa ovarii was removed, the reception and transportation of the ova in the tube were not affected. The number of ova recovered corresponded to the number of ovulation points, that is, there was no loss of ova. In two cases, however, 11 and 33 per cent of the ova shed were not recovered in the flushings. This could not be ascribed only to faults of flushing or manipulation. It may have been due to the loss of ova in the peritoneal cavity through the fimbriated end of the tube or to the absence of ova in the follicles.

When the suspended loop was attached to the mesosalpinx, the transportation of ova from the ampulla to the uterus was normal. Ova reception was affected and ova loss increased, ranging from 13 to 33 per cent of ova shed. This may be due to the adhesions which were induced by the experimental treatment. It is possible that the pendulous structure of the suspended loop helps in the reception of the ova.

There were no significant differences between the size of blastocysts in the two experimental treatments. There were wide individual variations in the intrazonal diameters and outside diameters of blastocysts. This may be partly due to differences in the cleavage-rate of fertilized ova in the same animal.

Exp. 2: When 1-cell fertilized ova were transferred to the ovarian bursa, ova pick-up by the fimbria occurred in 57 per cent of the cases. The percentage of transferred ova picked up varied from 0 to 35, with an average of 10. The rate of passage of the alien ova was similar to that of the recipient's own native unfertilized ova. This may indicate that the ova were picked up from the ovarian bursa in a relatively short time. The ova which were transferred to the ovarian bursa had normal subsequent development and implantation. The young delivered were also normal.

When 8–16-cell ova were transferred into the ovarian bursa of synchronous recipients, ova pick-up was recorded in 88 per cent of the cases. The percentage of transferred ova which were picked up varied from 0 to 25 with an average of 12. When the 1-cell or 8–16-cell ova were transferred to different parts of the tube, the percentage ova pick-up ranged from 41 to 44 with an average of 42. Ova were not picked up when transferred to the peritoneal cavity very close to the fimbria in 1-day pseudo-pregnant recipients (Hafez, unpublished work).

The present work and other comparative investigations have elucidated, to a certain degree, the physiological importance of the tubo-ovarian anatomic arrangement. The physiological significance of the suspended loop of the tube is not exactly known. It may be the agency by which the ovum is transported rapidly in this portion of the tube, as compared with the rate of ovum transport of the isthmus. The relative physiological significance of such anatomical structure in other species is also of academic significance.

Apparently, the membrane attached to the suspended loop which makes the ovarian bursa was of little importance in reception of ova. Adhesions induced in the suspended loop increased the percentage of ova lost in the peritoneal cavity. The fimbria was able to pick up 1-cell or 8–16-cell ova if transferred to the bursa ovarii of synchronous recipients. The percentage pickup was lower than when ova were transferred to the tube. Detailed results of this work will appear elsewhere.

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Seal Tracks in the Taylor Dry Valley

The mummified carcasses of the crab-eater seal (*Lobodon carcinophagus*) are found in many places in the ice-free regions around McMurdo Sound in the Ross Dependency, Antarctica[1]. All these carcasses appear to be old. Some have only fragments of tissue on the bones, but others, although desiccated and wind blasted, are well preserved. One carcass has been dated[1] giving an age of 1,600–2,600 yr.

During a soil survey in the summer of 1959 of the Taylor Dry Valley I observed many carcasses, probably including a number of those observed by Scott[2], Griffith Taylor[3] and Péwé's party[1]. Most of these carcasses also appeared to be old, wind blasted and in some cases only scattered bones were observed. Three carcasses were observed on the ice of Lake Bonney at the western end of the Dry Valley although more were observed on the ice of other lakes in the Valley. It is difficult to estimate the age of the carcasses, because of the very low rate of organic decomposition in the arid, cold climate of the valley, but casual observation leads to the conclusion that most of the carcasses are of similar age—probably as old as the carcass the age of which was determined by Péwé *et al.*[1]. However, on a small alluvial plain at the eastern end of a large lake in the Taylor Valley, the track of a seal was observed. This was a furrow in the sand, about 1 in. deep and 6 in. wide, following an irregular course across the plain. It showed flipper marks and places where the seal had rested, and changed direction. Similar tracks were observed on beaches at Cape Evans and Cape Royds. The track disappeared under snow-drifts at either end, so that it must have been made before the winter of 1959. The Valley has been visited many times in the past few years, and footprints, helicopter wheel impressions and other traces of human activity are quite common, including some that must have been made during the autumn of 1956, when a meteorological station in the Valley was occupied. This means that such minor impressions are not removed rapidly by climatic influences and persist for at least 5 yr. Judging by the state of preservation of the track and comparing it with footprints in the neighbourhood, the track appears to be less than 5 yr. old. A search was made for the seal, but it was not found. A lake was not far distant, and the seal could have reached it and entered the water at the thaw-moat that appears during the summer.

The site where the tracks were observed lies about 6 miles from the coast, and the intervening country is covered with ridges of moraine several hundred feet high. During the summer period the ground was bare except for occasional small patches of snow.

No seals have been observed in the act of crawling inland. If all the carcasses are of similar age, as they appear to be, then the conditions that cause seals to move inland may have existed at some time about 2,000 yr. ago and the presence of seals on any land surface must mean that the surface is older than about 2,000 yr. However, if the track observed indicates that seals do still travel inland occasionally, then the age of each carcass must be measured to determine a minimum age for the land-form. Observations in the Taylor Valley seem to indicate a rather complicated history of ice-movements. Some figures on the age of land-forms may help to sort out this history.

If the twenty or so carcasses observed in the Taylor Valley represent all the seals that have travelled up the Valley in the past 2,000 yr. and that there has been the same tendency to wander inland during this period, then the rate is approximately one every hundred years. Even if more carcasses are present, but not observed, and if others have crawled into moats at the edges of frozen lakes, then the rate is still very low. The carcass dated by Péwé *et al.* may not be the oldest in the Valley and the rate would then be still slower. It is possible that carcasses of seals dehydrate rapidly in the arid climate and after a short period reach a stage of decay which does not proceed further, and thus all observed carcasses appear of similar age.

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ENTOMOLOGY

Resistance to Dieldrin in *Anopheles* in Trinidad

Dieldrin has been in use as a larvicide against *Anopheles neomaculipalpus* Curry for several years in the Piarco Airport area of Trinidad. This species is of secondary importance to *Anopheles aquasalis* as a vector of malaria in the coastal areas of the Colony.

Lack of proper control recently in the Piarco area raised suspicions that the species was showing some degree of resistance to dieldrin. Susceptibility tests with the standard World Health Organization mosquito adult test kit using freshly blooded wild-caught specimens taken from calf-baited Shannon dawn traps gave the results with DDT and dieldrin shown in Table 1.

It is to be noted that about 27 per cent of the mosquitoes tested survived 1·6 per cent dieldrin papers for 8 hr. and there was a 5 per cent survival

Table 1. *Anopheles neomaculipalpus* CURRY. LOCALITY: PIARCO AIRPORT AREA, TRINIDAD.

Date of test 1960	No. of tubes	Concentration (per cent)	Exposure time (hr.)	No. of mosquitoes tested	Dead (per cent)	Control	
						No. tested	Dead (per cent)
			DDT Preliminary, 1960				
July 26	1	DDT, 0·25	1 hr.	23	17·4	26	0
July 26	1	DDT, 0·5	1 hr.	26	34·6	26	
July 27	1	DDT, 1·0	1 hr.	26	73·0	26	
July 18	1	DDT, 2·0	1 hr.	24	87·5	26	
July 28	1	DDT, 4·0	1 hr.	26	100	26	
			DDT Replicates, 1960				
July 29	4	DDT, 0·5	1 hr.	102	25·5	24	0
August 3–4	4	DDT, 1·0	1 hr.	102	56·0	26	0
August 5–6	4	DDT, 2·0	1 hr.	101	90·0	26	0
August 8–9	4	DDT, 4·0	1 hr.	102	100	27	0
			Dieldrin, 1959				
September 5–12	7	Dieldrin, 1·6	1 hr.	98	58·2	28	0
December 14–17	5	Dieldrin, 4·0	1 hr.	90	84·9	26	0
			Dieldrin, 1960				
August 15–17	4	Dieldrin, 1·6	1 hr.	99	35·3	25	4
August 12–13	4	Dieldrin, 1·6	2 hr.	101	43·5	26	0
August 11–12	4	Dieldrin, 1·6	4 hr.	102	63·7	25	0
August 10	4	Dieldrin, 1·6	8 hr.	103	72·8	25	8
August 23–26	4	Dieldrin, 4·0	1 hr.	95	85·3	25	0
August 25–30	4	Dieldrin, 4·0	8 hr.	105	95·1	24	0

even after 8 hr. exposure to 4 per cent dieldrin papers, indicating that the species is clearly resistant to dieldrin.

The tests with DDT show that despite DDT residual house spraying for several years in the nearby villages for malaria control, the species is still susceptible to this insecticide. The LD_{50} as determined on logarithmic-probability graph paper is about 0·8 per cent DDT.

This is the second anopheline to show resistance to dieldrin in Trinidad, the other being *Anopheles aquasalis*, which was first shown to be resistant in 1958.

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A Differentiation Centre in the Embryo of *Dacus tryoni*

IN several insects the cells of the embryonic blastoderm have been shown to depend for their further development on a stimulus emanating from a ventral prothoracic differentiation centre[1,2]. In cyclorrhaphous Diptera a similar centre has so far escaped detection, although Yao[3] has proposed one for *Drosophila melanogaster* on the basis that alkaline phosphatase activity is first detectable in the ventral prothoracic region during contraction of the germ band and spreads both anteriorly and posteriorly as histo-differentiation proceeds. Further results bearing on this question have now been obtained by the application of ligatures to the early embryos of *Dacus tryoni* (Diptera, Trypetidae (= Tephritidae)).

Methods for maintaining breeding stocks of *D. tryoni* in the laboratory are described by Bateman[4]. Development of the eggs, an account of which will be published in due course, follows the typical dipteran pattern, with cleavage leading to the formation of a blastoderm by 7½ hr. at 25° C. and subsequent gastrulation, elongation and shortening of the germ band, involution of the head and completion of histo-differentiation resulting in hatching at 42–48 hr.

In the present work nine batches of 20 eggs were used. In each batch 10 eggs were set aside as controls, and the remaining 10 identically ligatured at a known time after oviposition. Three batches were treated at 2–3 hr. (cleavage nuclei invading the cortex), three at 4½–5½ hr. (syncytial blastoderm) and three at 6–7 hr. (cellular blastoderm). For each the ligatures were so placed as to separate, in the first batch, the anterior third of the embryo from the posterior two-thirds, in the second, anterior and posterior halves and in the third, the anterior two-thirds from the posterior third. The eggs were then allowed to develop until the normal time of hatching and examined after removal of the chorion by immersion for 30 sec. in a commercial solution of sodium hypochlorite.

Control eggs developed normally. Of the ligatured eggs, approximately half of each batch was incompletely divided by the ligature, the two parts of the embryo being joined by a narrow neck. Development in these also proceeded normally. Where successful ligature completely separated the two parts, however, the following observations were made:

(*a*) Ligature at 2–3 hr. at the border of the anterior third resulted in normal development of the posterior two-thirds, but in cessation of development of the anterior third at the blastoderm stage. Ligature at or behind the equator at this time, conversely, allowed normal development of the anterior part. but caused cessation of development of the posterior part at the blastoderm stage.

(*b*) Ligature at 4½–5½ hr. either at the border of the anterior third or at the equator had no effect on the development of either part of the embryo, but ligature at the border of the posterior third again caused cessation of development of the posterior part at the blastoderm stage.

(*c*) Ligature at 6–7 hr. allowed both parts of the embryo to develop normally, irrespective of the position of the constriction.

It can be concluded, therefore, that a differentiation centre lying between the border of the anterior third and the equator of the egg begins to produce at 3 hr. after oviposition a stimulus, presumably chemical, essential to the further development of the blastoderm, and that the stimulus spreads both forwards and backwards, reaching beyond the equator of the egg by 4½ hr. and into the posterior third by 6 hr. Descriptive studies confirm that the site of the differentiation centre is the prothoracic segment, as in other insects, and also reveal that subsequent major events in gastrulation begin at this point. Further investigations are now being pursued to localize the differentiation centre of *D. tryoni* more precisely.

The present work also confirms that determination in the cyclorrhaphan egg is completed at a very early stage[5,6], since none of the developing parts here separated by ligature showed regulation.

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Distribution of *Acarapis woodi* (Rennie)

Mites determined as *Acarapis woodi* (Rennie) were found recently on one of the escorts with a queen bee (*Apis mellifera* L.) from California. This suggested a wider distribution than previously recorded, and a survey of commercial apiaries in Queensland was commenced. The mite is widely spread on bees in this State.

In the meantime *A. woodi* was found on bees from other American States, New Zealand, and also from Italy.

These findings certainly extend the known distribution of the species.

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Numerical Regulation of Populations of the Codling Moth, *Cydia pomonella* (L.)

The numerical regulation of the codling moth has been investigated in the Australian Capital Territory. Each year, part of the population undergoes two complete generations and begins a third, most individuals of which fail to survive. The other part of the population enters diapause, and the species overwinters as mature, diapausing larvæ produced mainly in the second generation.

The level of infestation is always very high in unsprayed orchards, irrespective of size of crop. It was found that the pest multiplies each year to densities which are limited by the supply of larval food, that is, pome fruits, particularly apples.

The extreme suitability of the local environment for codling moth is determined by: (1) a very long season, extending from mid-October to late March, during which weather conditions are almost continuously favourable; (2) the extensive use of a very late maturing apple variety (Granny Smith); and (3) the absence of effective antagonists.

Intraspecific competition both for larval food and for cocoon shelter is the essential mechanism for numerical adjustment of the species in this environment.

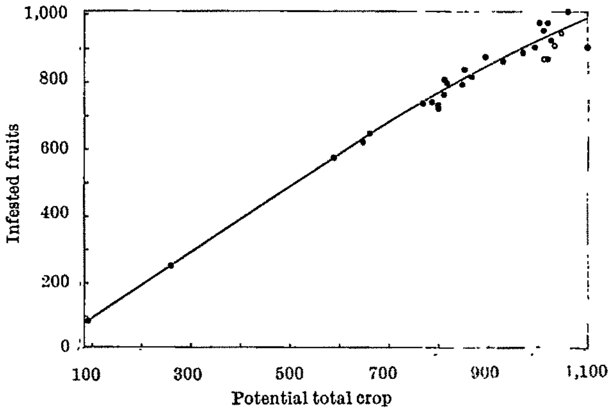

Fig. 1. Relationship between the potential number of fruits and the number of fruits penetrated by codling moth per tree. Dots, observed values; line, calculated values

It is known that larvæ may feed successively in several fruits, and that several larvæ may develop from the same fruit. However, these occurrences are exceptional. In general, only one individual survives after simultaneous infestation of the same fruit by several larvæ and one mature larva is produced for almost every infested fruit. Competition for larval food is thus a form of contest[1].

In a homogeneous plot of twenty-nine apple bushes, the relation between the potential total crop and the number of penetrated fruits per tree was described satisfactorily by the equation:

$$Y = X\left(1 - e^{-\frac{N}{X}}\right)$$

where Y is observed number of penetrated fruits per tree; X is potential number of fruits per tree (estimated from total numbers of fruit buds in spring); N is number of penetrations per tree (Fig. 1).

This equation is a variant of that used by Nicholson and Bailey[2] to describe random searching. Random search implies that all fruits are equally liable to penetration and that newly hatched larvæ are incapable of avoiding fruits which are already infested or otherwise unsuitable as food. This lack of discrimination intensified contest as population density increases, and makes complete infestation of a crop improbable. Furthermore, the equation postulates that the number of penetrations is similar on all trees of the plot irrespective of size of crop. This does in fact occur when the trees provide a comparable supply of cocoon shelter.

Larval development is completed in the fifth instar. Mature larvæ leave the fruits and seek sites in which to spin a cocoon and pupate. Whereas non-diapausing larvæ are often content to spin a flimsy cocoon in very poor shelter, overwintering individuals search their environment intensively for favourable sites. These occur mainly on the trees, under loose bark and in cracks, wounds and debris. However abundant such sites may be, they are not as a rule found easily by larvæ. Many larvæ fail completely to find adequate shelter and are lost, usually by predation. The favourable overwintering sites are potentially accessible to the remainder, some of which are successful and establish their hibernacula, while the others are eliminated by competition. This competition is also a form of contest. Except when the density of diapausing larvæ is extremely high, it is less intense than contest for larval food.

The very high levels of crop infestation which prevail in unsprayed orchards can result from remarkably small populations of overwintering larvæ, often no more than ten individuals per mature tree. These populations do not vary much from year to year, because the amount of cocoon shelter available remains fairly constant. Any variations which do arise in overwintering populations, following fluctuations in the size of the preceding crop, are damped by the contest for larval food resulting from random searching. Very dense populations in the early summer are thus prevented from destroying the fruit crop prematurely, and, consequently, from suppressing completely the diapausing larvæ which are produced later in the season. In this way, codling moth populations can very nearly exhaust their seasonal supply of larval food each year without the risk of attaining self-destructive densities.

The principal density-governing factor regulating codling moth abundance is the availability of previously uninfested fruits to newly hatched larvæ. The availability of favourable shelter to diapausing larvæ acts as a complementary governing factor[3].

MacLellan's[4] work on the destruction of overwintering codling moth populations by woodpeckers in eastern Canada and the resulting low levels of summer infestation suggests that similar effects could be achieved in Australia in a different way, namely, by bringing cocoon shelter into very short supply. For this to be effective, cocoon shelter would have to be reduced to such an extent that it became the principal governing factor in the numerical regulation of codling moth. Experiments are in progress to test the feasibility of this approach for Australian conditions.

The help obtained from Mr. G. A. McIntyre, in the Division of Mathematical Statistics of this Organization, is gratefully acknowledged.

A full account of this work will be published elsewhere.

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BACTERIOLOGY

Aspects of the Conversion of Glucose to α-Ketoglutarate in *Acetobacter melanogenum*

GLUCOSE oxidation in extracts of *Acetobacter melanogenum* has been shown to result in the formation of pyruvate[1] through reactions involving the pentose phosphate cycle and the Entner–Doudoroff splitting reaction[2]; glucose is also oxidized to 2,5-diketogluconate[3] and then to α-ketoglutarate[4]. In a previous paper[5], evidence was given for the functioning of only certain portions of the tricarboxylic acid cycle in *A. melanogenum*. It was also shown that these cells did not synthesize four carbon compounds from one-, two- or three-carbon precursors. It was, therefore, of interest to study the contribution of the various pathways in the synthesis of α-ketoglutarate from glucose in washed cell suspensions of *A. melanogenum*.

The isolation and assay of radioactive compounds and methods of preparing washed cell suspensions have been described[5]. Cells oxidizing specifically labelled glucose-^{14}C release amounts of $^{14}CO_2$ which differ according to the position of the labelled carbon (Table 1). The $^{14}CO_2$ values are consistent with the operation of a pentose phosphate cycle, Entner–Doudoroff splitting reaction and part of the glycolytic pathway[1]. Dinitrophenol almost completely inhibits the release of $^{14}CO_2$ from the differently labelled ^{14}C-glucose (Table 1), whereas the oxygen uptake in this experiment is only inhibited to the extent of 22 per cent. These results indicate a dichotomy in the oxidative degradation of glucose. As dinitrophenol is an inhibitor of oxidative phosphorylation[6], its presence decreases the supply of adenosine triphosphate necessary for the breakdown of glucose by enzymes of the phosphorylated pathways. This results in nearly maximal inhibition of the conversion of glucose-^{14}C to $^{14}CO_2$. The small amount of $^{14}CO_2$ released in the presence of dinitrophenol may be due to endogenous supplies of adenosine triphosphate regenerated by glycolytic enzymes and to the conversion of glucose to 2,5-diketogluconate, followed by its slow decarboxylation[7] with the eventual formation of α-ketoglutarate[4]. The latter sequence of reactions is not inhibited by dinitrophenol in extracts of *A. melanogenum*[4], and seems to be the main route of oxygen uptake during glucose oxidation by cells.

Table 1. EFFECT OF DINITROPHENOL ON THE OXIDATION OF GLUCOSE-1-, -2-, -6-^{14}C AND -U-^{14}C BY *A. melanogenum*

System	$^{14}CO_2$ formed (counts per min.) Dinitrophenol absent	Dinitrophenol present	Inhibition due to dinitrophenol (per cent)
Glucose-1-^{14}C	14,650	710	95
Glucose-2-^{14}C	6,330	140	98
Glucose-6-^{14}C	7,420	400	95
Glucose-U-^{14}C*	12,690	210	98

* Uniformly labelled glucose.

Components of reaction mixtures in 2·4 ml.: potassium phosphate buffer, *p*H 7·4, 120 μmoles; ^{14}C-labelled glucoses, 10 μmoles. (20,000 c.p.m./μmoles); dinitrophenol, 12 μmoles; cells, 0·8 gm. wet weight. Duration of experiment, 10 min. Temperature, 30° C.

Cells oxidizing glucose-U-^{14}C rapidly incorporate radioactivity into α-ketoglutarate as compared with pyruvate (Fig. 1). The specific activity of α-ketoglutarate reaches a maximum value which is similar to that obtained on a theoretical basis, assuming that it is formed from glucose with the loss of one carbon atom. It is interesting that the specific activity of pyruvate is low. This may be due to non-equilibration of the pyruvate pool with radioactive pyruvate, a phenomenon which has been demonstrated[5] when *A. melanogenum* cells were incubated with pyruvate-2-^{14}C.

Fluoroacetate (3×10^{-3} *M*) and dinitrophenol (5×10^{-3} *M*) had no effect on the kinetics of formation of radioactive α-ketoglutarate and pyruvate (Fig. 1). Fluoroacetate is known to inhibit aconitase in animal

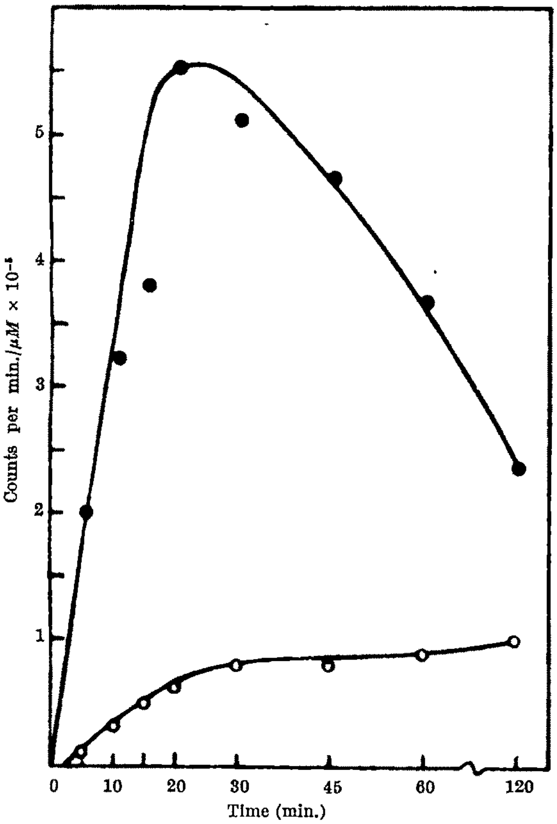

Fig. 1. Variation with time of the specific activities of α-ketoglutarate (—●—) and pyruvate (—○—) formed from glucose-U-^{14}C by resting cell suspensions. Reaction mixture contained in 10 ml.: potassium phosphate buffer, *p*H 7·4, 500 μmoles; glucose-U-^{14}C, 15 μmoles (666,000 counts per min./μmole); cells, 3·3 gm. wet weight. Temperature 30° C.

tissues[8]. In *A. melanogenum* its use results in a two-fold increase of radioactive citrate in cells oxidizing acetate-1-^{14}C.

From the results of these experiments, the conclusion may be drawn that α-ketoglutarate is formed in washed cell suspensions of *A. melanogenum* by the pathway involving non-phosphorylated intermediates (that is, gluconate and 2,5-diketogluconate[3,4]). This synthetic route is also consistent with the observations[5] on the lack of synthetic enzymes for the net formation of α-ketoglutarate from pyruvate in the tricarboxylic acid cycle.

I wish to thank Dr. R. M. Hochster, in whose laboratory in the Microbiology Research Institute, Department of Agriculture, Ottawa, Canada, the major part of this work was carried out, while I held a postdoctoral fellowship of the National Research Council of Canada.

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Folinic Acid Requirement of Strains of the Genus *Pediococcus*

Until some ten years ago, the genus *Pediococcus* was recognized only in the brewing industry, but strains of *Pediococcus cerevisiae* are now known to occur commonly in other food products[1,2]. It has been suggested that strains of the genus isolated earlier were not always correctly identified, and were classified as either leuconostocs or micrococci, according to their catalase reaction[3]. Therefore, any test which would help to identify the genus or the species *Pediococcus cerevisiae* would be useful. Felton and Niven[4] suggested that a requirement for folinic acid might be a generic character of the pediococci. However, they examined only typical strains of *Pediococcus cerevisiae* and, while it is true that a majority have this requirement, strains without it are known[5].

Recently four strains of *Pediococci* (*N.C.D.O.* 1247, 1248, 1249 and 1250; that is, *L*354, *S*190, *L*22 and *S*344 respectively) were deposited in the National Collection of Dairy Organisms by Dr. H. L. I. Gunther, of Queen Elizabeth College, University of London, and these were reported to vary in several respects from the typical *Pediococcus cerevisiae*[6].

They were therefore examined for their folinic acid requirement in the synthetic medium used for the assay of folinic acid with *Pediococcus cerevisiae* *ATCC* 8081[7]. All four strains failed to grow even when folinic acid was present, and under conditions which supported normal growth of *ATCC* 8081. It was found that certain additions to the assay medium and sometimes modified cultural conditions were necessary before all the strains would grow.

The strains were sub-cultured in tomato glucose broth in which three of them, *N.C.D.O.* 1247, 1248 and 1249, grew well aerobically and also anaerobically. The fourth strain, *N.C.D.O.* 1250, failed to grow except under anaerobic or reducing conditions, and good growth of this strain has not been obtained aerobically in any medium. Therefore, for *N.C.D.O.* 1250, 0·5 ml. of a 1 per cent w/v solution of ascorbic acid, sterilized by Seitz filtration, was added to each 10 ml. of medium before inoculation, and the tubes incubated aerobically.

'Tween 80', an ingredient of the tomato glucose broth, was found to be essential for the growth of all four strains, although Kitay and Snell[8] found that *ATCC* 8081 did not require it. Two of the strains, *N.C.D.O.* 1247 and 1250, grew well when 'Tween 80' (0·001 ml./10 ml.) was added to the Sauberlich and Baumann medium containing folinic acid.

Charcoal-treated tryptic digest of casein solution was prepared according to Roberts and Snell[9] and added to the assay medium, at a level of 1 ml./10 ml. Under these conditions all the strains grew well. It appears that, as with other strains within the Lactobacilliaceae[10], *N.C.D.O.* 1248 and 1249 require a peptide in addition to the amino-acids and other growth factors supplied.

A solution containing vitamin-free acid-hydrolysed casein, tryptophan, phenylalanine, histidine, tyrosine and cystine could replace the amino-acid mixture in the original medium, but the addition of the tryptic digest of casein was still necessary.

The folinic acid requirement of the strains was examined in this modified medium, adding only one level, 0·1 μgm./10 ml. The composition of the medium and the techniques used for the growth tests have been described by Garvie, Gregory and Mabbitt[11]. Since a variety of strains and possibly more than one species were being examined, the growth of each strain was observed after 24- and 48-hr. incubation at 30° C. in the medium with and without folinic acid. Two of the strains, *N.C.D.O.* 1250 and 1247, grew equally well in all tubes and had no requirement for folinic acid. The other strains, *N.C.D.O.* 1248 and *N.C.D.O.* 1249, gave excellent growth in the presence of folinic acid at 24 hr., but none in its absence. However, at 48 hr. in the absence of folinic acid, growth sometimes occurred. Thus, with these strains, folinic acid has a marked stimulatory effect on growth, although under conditions at present undefined, they grew in its absence, but only after a prolonged lag phase. The control, *ATCC* 8081, showed no growth in the absence of folinic acid after 48 hr. incubation.

These results show that a requirement for folinic acid is not restricted to typical strains of *Pediococcus cerevisiae*. Until more strains have been examined, it is not possible to decide if the growth requirements which have been discussed are useful in classification, or if they demonstrate merely variations within the species *Pediococcus cerevisiae*.

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A Bacteriophage for Motile Bacteria

IN the first stage of phage infection, the phage particle is adsorbed to a specific receptor which is usually on the bacterial body[1]. However, an exceptional phage has been reported[2] the host-range of which appeared to be determined by flagella, and these are structurally distinct from the bacterial surface proper[3].

Four conditions have now been found to determine bacterial sensitivity to this *Salmonella* phage, named χ.

(1) The bacteria must be flagellated. Non-flagellated strains are invariably resistant, and resistant non-flagellated variants are selected from sensitive strains by the phage.

(2) The flagella must carry certain antigens. Sensitivity was shown to depend on the flagellar (H) antigen, and not on the somatic (O) antigen, by tests on (a) 524 naturally occurring motile *Salmonella* strains belonging to 27 different O groups and carrying most of the known H antigens; and (b) sets of artificial derivatives of single strains, differing in their H antigens, which had been prepared by phage-mediated transduction[4]. Strains with most H antigens could be sensitive, but all 53 naturally occurring strains with antigens of the g complex (g,m, g,p, g,s,t, etc.) were resistant: transduction of antigen g . . . to sensitive strains made them resistant, and resistant strains naturally carrying g . . . were often sensitive when another antigen was substituted. The wild-type phage did not attack strains with H antigen l . . . (l,z_{13}, l,v, or l,w), but could mutate to a form able to do so. Host-range mutants were also isolated for bacteria with H antigens e,h and Arizona 13. Diphasic strains carrying g . . . , l . . . or e,h in one phase could be sensitive to wild-type phage in the alternate phase.

(3) The flagella must be active: 10/10 naturally occurring paralysed (that is, flagellated but non-motile) strains were resistant while their motile derivatives were sensitive. Also, 8/80 resistant variants selected by the χ phage from sensitive strains turned out to be paralysed. Reversion to motility was accompanied by return of sensitivity.

(4) The bacterial strain itself must be suitable, for not all motile bacteria with appropriate H antigens were sensitive. The importance of the bacterial strain was clearly seen with transduction of an antigen, for the strain from which the antigen had been transduced often reacted differently from the recipient.

Resistance due to absent or inactive flagella, or to flagella of inappropriate antigenic type, could be shown to be associated with impaired adsorption of the phage, by using sets of variants of suitable strains. Adsorption was prevented by detaching the flagella from sensitive bacteria, and returned progressively to normal as the flagella were regenerated. Adsorption was also impaired when sensitive bacteria were artificially paralysed by treatments such as thorough washing, or exposure to 2,4-dinitrophenol.

No adsorption to detached flagella was detected, probably because they were inactive.

Electron micrographs, kindly prepared by Mrs. H. Ozeki and by Dr. E. H. Mercer, showed phage particles attached by the tips of their tails all along the length of the flagella; none was seen on flagella carrying antigens associated with resistance and few or none were found on paralysed bacteria. Other experiments showed that infection could follow adsorption to distal as well as proximal parts of a flagellum.

If, as it appears, isolated[5] and paralysed[6] flagella are structurally similar to active flagella, then the latter do not seem to possess any unique structure that might be the phage receptor. The necessity for flagellar movement to promote phage infection may thus mean that the activity is necessary in itself and not merely a concomitant of a new flagellar structure. The means by which the phage genome passes from its primary site of adsorption on a flagellum to the bacterial body is not clear, but the flagella do not seem to act merely by 'activating' the phage so that it can attach itself to the bacterial body afterwards. These points and the detailed results are discussed elsewhere.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, May 8

University of London (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital, Paddington, London, W.2), at 5 p.m.—Dr. S. Brenner: "Bacteriophage Growth".*

University of London (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. A. Neuberger, F.R.S.: "The Biosynthesis of Porphyrins and Chlorophylls".* (Further lecture on May 11.)

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Scientific Papers.

University of London (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. L. W. Busse (Wisconsin): "Some Aspects of Percutaneous Absorption".*

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Basil Taylor: "The Presentation of Visual Arts". (Second of three Cantor Lectures on "The Presentation of Science and the Arts on Television".)

Royal Aeronautical Society, Historical Group (at 4 Hamilton Place, London, W.1), at 7 p.m.—"A Quiz on Little Known Early Aircraft".

Tuesday, May 9

Society of Chemical Industry, Agriculture Group (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Annual General Meeting, followed by a full-day Meeting on "Manuring of Tropical Crops".

Aslib (in the Lecture Theatre of the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 2.30 p.m.—Discussion on "The National Reference Library of Science and Invention".

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

University of London (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. L. W. Busse: "Electronic Analysis of Emulsion Globules".*

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. M. E. Burt: "Structural Weight Estimations for Novel Configurations".

Wednesday, May 10

University of London (in Lecture Room 102, Chemical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 4 p.m.—Prof. Gabriel Stein: "Recent Advances in the Radiation Chemistry of Aqueous Systems—Part II, Some Systems of Biochemical Interest".*

Institution of Electrical Engineers, Supply Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. M. Datta: "Electricity Supply in India and its Future".

University College London (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. P. Bálint (University of Budapest): "The Reliability of Clearance Tests in the Evaluation of Renal Blood Flow".*

University of London (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. P. D. Bartlett (Harvard): "Peresters and Homolytic Fragmentation".*

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. J. S. Fulton: "The Future Pattern of University Education in the United Kingdom".

Wednesday, May 10—Thursday, May 11

Association for the Study of Medical Education (in the New Hall of the Students Hostel, London Hospital Medical College, Ashfield Street, Whitechapel, London, E.1)—Symposium on "Closed-Circuit Television in the Medical School".

Thursday, May 11

University of London (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. P. D. Bartlett (Harvard): "Autoxidation and Chain Termination".*

Society of Instrument Technology, Control Section (at Manson House, 26 Portland Place, London, W.1), at 6.15 p.m.—Annual General Meeting. 7 p.m.—Dr. W. Grey Walter: "Control Mechanism in the Human Nervous System".

Friday, May 12

British Interplanetary Society (in the Council Room of the Federation of British Industries, 21 Tothill Street, London, S.W.1), at 10 a.m.—Symposium on "Communications Satellites".

Institution of Electrical Engineers, Education Discussion Circle (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "The Place of Transistors in National Certificate Courses", opened by Mr. B. F. Gray and Mr. W. B. K. Ellis.

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. T. Stanley Westoll, F.R.S.: "A Crucial Stage in Vertebrate Evolution: Fish to Land Animal".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or Assistant Lecturer (preferably with special interests or qualifications in igneous and metamorphic petrology) in the Department of Geology—The Registrar, The University, Leeds 2 (May 12).

Postdoctoral Fellow in Organic Chemistry, to carry out researches into the behaviour of olefinic acids in strong acid media in collaboration with Dr. M. F. Ansell—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (May 12).

Lecturer in the Department of Metallurgy—The Registrar, The University, Liverpool (May 15).

Assistant Lecturer in Applied Mathematics—The Secretary, The Queen's University, Belfast (May 20).

Harry Bolus Chair of Botany in the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, May 20).

Lecturer in Chemical Engineering—The Registrar, University College, Singleton Park, Swansea (May 20).

Lecturer (preferably with an interest in ecology and/or meteorology and climatology) in the Department of Geography - The Secretary, The University, Aberdeen (May 20).

Senior Lecturer in the Department of Physics—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (May 24).

Lecturer (preferably with research experience in plant-insect relations or a related field) in the Department of Horticulture, to teach biological aspects of horticultural crop protection - The Registrar (Room 22, O.R.B.), The University, Reading (May 31).

Lecturer (with a higher degree, preferably a Ph.D., and preferably experience in university teaching) in Chemistry at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 31).

Research Fellow in Chemical Crystallography in the Department of Chemistry in the Faculty of Science to undertake investigations into the structures of complex natural products of biological importance and novel structural interest—The Registrar, University of Manchester, Manchester 13 (May 31).

Senior Lecturer in Inorganic Chemistry—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1 (May 31).

Senior Principal Scientific Officer (research worker with specialized knowledge and experience in animal research)—The Secretary, Hill Farming Research Organization, 48 Palmerston Place, Edinburgh 12 (May 31).

Lecturer (preferably with experience in plant physiology or developmental anatomy) in Botany at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, June 2).

Lecturer/Senior Lecturer (interested and qualified in mineralogy and crystallography, and preferably with experience in X-ray techniques) in Geology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 9).

Lecturer in Education; and Lecturers (2) in Geography at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, June 15).

Chair of Physics (High Energy Nuclear Physics) in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

Biochemist (non-medical) in the Department of Medicine, Makerere University College, East Africa, to participate in the current programme of research—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Chair of Physics in the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Chemist or Biochemist (honours graduate), to investigate fundamental aspects of the dehydration of meat—The Director, Low Temperature Research Station, A.R.C., Downing Street, Cambridge.

Head, Senior Scientific or Principal Scientific Officer (with a good honours degree in science or equivalent and previous research experience), of the Pig Section of the Applied Nutrition Department—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen.

Lecturer, and a Lecturer (Grade B) in the Department of Mathematics—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Lecturer in Psychology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1.

Medical Laboratory Technician (qualified A.I.M.L.T., science degree or equivalent)—The Administrative Office, North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex.

Otto Beit Chair of Physiology; Senior Lecturers in the Department of Physiology; Lecturer in the Department of Pathology and Microbiology; a Senior Lecturer and Lecturer in the Department of Mathematics; and a Senior Lecturer and Lecturer in Physical Chemistry at the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1.

Research Assistant (graduate) in the Mathematics Department, to work either in computing, mathematics or statistics—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Research Fellow or Assistant (with a good honours degree in mathematics or engineering, and preferably experience in programming for a digital computer) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS for work in the field of hypersonic gas dynamics—The Secretary and Registrar, The University, Southampton.

SENIOR LECTURER (graduate in botany, agriculture or horticulture, with teaching and research experience at university level, and preferably a knowledge of tropical crops) IN CROP HUSBANDRY at the University College of the West Indies (Imperial College of Tropical Agriculture, Trinidad)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

SENIOR LECTURER or LECTURER IN ORGANIC CHEMISTRY; a LECTURER IN INORGANIC CHEMISTRY; and an ASSISTANT LECTURER IN INORGANIC CHEMISTRY—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 9/G.

SENIOR LECTURER (qualified to teach microbiology to final degree and Diploma of Technology standard) IN MICROBIOLOGY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 16: Feeding Dairy Cows. Pp. 15. Leaflet No. 84: Lime and Liming. Pp. 7. Leaflet No. 100: The Cultivation of Carrots. Pp. 8. Leaflet No. 107: Mineral Mixtures for Stock-Feeding. Pp. 4. Leaflet No. 142: Lead and Copper Poisoning. Pp. 4. (Belfast: Ministry of Agriculture, 1960 and 1961.) [72

Notes on Process Selection and Filler/Electrode Materials. (Addendum 2 to Information Bulletin 19.) Pp. 7. (London: The Aluminium Development Association, 1960.) [72

Agricultural Research Council. Postgraduate Studentships and Fellowships offered in 1961. Pp. 20. (London: Agricultural Research Council, 1961.) [72

Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom. Third Report of an *ad hoc* Committee on Procedure for Admission of Students. Pp. v+41. (London: Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom, 1961.) 2*s*. 6*d*. [72

Department of Scientific and Industrial Research: National Physical Laboratory. Notes on Applied Science No. 20: Small-Scale Preparation of Fine-Grain (Colloidal) Photographic Emulsions. By B. H. Crawford. Pp. iv+13+1 plate. (London: H.M. Stationery Office, 1960.) 1*s*. 6*d*. net. [72

The Engineer Buyers Guide, 1961. Pp. 984. (London: Morgan Brothers (Publishers), Ltd., 1961.) 10*s*. [82

English Electric Valve Co., Ltd. Abridged Valve Data. Pp. 38. (Chelmsford: English Electric Valve Co., Ltd., 1961.) [82

The Consolidated Zinc Corporation, Ltd. Descriptive Booklet. Pp. 96. (London: The Consolidated Zinc Corporation, 1960.) [92

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1028, Vol. 253 (9 February, 1961): Ionization Fronts in Interstellar Gas and the Expansion of H II Regions. By F. A. Goldsworthy. Pp. 277–300. 7*s*. 6*d*. No. 1029, Vol. 253 (9 February, 1961): Ionization Fronts in Interstellar Gas: The Structure of Ionization Fronts. By W. I. Axford. Pp. 301–333. 10*s*. 6*d*. (London: Royal Society, 1961.) [92

Liverpool Observatory and Tidal Institute. Annual Report, 1960. Pp. 20. (Liverpool: Liverpool Observatory and Tidal Institute, 1961.) [102

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 82: The Production of Clean Milk. Pp. 19. Leaflet No. 146: The Growing and Harvesting of Ryegrass Seed. Pp. 4. (Belfast: Ministry of Agriculture, 1961.) [102

Bulletin of the British Museum (Natural History). Botany. Vol. 2, No. 10: New Species of *Taraxacum* from the Himalayan Region. By J. L. van Soest. Pp. 261–273+plates 22–29. 10*s*. Zoology. Vol. 7, No. 4: A Revision of the Genus *Dinotopterus* Blgr. (Pisces, Clariidae), with Notes on the Comparative Anatomy of the Suprabranchial Organs in the Clariidae. By P. H. Greenwood. Pp. 215–241. 10*s*. (London: British Museum (Natural History), 1961.) [102

Building Research Station Digest (Second Series), No. 7: Noise in the Home. Pp. 4. (London: H.M. Stationery Office, 1961.) 4*d*. [102

University of Birmingham. Report of the Proceedings of the Council of the University for the year ended 31st December, 1960. Pp. 19. Accounts for the year ended 31st July, 1960. Pp. 35. Report of the Vice-Chancellor and Principal for the calendar year 1960. Pp. 35. University Research Committee—Thirty-first Annual Report (Session 1959–60). Pp. 137+1 plate. (Birmingham: The University, 1960, and 1961.) [102

Agents of Cultural Advance. By Sir Aubrey Lewis. (L. T. Hobhouse Memorial Trust Lecture No. 30, delivered on 30 April, 1960, at University College, London.) Pp. 29. (London: Oxford University Press, 1961.) 5*s*. net. [102

Other Countries

Ministry of Agriculture and Lands, Jamaica, W.I. Bulletin No. 57 (New Series): Investigations, 1956–1957. Pp. iii+139. 3*s*. 6*d*. Black Pepper: a Review of Cultural Practices and their Application to Jamaica. By J. F. Ward. Pp. i+13. Veterinary Division Extension Circular No. 61 (August, 1959): Swine Erysipelas. Pp. 7. (Kingston: Government Printer, 1959 and 1960.) [311

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CONQUEST OF SPACE

"ALL this world is heavy with the promise of greater things, and a day will come, one day in the unending succession of days, when beings, beings who are now latent in our thoughts and hidden in our loins, shall stand upon this earth as one stands upon a footstool, and shall laugh and reach out their hands amidst the stars." So H. G. Wells predicted in his discourse, "The Discovery of the Future", delivered at the Royal Institution on January 24, 1902 (*Nature*, **65**, 326; 1902).

Almost sixty years later the Academy of Sciences of the U.S.S.R. has given point to that prophecy. Man is entering a new régime of endeavour. Risen from intertidal slime, he now cleaves interstellar plasma. The astronautical venture has begun. For those who, from innate scepticism or lack of knowledge, have underrated this epic achievement as being no more important than rocketing instruments into space, some guidance is necessary. Apart from the sheer poetry of a human being undertaking such a Ulyssean journey, the scientific value is vast. Instruments, however ingenious, can only yield what they have been set to do. They are precluded from the realm of discovery. Man, with his natural universality and ability to reason, is at the very pinnacle of all discovery.

Unfortunately, the perspective received by the public is a trifle confusing. The reason is that astronautical programmes have given the impression of being somewhat of a random process—not wholly untrue this side of the Iron Curtain. However, Albert Ducrocq, a savant of note in astronautics and cybernetics, has managed in his book, *Victoire sur L'Espace**, to describe with much clarity the achievements and aims of space science. The book does not deal in detail with the development of space-vehicles, but provides an excellent review of performance and accomplishment. With Gallic limpidity of expression (not lost in the translation) he shows how the chief outcome of the astronautical revolution has been to make the scientific description of Nature more elegant.

Merely a few measurements made outside our planet have produced immense strides forward in certain branches of science. For example, as described in the famous report (March 28, 1959) by Academician A. N. Nesmeyanov, president of the Academy of Sciences of the U.S.S.R., the first *Sputniks* revealed that the density of the upper atmosphere is greater than was thought and composition is very different too, molecular forms of nitrogen and oxygen being essentially non-existent. Also, extreme turbulence with enormous variation in temperature and pressure appears to characterize this home of Vulcan. The very basis of meteorology has been transformed with such knowledge. Human intervention in directing the course of winds, rains and hurricanes may be envisaged. By analyses of the orbits, through application of Kepler's laws, the radius of the Earth has been estimated precisely. There appears to be a gravitational asymmetry suggesting a bulge in the southern part. Geophysical measurements have acquired a remarkable accuracy.

The existence of intense radiation at great height was indicated when the American *Explorer I* followed an eccentric orbit not planned by the technicians. It was an error on the plus side, since it led to identification, chiefly by Van Allen, of belts of radiation encircling our globe. It appears that the Earth is submerged within the Sun's atmosphere. These belts of plasma material having their origin in solar rays provide a link between solar events and their effects on Earth. For the first time outer space was used as a laboratory in an experiment known as 'Operation Argus'. An atom bomb was fired far out in space. Liberated electrons assembled almost instantaneously in the Earth's magnetic field to produce an artificial aurora. There is a danger in such experiments. Apart from causing the Earth's rotational speed to vary, repeated explosions could shatter the electrical equilibrium of the upper atmosphere, so playing havoc with our climates. This planet of ours seems to be rather delicately balanced.

What has distinguished the Soviet research in space is its direction toward the future. One investigation leads elegantly to another which, in turn, gives impetus to more extended work. While preparing for the future on a very large scale, Soviet scientists restrict the number of operations and make each as fruitful as possible. For example, it was puzzling that all three *Sputniks* should have been placed in orbits with low perigee, until one realizes that such satellites are at once atmospheric and geodetic, so yielding the greatest range of information. This powerful, ordered way of thinking is an interesting counterpoint to the impression one gets from the literature of Tolstoi, Dostoievsky and Bryúsov, for example, of the Russian mind, so passionately unrestrained. Therein probably lies their success.

Regrettably, one often finds naïve dismissal of Soviet scientific achievement as being another Machiavellian device for propelling the propaganda machine and making overtures to the 'uncommitted'. To be sure, the Russians do relate political prestige to their scientific endeavour. But this should not compel us to denigrate the sheer scientific attainment itself.

M. Ducrocq discusses the categories of problems encountered in space travel and how some of these have been surmounted. First, stabilization of various kinds was required to prevent space-vehicles from tumbling. This was done by creating an initial spin or by using ancillary fluid-ejection devices. He reviews problems related to re-entry. When a satellite returns into the atmosphere, it takes up a streamlined attitude according to aerodynamic influences.

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* The Conquest of Space: Moon Probes and Artificial Satellites—Their Impact on Human Destiny. By Albert Ducrocq. Pp. 300+8 plates. (London: Putnam and Co., Ltd., 1961.) 30s. net.

But, on crossing the interface, it is likely to experience tremendous oscillations, comparable with the flapping of a flag. Uncontrolled kinetic heating can have dramatic consequences, as in the case of the early *V*2's, which exploded, in mid-air, despite their relatively low speed. A human occupant of a space-vehicle has to contend with a range of gravitational effects, and, for interplanetary voyages, there is the hazard of the radiation belts.

In travel to the Moon or planets the traditional notion of a journey no longer applies, since both the point of departure and the destination are moving. Such a voyage must be conceived in four-dimensional space. An advance without precedent in the 'conquest of precision' occurred when *Lunik II* was navigated to reach the Moon. The *Sputniks* had attained a precision of 1 per cent. For a shot to the Moon this represents an uncertainty of several tens of thousands of miles (with a régime close to unity). In January 1959, *Lunik I* took off at an initial speed controlled to within 0·1 per cent, already a ten-fold increase in precision since the previous year. It passed at 4,000 miles from the Moon. In September 1959 the staggering success of a precision of 0·01 per cent was gained when the second lunar probe landed on its target.

Thus for the first time spatial guidance was introduced. Using an ingenious iterative technique designed by Prof. Koteinikov and Prof. Chtchoukino deviation from an ideal ballistic path is measured *en route*, then small corrections are made. The vehicle is virtually steered to the Moon, which subtends an angle of only 0·5 degree as seen from the Earth. This amazing feat of gaining on the average one decimal place per annum attests to the directing influence of the Soviet astronautical programme, namely, to combine power with accuracy, each step paving the way for its successor.

It seems scientists may have attained yet higher orders of precision; at present a Soviet missile is in hot pursuit of the goddess of sweet intimacy. Sadly, perhaps, she will have to reveal her mysteries. M. Ducrocq discusses possible voyages to Mars as well and provides a very good summary of the elements of ballistic theory in an appendix.

It is indeed wondrous to witness how mankind, though thwarted and frustrated, often by its very nature, can, like Ulysses, ever feel that force

> "To follow knowledge like a sinking star
> Beyond the utmost bound of human thought."

A FURTHER TRIUMPH FOR SCIENCE

As is now universally known, American scientists and technologists achieved a great victory when, on May 6, they were responsible for the projection of a rocket carrying a man (Commander Alan Shepard) into sub-orbital space, the capsule being recovered intact with Commander Shepard alive and well. This is a record of a controlled flight 115 miles high and 302 miles down-range.

The congratulations of all must be extended to the American scientists and technologists who made this feat possible; and we express great admiration to Commander Shepard for his valour and devotion to duty.

This American achievement follows closely on that of the Soviet scientists, who, on April 12, succeeded in placing a controlled space-vehicle in orbit containing a man (Major Gagarin) and also recovered him alive and well (see *Nature* of April 22, p. 291).

In announcing their achievement, the Soviet political authorities stated: "We gladly place them [the scientific results] at the service of all peoples in the name of progress, happiness and the well-being of all human beings on Earth". Similarly, from the White House, the President of the United States, commenting on this most recent American space victory, stated: "Important scientific material has been obtained during this flight and this will be made available to the world's scientific community".

Here are two great scientific feats achieved by two Powers more or less diagonally opposed in political creed. While the world's scientists must share satisfaction in the almost fantastic progress thus revealed, all and sundry should view with grave concern and no little apprehension the politics behind it.

As pointed out in our previous comment (*Nature* of April 22), it still seems that only lip service is being paid to freedom for scientific research and, above all, freedom to publish and discuss scientific results—to the ultimate benefit of man. We are glad to read the Soviet and American promises to release these, their latest, results, but shall feel happier when such promises are implemented, for until then scientific progress is shackled and world peace possibly threatened. To-day, far too much science is censored, and this is bound to cause international bad faith and suspicion.

Commenting on the great American achievement, a spokesman in the Soviet Embassy in London said: "This stage of development was reached several years ago in the Soviet Union". Most men of science, and indeed others, will be prepared to accept this as a fact; but it is significant that the Russians, so far as we know, made no announcement at the time or published any scientific results afterwards. On the other hand, the Americans have not only published immediately the actual fact of their success, but also did everything they could (through radio, television, etc.) to allow the world to follow the last stages of their gigantic experiment (for there must have been many stages before the actual launching of the rocket)—even at the risk of enacting a failure.

It is relevant here to reveal that for some years *Nature* has tried to persuade the Soviet authorities (through the media of some of their scientists) to allow their magnificent scientific results (not confined to space research) to be published for all to read; and very recently approaches have been made direct to political authorities in Moscow and to the Soviet Ambassador in London; but, alas, so far with negative results.

To most non-scientists, the successful pioneers in these recent space researches are Gagarin and Shepard. Yet, with all due respect to these brave men, they themselves were actually part of certain scientific equipment. The fundamental work was done by, and therefore praise is also due to, the scientists who made possible the heroic feats of these two men. In fact, these journeys into space are the results of the work of many people over many years, culminating in team work among scientists and non-scientists. Alas, however, thanks mainly to the fundamental defects in the world's means of mass information and education, the scientists are relegated by the layman to an almost intangible, certainly generally incomprehensible, position, and have to suffer such vulgar epithets as 'back-room boys' or 'boffins'.

Such men as Gagarin and Shepard have had, and will continue to receive, public acclaim and fêting which is their due, though much of this may well verge on hysteria stimulated by political propaganda and national patriotism. Meanwhile, the scientist goes quietly on with his work.

One sometimes wonders if such national and international acclaim would be accorded to a man who discovered an over-all cure for cancer or who made two blades of grass grow where only one or none grew before. Certainly no such world-wide publicity and praise were meted out to those scientists who were responsible for the discovery and development of penicillin in the late 'thirties and onwards: yet to-day, as a result of this work, millions of lives are being saved, much suffering alleviated and a great industry of antibiotics established in many parts of the world.

We would be the last to wish to denigrate the contributions Major Gagarin and Commander Shepard have personally made to the advancement of space science; but these two achievements, and especially the way in which they are being presented to the world, help to emphasize what *Nature* has been stressing continuously for some years, that is, the great danger inherent in allowing science to become the tool of political and diplomatic intrigue; for from scientific endeavour coupled with diplomatic intrigue it is only a short step to scientific success possibly coupled with political threat. Neither is it wise that scientific achievements be used for the enhancement of political prestige.

Until we have a world where all or most of its peoples are sufficiently educated or informed about things scientific, we shall travel further and further into that dread arena where politicians are playing ball with bombs—a dangerous game having no referee, not even the United Nations, who are certainly not yet wholly united in scientific endeavour.

MORE RECENT ADVANCES IN BIOCHEMISTRY

Recent Advances in Biochemistry

By Prof. T. W. Goodwin. Fourth edition. Pp. vi+301. (London: J. and A. Churchill, Ltd., 1960.) 50*s*. net.

It will probably come as a surprise to most readers of this book to find it described as the fourth edition of *Recent Advances in Biochemistry*. The third edition of a book with this title appeared in 1931. It was written by Prof. J. Pryde and was a landmark in biochemical literature. The present book is by a different author and the whole subject of biochemistry has, of course, become transformed in the intervening thirty years. Two consecutive editions of a book could scarcely be more different; yet the present one is likely to take a similarly outstanding place in the literature. The aim of the book is to bridge the gap between the general text-book and the specialized review article, and to introduce senior undergraduate students and research workers to some of the present-day frontiers of biochemical discovery. In a book of eight chapters, it has been possible to deal only with selected topics, and emphasis has been placed on mechanisms of biosynthesis, with a leaning towards the chemical rather than the physiological aspects of biochemistry.

Chapter 1 deals with electron transport and oxidative phosphorylation. Here, as elsewhere in the book, the author describes not only the known facts but also their experimental basis. Chapter 2 is concerned with carbon dioxide fixation in higher plants and micro-organisms and in animal tissues. It includes a discussion on the Krebs urea-cycle. The chemistry and biosynthesis of phospholipids is covered in Chapter 3, and the biosynthesis of aromatic and heterocyclic ring compounds in the next two chapters. These are followed by a discussion of the nucleic acids: "The compounds which perhaps more than any dazzle biochemists and biologists alike with the glittering prospect of an explanation of the ultimate mysteries of living matter". The subject is treated from the synthetic rather than the functional aspect, but there is an interesting discussion of recent work on the infection of *E. coli* by different strains of bacteriophage. Chapter 7 deals with biosynthesis of terpenes and related compounds, and contains a detailed description of the steps leading to the biosynthesis of cholesterol. Carotinoids are included in this chapter, and here the author is on his home ground for he has contributed much to our knowledge of the biochemistry of these compounds. The book ends with a short but most valuable chapter on the regulation of cell metabolism, a subject of great interest and importance, but one which has scarcely reached the text-books yet.

Throughout the book there are numerous references to the more significant original articles and there is a useful index. The author makes very free use of abbreviations for the names of compounds: indeed, some pages look as if they might have been written in code. Some of these abbreviations are not in common use; others, such as DPN, may soon become obsolete (see *Nature*, 188, 464; 1960). It would save readers a lot of unnecessary searching if, in future editions, an alphabetical key to abbreviations were supplied at the beginning or end of the book. The standard of production is good, and the numerous diagrams of formulæ and reaction scheme-are clearly set out. This book will be greatly valued by research workers and teachers and, perhaps above all, by undergraduates in their final year of honours biochemistry. Organic chemists will find much to interest them too. It is not easy reading because of the complexity of the subjects discussed, but the chosen ground could scarcely have been covered better in a book of this size. It is much to be hoped that Prof. Goodwin will have the time and patience to bring out further up-to-date editions of this book from time to time. D. C. HARRISON

ANIMAL BEHAVIOUR

Current Problems in Animal Behaviour

Edited by W. H. Thorpe and Prof. O. L. Zangwill. Pp. xiv+424+2 plates. (Cambridge: At the University Press, 1961.) 45*s*. net.

THE phenomena of animal behaviour are being studied by a rapidly increasing number of scientists. Few other biological phenomena have attracted such a variety of workers and have led to so many differently oriented disciplines. After a period in which these various disciplines ignored each other, a tendency grew towards establishing contact; this at first expressed itself in mutual criticism, but it is gradually developing into real co-operation. Because the subject is so complicated, because it also offers more obstacles to consistent scientific inquiry than most other life processes, and further because each class of workers has developed its own concepts, terminology and theories during periods of isolation, the growth of co-operation is a difficult and painful process. In spite of this, and notwithstanding a growing tendency towards specialization, an increasing number of workers is seriously attempting to build at least a common foundation for a unified, broad and systematic attack on problems of animal behaviour. These attempts naturally require much discussion, and in many research centres there have been formed interdisciplinary discussion groups in which problems, methods, concepts and concrete research plans are critically considered.

The present book gives us an insight into the kind of activities of such seminars. Each member of Dr. Thorpe's and Prof. Zangwill's alert and able group "was asked to write an essay on an aspect of his own work or discipline which appeared to him interesting and vital for the study of behaviour as a whole"; these essays were then critically discussed and rewritten.

The result is a series of extremely worth-while chapters, loosely bundled into four major parts. The editors jointly wrote a general introduction, and each part is again introduced by one of them.

Part 1 ("Neural Mechanisms and Behaviour") contains a chapter by D. M. Vowles in which differences between the nervous systems of insects and vertebrates are traced, and constructive chapters by L. Weiskrantz and O. L. Zangwill. Part 2, not quite appropriately called "Experimental Studies of Animal Behaviour", contains chapters on ritualization (A. D. Blest), on flock organization in birds (J. H. Crook) and on filtering of external stimuli (P. Marler); Part 3, on "Problems common to the Psychology of Animals and Men", has 5 chapters (R. A. Hinde, W. H. Thorpe—whose contributions overlap a little—M. A. Vince, D. E. Broadbent and A. J. Watson) and the last part deals with "Theoretical Approaches" (R. L. Gregory, H. B. Barlow and V. Cane).

While such a book can of course not be expected to be an exhaustive review, it is amazing how many aspects of the field are given critical attention, and how many chapters present really new points of view. Not unexpectedly, there is a great deal of trial-and-error thinking in print, and if the trend of some contributions is a little over-destructive, this reflects the soul-searching state our science is in. Nor should it be forgotten that there has been a considerable delay in publishing.

The title, and particularly the sub-title, perhaps suggest a more exhaustive covering and more unity of approach than are really presented. When the editors state that "the psychologist is . . . interested in . . . the explanation of behaviour in general" (p. x) they are, I feel, underrating the facts that the narrow descriptive basis of psychology makes it at times difficult to distinguish the general from the special, and that the undoubtedly general problems of survival value and evolution are still neglected in psychology. Furthermore, when they say that "biology is *still* very largely dominated by the concept of evolution" (p. xii, italics mine), do they mean that this is a passing stage, which biology will outgrow, and which psychology has already outgrown? N. TINBERGEN

AXIOMATICS AND FOUNDATIONS

The Axiomatic Method, with special reference to Geometry and Physics

Proceedings of an International Symposium held at the University of California, Berkeley, December 26, 1957–January 4, 1958. Edited by Prof. Leon Henkin, Prof. Patrick Suppes and Prof. Alfred Tarski. (Studies in Logic and the Foundations of Mathematics.) Pp. xi+488. (Amsterdam: North-Holland Publishing Company, 1959.) 90*s*.

The Foundations of Mathematics

A Study in the Philosophy of Science. By Evert W. Beth. (Studies in Logic and the Foundations of Mathematics.) Pp. xxvi+741. (Amsterdam: North-Holland Publishing Company, 1959.) 100*s*.

THESE two books, different in emphasis but alike in purpose, present an inspiring picture of modern thought concerning the basis of mathematics. The drilling into the sub-structure of the subject goes on with relentless vigour. The whole field is becoming so vast that expertize within expertize is beginning to appear, which means that the student needs instruction of course, but guidance as well, through the mass of highly specialized literature. All this is provided in good measure.

Axiomatic Method is the outcome of a conference held in 1958. Its general theme, as the title suggests, is the formal structure of mathematics. The deliberations are divided into three parts: (1) foundations of geometry; (2) foundations of physics; (3) general problems; and applications of the axiomatic method. In the first of these the order is that of subject, in the second of historical development, and in the third (broadly) from the more specific to the more general. The reader will appreciate this plan, but the purpose behind the arrangement is worth noting. Geometry has been the home ground of axiomatics for more than two thousand years, and in that field the method originated. It would be quite unnatural if any sequence other than by subject were adopted. But physics has not developed like that, and so the chronological treatment is the better. In fact, to-day physical research is often curiously informal (in the technical sense), and its theoretical side is frequently kept lively and progressive by the need to resolve some inconsistency or to remove some apparent paradox thrown up by experiment. Newton's *Principia* has indeed more than a touch of Euclidian methodology. But of that, more anon.

There are at least two classic examples in which the axiomatic principle worked well in physics under the hand of a great master. The first was Archimedes, and the second Carathéodory, who in 1908–9 set up a system of thermodynamics on the basis of two axioms. Neither of these receives comment. The omission is by no means serious, since much of the work discussed is—very properly—modern. But it would have been interesting to have had the editors' reactions to the contributions made by such outstanding minds separated by such a long period of time.

Concerning the particular 'atmosphere' of the Newtonian period, and the seeming lack of postulates or any other kind of 'common notions', it looks very much as if the influence of Francis Bacon was too dominant, and that stress was laid very firmly on the power of science (in general) as an instrument of progress. If this should be a correct interpretation of events, it would follow that induction rather than deduction was *de rigueur* in the prevailing intellectual climate.

Turning to applications of the axiomatic method (Part 3), Prof. R. L. Wilder, of the University of Michigan, has a most intriguing paper on the innovations which he brought about in teaching his postgraduate students to use such a powerful tool for themselves in the establishment of proofs, sufficiently refined and complete to satisfy twentieth-century standards of consistency and adequacy. In this way (and occasionally even at undergraduate-level) he claims to have discovered creative talent otherwise dormant or perhaps frustrated.

The Foundations of Mathematics has a somewhat different aim. It is essentially a treatise, covering almost all aspects of foundation theory, but axiomatic in spirit, though this aspect is confined to Parts 2 and 3. Part 1 provides an exceptionally lucid study of the historical background of foundations, with particular reference to Aristotle's Theory of Science, and the rise of symbolic logic—which must, almost of necessity, include a brief outline of formalized axiomatics. Further parts deal with such topics as non-elementary mathematics, the existence of mathematical entities, paradoxes, set-theory and topology, recessive functions, sets and predicates. In conclusion come some comprehensive remarks on metamathematics and philosophy. Prof. E. W. Beth has some shrewd observations to make on the countless—often amateurish—efforts to resolve the more important paradoxes. Having expounded the orthodox views on such matters, he implies, very gently, that they have too often suffered from the 'fools and angels' type of attention. It is all too easy to under-estimate the methodological significance of the paradoxes, as well as to fall short of present-day standards of rigour in any approach to clarify their more intractable features.

The author's method is, in principle, analytic. He is thus enabled to deal with questions as they arise, rather than to ask his readers to accept assertions which may be far from obvious. Again, he recognizes that even logic may be a matter of degree: beginning at an "average level", he is able to scale the heights as the work proceeds. From the students' point of view, the realization of this dynamic process is important.

Reference to sections would be facilitated if the section numbers could be printed at the top of each page. As it is, much searching is involved to find such necessary information, especially when an equation or formula is first encountered, and then mentioned again many pages ahead.

F. I. G. Rawlins

GROUPS, PURE AND APPLIED

The Theory of Groups

By A. G. Kurosh. Translated from the Russian and edited by K. A. Hirsch. Second English edition. Vol. 1: Pp. 272. Vol. 2: Pp. 308. (New York: Chelsea Publishing Company, 1960.) 4.95 dollars each volume.

Abelian Groups

By L. Fuchs. Pp. 367. (London and New York: Pergamon Press, 1960.) 63*s*. net.

Seminar on Transformation Groups

By Armand Borel. With contributions by G. Bredon, E. E. Floyd, D. Montgomery and R. Palais. (Annals of Mathematics Studies, No. 46.) Pp. viii+245. (Princeton, N.J.: Princeton University Press; London: Oxford University Press 1960.) 36*s*. net.

THE two volumes by A. G. Kurosh, in their American translation by K. A. Hirsch, as well as the book by L. Fuchs, are photographic reprints of the first editions that appeared in 1955 1956 and 1958 respectively. A few misprints have been corrected in this second American edition of Kurosh's book; the Pergamon Press has appended a short list of errata to Fuchs's book which was first published by the Hungarian Academy of Sciences, but the present reprint is not even described as a second edition. The short space of time that has elapsed since their first appearance is a measure of the success and the importance of these books. But a glance at their history will explain why this time is too long for them to re-appear unchanged: it would have been more in keeping with the spirit of these books if some of the recent advances that they themselves have done so much to stimulate had been included.

The theory of groups, now one of the most important branches of abstract algebra, has seen a spectacular burst of activity during the past few decades. Kurosh's book is remarkable for the way in which its various editions are in turn result and cause of this development. Kurosh first wrote *The Theory of Groups* in 1940. It was then the first book on just groups, rather than finite groups, and the stress was on infinite groups. The War delayed publication until 1944, and the book became available to a wider public only in 1953 when the Akademie-Verlag, Berlin, published a German translation. By that time the stimulus provided by the Russian original had led to numerous new discoveries, some of which were added to the German translation in an appendix by B. H. Neumann. In the meanwhile, Kurosh himself had completely recast and revised his book in the light of the rapid advances, especially in the theory of infinite nilpotent groups, made by the powerful school of Russian group theorists. Thus, in the same year 1953, the second Russian edition appeared, virtually a new book compared with the first edition. It is from this second edition that Hirsch's translation was made. Once again Hirsch supplemented the translation by notes reporting on more recent developments. He also brought the bibliography up to date—that is, up to 1956—so that it now includes more than 800 titles.

The theory of abelian groups, though part of general group theory, has its own methods and its own history of growth. A first account of the theory of infinite abelian groups appeared in 1954, in a well-known monograph by I. Kaplansky. Here again further progress was rapid, and Fuchs's book in 1958 came none too early. Naturally, so far as the basic structure theory is concerned the books overlap. Beyond that much of the characteristic flavour of Fuchs's book is due to the fact that it, too, comes from the pen of one of a lively school of research workers in the subject, this time the Hungarian school of group theorists. Indeed, a glance at the extensive bibliography and at the numerous examples at the end of each chapter, many of them provided to give additional information on recent results in concise form, reveals the vigour of the (then) current research.

Both books start from the beginnings of the subject, assuming no more than a certain maturity of mathematical reasoning and familiarity with some basic mathematical tools. Both lead to the frontiers of knowledge in their time, giving not only the latest results but also pointing again and again to the unsolved problems and open questions. Thus both books are suitable for, in fact strongly recommended to, the bright undergraduate and the young research student; at the same time their depth and scope make them indispensable for the expert. Some of the most striking discoveries made since their appearance owe much to the influence of these books; many of the problems they state are, in fact, now solved. Let us hope that a further edition may soon be possible.

By contrast, the book by Armand Borel and his collaborators deals with a highly specialized application of group theory and homology theory. It embodies the results of a seminar held by Borel at the Institute of Advanced Studies at Princeton, N.J.; several chapters are written by members of the seminar presenting their own contribution to its work. The topic is the application of cohomological methods to the study of the action of compact transformation groups on topological spaces; the tools, ranging far and wide over recent work in algebraic topology, are mainly taken for granted, though a general reference to the sources is provided. At this different level of specialization the presentation is again clear and well organized, but naturally concise. Each chapter carries its own short bibliography, not aiming at completeness but containing only the most relevant papers. This book is clearly for the expert only.

HANNA NEUMANN

POWDER METALLURGY

Fundamental Principles of Powder Metallurgy

By Dr. W. D. Jones. Pp. xii+1032. (London: Edward Arnold (Publishers), Ltd., 1960.) 147*s*. net.

POWDER metallurgy is becoming an increasingly important metallurgical process, and, though a number of comprehensive books on the subject have been published in the United States, it is very useful to have this long and detailed book published in Great Britain and written by an author with such wide experience in powder metallurgy. Dr. Jones is the author of the well-known book *Principles of Powder Metallurgy* published in 1937.

The new book starts with a long chapter of 239 pages on the preparation of powders and then has three chapters, totalling 350 pages, on "Pressing", "Shaping without Pressing" and "Sintering". These are followed by another very long chapter on "Attainment of Specific Qualities", which deals mainly with the properties required in products made by powder metallurgy, but which also contains a section on factory management. The book ends with two short chapters on "Continuous Powder Metallurgy" and "Methods of Control"; the former covers the three main methods of continuous compacting of powders; the latter is very short and merely lists the various methods of testing powders and compacts and states where details of the actual tests can be found.

The author states in his introduction that he intends to cover principles rather than practice and so detailed descriptions of plant and processes are not included. Apart from this omission the book is very comprehensive; very little, if anything, appears to have been left out. The book is written in a clear and simple manner with good and relevant illustrations.

The book will be essential for all metallurgical libraries, since some at least of the matters described will be of importance or interest to all metallurgists. It will also be useful to others who are not metallurgists, so important are powders in modern technology.

It is possible that with the development of processes such as the Sherritt–Gordon process for the reduction of metals from their ores, the final products of which are powders, powder metallurgical products will no longer be used only for small or specialized components but will also be used for large-scale processes.

As has been said, the book is long, more than a thousand pages, and at seven guineas is expensive. The length and cost could have been reduced by omitting parts which do not fall strictly within the province of powder metallurgy or which can be found in standard text-books. Examples are the descriptions of the properties of metal whiskers and of general magnetic properties. The section on "Powder Metallurgy as a Competitive Process" does not, as might be expected from its title, show when it is economical to use powder metallurgy rather than other processes, but describes the organization of a factory producing powder metallurgy products. The section is interesting, but it is doubtful whether the organization of such a factory differs in its essentials from any other factory producing small parts.

J. H. RENDALL

The Brittle Fracture of Steel

By Dr. W. D. Biggs. Pp. xxvi+420. (London: Macdonald and Evans, Ltd., 1960.) 70*s.* net.

IN this book the author gives a very thorough account of all the factors related to the brittle fracture of structural steel. Thus he details a vast amount of accumulated knowledge on deformation and fracture, and discusses service and design aspects as well as methods of testing and the effect of metallurgical variables on behaviour. In addition, he deals with the relation between welding and brittle fracture, and the significance of residual stresses. Although cast irons are briefly mentioned, the coverage on steel does not extend to alloy steels as such.

The text is authoritative and comprehensive. However, as a result of the wide treatment the book does not readily yield a concise overall picture. The diffuseness in increased by the fact that in places the style is rather involved. This tendency is most noticeable when the author is generalizing rather than when he is giving specific information. There is indeed a useful summary chapter of fourteen pages, but it would have been better if this had been subtitled and set down in a crisper style.

In conclusion, it must be emphasized that this is a sound and informed book providing a thorough development of the subject-matter, and in this respect it deserves recognition as a valuable reference work. Regarding the style, the remarks here of course merely indicate my own reaction. Nevertheless, it does seem that the book would have been much easier to assimilate had the author cut through the subject in a more incisive manner.

A. R. BAILEY

Proceedings of the Fourth International Conference on Ionization Phenomena in Gases

Uppsala, 17–21 August, 1959. Edited by N. Robert Nilsson. (Series in Physics.) Vol. 1: Pp. xii+552. Vol. 2: Pp. viii+553–1210. (Amsterdam: North-Holland Publishing Company, 1960.) £12 6*s.* 0*d.* the two volumes.

THESE two volumes contain the majority of the 250 papers which were read at the conference by scientists actively engaged in the field of ionization physics in twenty-one countries throughout the world. The papers, by far the greater number of which are in English, cover a very wide range of topics, but they have been conveniently grouped so that Volume 1 contains those concerned with fundamental ionization processes and various types of gas discharges, and Volume 2 those dealing with various aspects of plasma physics. (More detailed information about the topics discussed under these general headings was given in a recent report on the conference[1].) It would have been helpful to the reader if the review papers, given to the plenary sessions of the conference, could have been distinguished in some way from the more detailed papers read to the four parallel sessions into which the conference was divided for much of the time. It is also a pity that the often lively and informative discussion of the papers had to be omitted for reasons of space. These, however, are minor criticisms, and both the editor and publishers are to be congratulated on providing such a very well-produced record of this important and extremely well-organized conference. All those with an interest in this field of work will wish to have ready access to these volumes in their departmental library, even if the inevitably high price precludes them from owning their own personal copies.

J. DUTTON

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Determination of the Mechanical and Technological Properties of Metals

By B. M. Gliner. Translated from the Russian. 2nd edition. Translation Editor: E. Bishop. Pp. ix+160. (London and New York: Pergamon Press, 1960.) 50*s.* net.

THIS book gives a useful résumé of Russian mechanical testing methods. On the assumption that it contains a reasonably modern exposition (no date is given in the translated preface) of the varied techniques, it reveals no startling divergences from Western practice, although there are two unusual features that are mentioned.

One of these features is the use of ring and splitring test pieces, for testing under short-time transverse tension, at room temperature, and transverse creep tension, at elevated temperature, respectively. It is not made clear why, or on what scale, such test pieces are used, or what information, other than an empirical guide to design, is gained from the tests.

The other unusual feature is the use of a triangular pyramid indentor for micro-hardness testing. It is difficult to see what particular advantage is gained by this form of indentor, other than to average out directionality effects in the material.

A statement is made (on page 14) that load measurement is most accurately achieved by means of a pendulum lever mechanism. This will certainly be questioned by Western manufacturers unless it is taken as a reflexion on Russian machine construction methods.

There are one or two minor errors to be found; for example, Fig. 61(*b*) is incorrect.

If the translation were less literal then the book would be easier to read. For example, on p. 16, "jockey" would read better than "trolley", on p. 17, "drum" would be better than "box", and "I-section" would be better than "Type 1", and on p. 93, "disk" is preferable to "shaft".

JAMES G. TWEEDDALE

Practical Heredity with Drosophila

By Dr. Gordon Haskell. Pp. xii+124. (Edinburgh and London: Oliver and Boyd, Ltd., 1961.) 10*s.* 6*d.* net.

IT is surprising that this useful little book has not been written before. Elementary practical genetics is studied in many schools and colleges, and, because of inexperience of the manipulators, many breeding errors occur which could have been avoided. Haskell's book provides valuable hints on the means of obtaining and rearing pure stocks of *Drosophila*, the apparatus required to do this and the right ways to use it, the selection of suitable mutants to demonstrate genetic principles, statistical tests for evaluation, the cytology of *Drosophila* and an introduction to biometrical investigation. Many workers using *Drosophila* who have tried in vain to mate already pregnant females will be glad of all the practical help which Haskell has compressed into quite a short space.

T. H. HAWKINS

SEASONAL VARIATION AND AGE OF RADIOACTIVE FALL-OUT

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A NUMBER of observers in the temperate zones of the northern hemisphere have reported a seasonal variation in the concentration of long-range fall-out material[1-3]. Thus both dust samples collected from surface air and rain-water samples have shown higher activities in the late spring than in the late autumn. It has been suggested by Martell[4] that the spring maxima are due to the descent of fall-out material injected into the atmosphere by tests in the previous autumn, while Stewart *et al.*[1] have suggested an explanation on meteorological grounds using the Brewer–Dobson model of atmospheric circulation[5,6]. On the latter thesis the cold air which forms above the Pole during the late winter sinks in the spring, thus bringing down stratospheric air and associated fission debris. More recently Feely and Spar[7] have postulated that the movement of fission debris from the stratosphere into the troposphere primarily occurs by a process of turbulent mixing in the region of the troposphere gap.

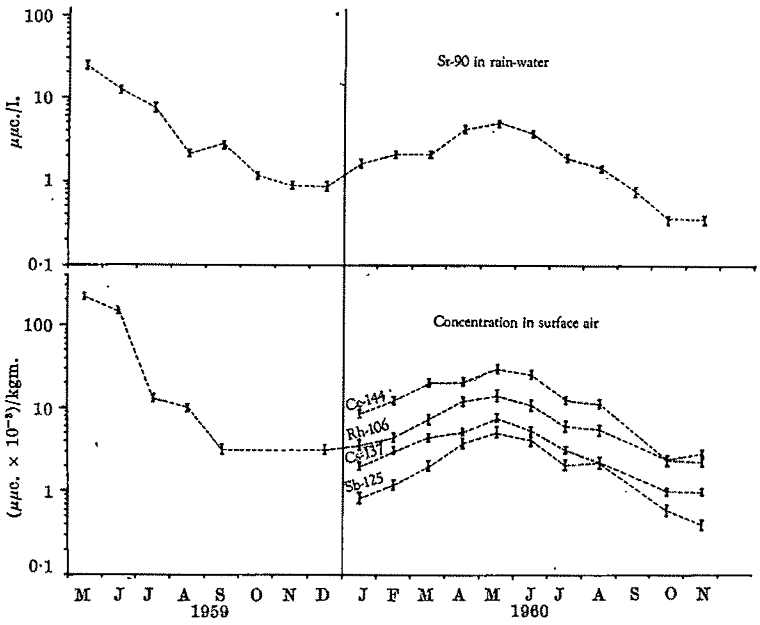

Fig. 1

In the past it has been difficult to assess the relative importance of these views owing to the periodic injection of fresh nuclear debris into the atmosphere. For example, Anderson *et al.*[2] have shown that while the fall-out pattern for 1959 was consistent with Martell's suggestion, it could not be taken to exclude a seasonal variation of the type suggested by Stewart. The spring of 1960 presented the first opportunity of studying this variation virtually free from the effect of recent tests. (The tropospheric fall-out from the 1960 French tests had only a transitory effect in Britain[8], and the yields were too small to produce a significant contribution to the total activity in the stratospheric reservoir.) We have already reported measurements for the early part of 1960[2] which indicate the beginning of a rise in the concentration of fall-out material, and more recently Peirson *et al.* have published measurements of the cæsium-137 content of surface air up to the middle of 1960, which show a maximum in the spring[3]. We present here measurements of the principal γ-emitting fission products in surface air in 1960, together with values for the strontium-90 content of rain-water during this period. As before[2,9], samples of dust collected from surface air by means of an electrostatic precipitator at our laboratory at Sutton, Surrey, have been analysed by means of γ-spectrometry.

The principal γ-emitting fission products present in our 1960 samples are cerium-144, cæsium-137, rhodium-106 and antimony-125, as expected for a period 12–24 months after fission[10]. In addition to the photo-peaks in the γ-spectrum which may be ascribed to the above nuclides, the identity of which has been corroborated by decay measurements on a number of samples, a peak occurs at an energy of 0·475 MeV. We have previously shown[8], on the basis of chemical and physical evidence, that this is in part due to 53-day beryllium-7, a product of the interaction of cosmic rays with atmospheric nitrogen and oxygen nuclei. The situation is now further com-

Table 1. CONCENTRATIONS (CURIE × 10^{-15} PER KGM. AIR) OF γ-EMITTING FISSION PRODUCTS IN SURFACE AIR

Mean date of collection	Period of collection (days)	Rh-106	Sb-125	Cs-137	Ce-144	$\frac{\text{Ce-144}}{\text{Cs-137}}$
2.1.60	22	3·2	0·8	1·7	7·5	4·4
30.1.60	8	4·6	0·9	2·7	11	4·1
17.2.60	10	5·0	1·3	3·2	13	4·1
4.3.60	6	6·2	1·9	3·9	16	4·1
18.3.60	22	7·4	2·1	4·8	21	4·4
3.4.60	10	11	3·2	5·4	25	4·6
14.4.60	13	12	3·4	5·7	22	3·9
23.4.60	5	13	4·4	7·2	27	3·8
29.4.60	6	14	6·2	8·1	34	4·2
9.5.60	8	17	6·1	8·1	35	4·3
21.5.60	7	15	4·2	7·1	29	4·1
4.6.60	5	20	6·4	12	48	4·0
14.6.60	14	8·9	4·4	5·2	22	4·2
29.6.60	15	8·9	2·6	5·4	18	3·3
14.7.60	15	5·2	1·9	3·9	11	2·8
1.8.60	8	5·5	2·0	3·7	12	3·2
15.8.60	7	5·6	2·4	3·1	11	3·5
11.10.60	13	2·4	0·6	1·0	2·4	2·4
9.11.60	14	2·3	0·4	1·0	2·8	2·8

Table 2. Concentration ($\mu\mu$c./l.) of Strontium-90 in Rain-water Samples, 1960

Period of collection	Sr-90
January	1·7
February	2·2
March	2·2
April	4·4
May	5·3
June	3·9
July	2·0
August	1·5
September	0·79
October	0·42
November	0·46

plicated by the presence of 210-day rhodium-102, recently reported to have been produced as a tracer in certain of the American 'Hardtack' explosions of April-August 1958[11,12]. Recently this nuclide has been stated to emit principal γ-rays of energy 0·475 MeV. (63 per cent) and 1·09 MeV. (6 per cent)[13]; but earlier decay schemes suggested a higher intensity for the 1·09-MeV. line. The rhodium-102 photo-peak at 0·475 MeV. cannot be resolved in our apparatus from the 0·478-MeV. line of beryllium-7, and, if we accept the new data, the intensity of the 1·09-MeV. line is too weak for it to be used in the identification of the small amounts of rhodium-102 which may be present in our samples. We are therefore carrying out further work to determine the relative amounts of beryllium-7 and rhodium-102.

Our measurements on the specific concentrations of rhodium-106, antimony-125, cæsium-137 and cerium-144, corrected for decay to the time of the collection, are given in Table 1. After averaging over calendar months, the results are plotted in Fig. 1, together with the strontium-90 concentrations in rain-water samples (Table 2). These measurements form a continuation of work previously reported[2].

The common feature of all these results is the pronounced rise to a maximum in May, with a fall during the summer months to low autumn and winter values. From June until October the values for the strontium-90 concentrations in rain are a close fit to an exponential curve of half-period 40 days, which is remarkably similar to our results for 1959[2]. The ratio between maximum and minimum in 1960 is approximately 5 : 1. This is in agreement with results for strontium-90 in rain during 1955–58[14], and with the finding that the poleward transfer of tungsten-185, liberated as a tracer in certain of the American tests near the equator, is seasonal[7,15]. It would therefore appear that our results may be explained in meteorological terms by the Brewer-Dobson or Feely-Spar models, since the Martell thesis is not applicable in 1960.

Information on the age of the debris collected may be obtained from the relative proportions of the various nuclide activities present in our samples. The yield of both cerium-144 and cæsium-137 is relatively independent of the type of fission process (Table 3)[10], and therefore the ratios of the specific activities of these nuclides provide a suitable method of determining age at the present time. The concentrations of nuclides of shorter half-life, such as strontium-89 and zirconium-95, are now too low for accurate measurement[8].

Table 3. Fission Yield (per cent) for Different Types of Fission (ref. 10)

Mass-chain	Half-life	U-235, n_f	Pu-239, n_f	U-238, n_f	U-238, n_{14}	U-235, n_{14}
106	370 days	0·47	6·17	2·61	3·11	1·40
125	2·4 years	0·12	0·26	0·05	1·00	1·36
137	30 years	6·18	6·14	6·45	5·71	5·10
144	285 days	4·76	3·66	4·42	3·32	3·30

n_f, fission spectrum neutrons; n_{14}, 14-MeV. neutrons.

Our experimental cerium-144/cæsium-137 ratios are listed in Table 1, and it is interesting to note that during the early part of 1960 they are in good agreement with values quoted for rain-water at Milford Haven[16]. Using the theoretical yield values (Table 3) the average age for each sample has been calculated, and the results are presented in Fig. 2. The error in any one value is estimated to be ± 20 per cent. If we take the mean of these estimated dates of origin, a time April–May 1958 is obtained.

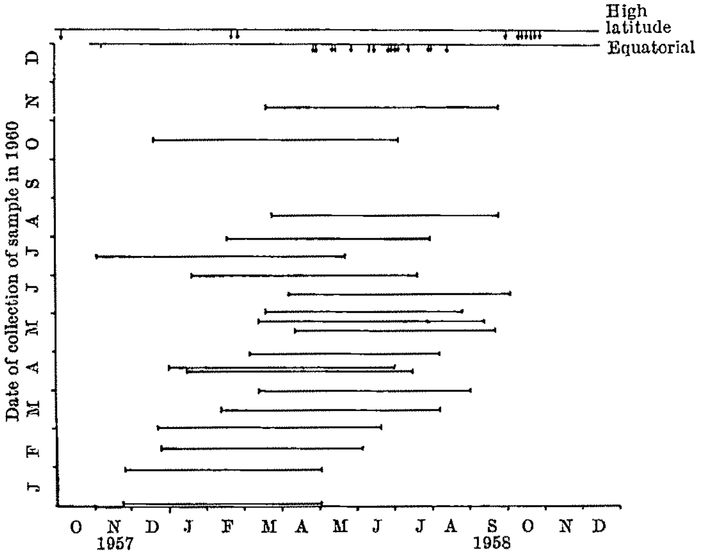

Fig. 2

This is compatible with available information on the dates of megaton explosions[17]; these are indicated by arrows at the top of Fig. 2. The above calculation is based on the fission yield for 14-MeV. neutrons, and in the unlikely event of all the yield being due to the fission of uranium-235 by fission neutrons the mean date of origin would be approximately four months earlier. Thus the majority of fall-out material collected at our site during 1960 probably originates from the American and British equatorial tests of 1958.

The antimony-125/cæsium-137 and rhodium-106/cæsium-137 ratios had experimental values in mid-1960 of approximately 0·6 and 1·9 respectively. These are commensurate with an age of 3–4 years if 14-MeV. fission yields are used. However, the yield of both antimony-125 and rhodium-106 is very sensitive to the type of fission process, as shown by Table 3. Thus the discrepancy may be explained if, in addition to the 14-MeV. fission of uranium-238, other fission processes are involved.

We wish to acknowledge the guidance and encouragement of Prof. W. V. Mayneord and Dr. W.

Anderson during this work. We also wish to thank Mr. R. W. Kent and Mr. D. Smith for their help in connexion with the electrostatic precipitator, and Mrs. A. Perry for assistance in the chemical analyses.

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AN UNSTABLE INTERMEDIATE CARRYING INFORMATION FROM GENES TO RIBOSOMES FOR PROTEIN SYNTHESIS

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A LARGE amount of evidence suggests that genetic information for protein structure is encoded in deoxyribonucleic acid (DNA) while the actual assembling of amino-acids into proteins occurs in cytoplasmic ribonucleoprotein particles called ribosomes. The fact that proteins are not synthesized directly on genes demands the existence of an intermediate information carrier. This intermediate template is generally assumed to be a stable ribonucleic acid (RNA) and more specifically the RNA of the ribosomes. According to the present view, each gene controls the synthesis of one kind of specialized ribosome, which in turn directs the synthesis of the corresponding protein—a scheme which could be epitomized as the one gene–one ribosome–one protein hypothesis. In the past few years, however, this model has encountered some difficulties: (1) The remarkable homogeneity in size[1] and nucleotide composition[2] of the ribosomal RNA reflects neither the range of size of polypeptide chains nor the variation in the nucleotide composition observed in the DNA of different bacterial species[2,3]. (2) The capacity of bacteria to synthesize a given protein does not seem to survive beyond the integrity of the corresponding gene[4]. (3) Regulation of protein synthesis in bacteria seems to operate at the level of the synthesis of the information intermediate by the gene rather than at the level of the synthesis of the protein[5].

These results are scarcely compatible with the existence of stable RNA intermediates acting as templates for protein synthesis. The paradox, however, can be resolved by the hypothesis, put forward by Jacob and Monod[5], that the ribosomal RNA is not the intermediate carrier of information from gene to protein, but rather that ribosomes are non-specialized structures which receive genetic information from the gene in the form of an unstable intermediate or 'messenger'. We present here the results of experiments on phage-infected bacteria which give direct support to this hypothesis.

When growing bacteria are infected with a virulent bacteriophage such as *T*2, synthesis of DNA stops immediately, to resume 7 min. later[6], while protein synthesis continues at a constant rate[7]. After infection many bacterial enzymes are no longer produced[8]; in all likelihood, the new protein is genetically determined by the phage. A large number of new enzymatic activities appears in the infected cell during the first few minutes following infection[9], and from the tenth minute onwards some 60 per cent of the protein synthesized can be accounted for by the proteins of the phage coat[7]. Surprisingly enough, protein synthesis after infection is not accompanied, as in growing cells, by a net synthesis of RNA[10]. Using isotopic labelling, however, Volkin and Astrachan[11] were able to demonstrate high turnover in a minor RNA fraction after phage infection. Most remarkable is the fact that this RNA fraction has an apparent nucleotide composition which corresponds to that of the DNA of the phage and is markedly different from that of the host RNA[11]. Recently, it has been shown that the bulk of this RNA is associated with the ribosomes of the infected cell[12].

Phage-infected bacteria therefore provide a situation in which the synthesis of protein is suddenly

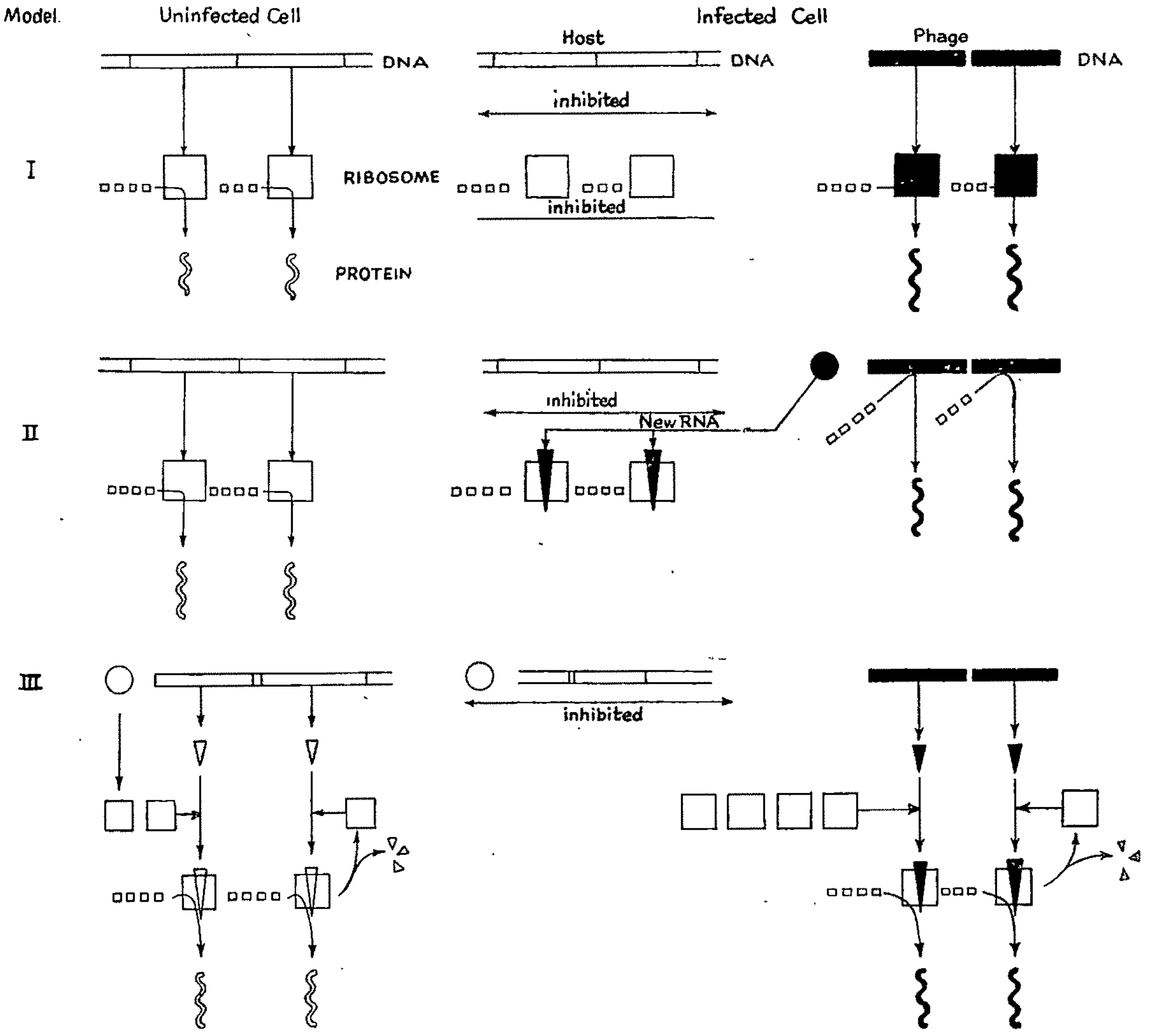

Fig. 1. Three models of information transfer in phage-infected cells

switched from bacterial to phage control and proceeds without the concomitant synthesis of stable RNA. *A priori*, three types of hypothesis may be considered to account for the known facts of phage protein synthesis (Fig. 1). Model I is the classical model. After infection the bacterial machinery is switched off, and new ribosomes are then synthesized by the phage genes. The *ad hoc* hypothesis has to be added that these ribosomes are unstable, to account for the turnover of RNA after phage infection. This is, in fact, the model favoured by Nomura *et al.*[12]. Model II assumes that in the particular case of phage the proteins are assembled directly on the DNA; the new RNA is a special molecule which enters old ribosomes and destroys their capacity for protein synthesis. At the same time, synthesis of ribosomes is switched off. Model III implies that a special type of RNA molecule, or 'messenger RNA', exists which brings genetic information from genes to non-specialized ribosomes and that the consequences of phage infection are two-fold: (*a*) to switch off the synthesis of new ribosomes; (*b*) to substitute phage messenger RNA for bacterial messenger RNA. This substitution can occur quickly only if messenger RNA is unstable; the RNA made after phage infection does turn over and appears, therefore, as a good candidate for the messenger.

It is possible to distinguish experimentally between these three models in the following way: Bacteria are grown in heavy isotopes so that all cell constituents are uniformly labelled 'heavy'. They are infected with phage and transferred immediately to a medium containing light isotopes so that all constituents synthesized after infection are 'light'. The distribution of new RNA and new protein, labelled with radioactive isotopes, is then followed by density gradient centrifugation[13] of purified ribosomes.

Density gradient centrifugation was carried out in a preparative centrifuge, and the ribosomes were stabilized by including magnesium acetate (0·01–0·06 *M*) in the cæsium chloride solution. Ribosomes show two bands, a heavier *A* band and a lighter *B* band, the relative proportions of which, for a given preparation, depend on the magnesium concentration used. The lower the magnesium concentration, the smaller the proportion of *B* band ribosomes and the larger the proportion of *A* band ribosomes.

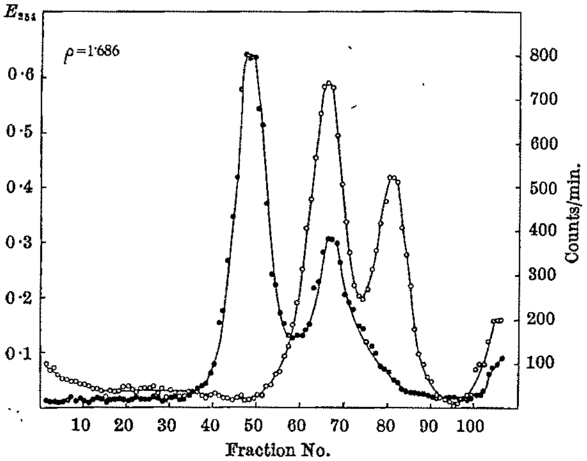

Fig. 2. Distribution of heavy and light ribosomes in a density gradient. *E. coli* B, grown in 5 ml. of a medium containing ^{15}N (99 per cent) and ^{13}C (60 per cent) algal hydrolysate and $^{32}PO_4$, were mixed with a fifty-fold excess of cells grown in nutrient broth, the ribosomes extracted by alumina grinding in the presence of 0·01 *M* Mg^{++} and purified by centrifugation. 1 mgm. of ribosomes was centrifuged in 3 ml. of cæsium chloride buffered to *p*H 7·2 with 0·1 *M tris* and containing 0·03 *M* magnesium acetate for 35 hr. at 37,000 r.p.m. in the *SW*39 rotor of the Spinco model *L* ultracentrifuge. After the run, a hole was pierced in the bottom of the tube and drops sequentially collected. Ultra-violet absorption at 254 mμ detects the excess of light ribosomes (○), ^{32}P counts detect the heavy ribosomes (●)

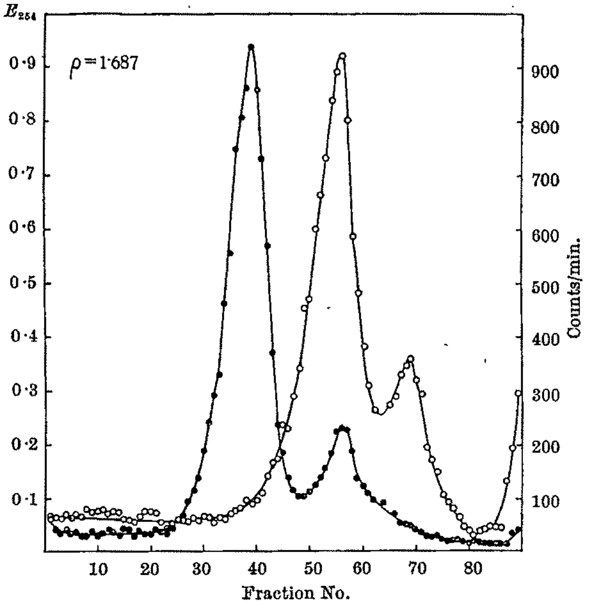

Fig. 3. Distribution of randomized heavy and light ribosomes in a density gradient. The mixture of $^{15}N^{13}C^{32}P$ and $^{14}N^{12}C^{31}P$ ribosomes was dialysed first for 18 hr. against 0·0005 *M* magnesium acetate in 0·01 *M* phosphate buffer *p*H 7·0, and then for 24 hr. against two changes of 0·01 *M* magnesium acetate in 0·001 *M tris* buffer *p*H 7·4. 1 mgm. of ribosomes was centrifuged for 38 hr. at 37,000 r.p.m. in cæsium chloride containing 0·03 *M* magnesium acetate. The drops were assayed for ultra-violet absorption (○) and ^{32}P content (●)

In order to show that there is no aggregation of ribosomes during preparation and density gradient centrifugation an experiment was carried out on ribosomes extracted from a mixture of $^{15}N^{13}C$ and $^{14}N^{12}C$ bacteria. The results are shown in Fig. 2, from which it can be seen that ribosomes of different isotopic compositions band independently and that there are no intermediate classes. The same preparation was then dialysed against low magnesium to dissociate the ribosomes into their 50 *S* and 30 *S* components and then against high magnesium to re-associate the sub-units[14]. This should have resulted in distributing heavy 30 *S* and 50 *S* subunits into mixed 70 *S* and 100 *S* ribosomes. Surprisingly enough, density gradient centrifugation of this preparation (Fig. 3) yields the same bands as found in the original ribosomes except for a decrease in the proportion of the *B* bands. This means that both bands contain units which do not undergo reversible association and dissociation and that the mixed 70 *S* ribosomes prepared by dialysis separate

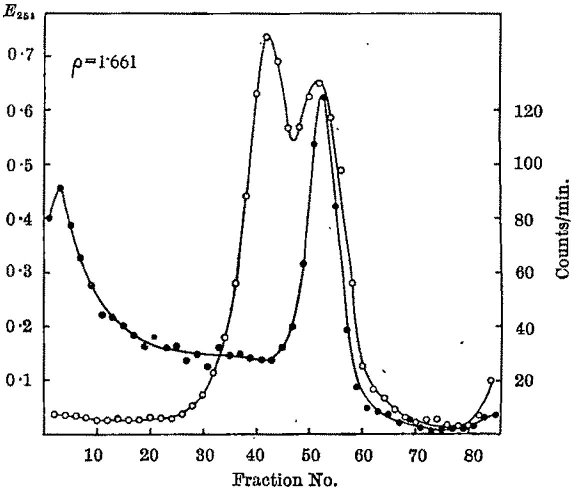

Fig. 4

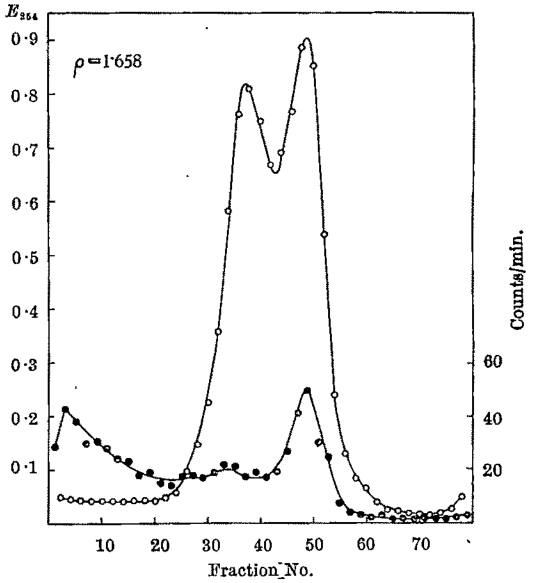

Fig. 5

Figs. 4 and 5. Distribution and turnover of RNA formed after phage infection. A 600 ml. culture of *E. coli B*6 (mutant requiring arginine and uracil) was infected with *T4D* (multiplicity 30) and fed ^{14}C-uracil (10 mc./m*M*) from third to fifth min. after infection. One half of the culture was removed and ribosomes prepared (Fig. 4). The other half received a two hundred-fold excess of ^{12}C-uridine for a further 16 min. and ribosomes prepared (Fig. 5). In both experiments approximately 3 mgm. of purified ribosomes were centrifuged for 42 hr. at 37,000 r.p.m. in cæsium chloride containing 0·05 *M* magnesium acetate. Alternate drops were collected in *tris*-magnesium buffer for ultra-violet absorption (○) and on to 0·5 ml. of frozen 5 per cent trichloroacetic acid. These tubes were thawed, 1 mgm. of serum albumin added, and the precipitates separated and washed by filtration on membrane filters for assay of radioactivity (●)

into their components in the density gradient. Other experiments to be reported elsewhere suggest that the *A* band is composed of free 50 *S* and 30 *S* ribosomes and that the *B* band contains undissociated 70 *S* particles.

The bulk of the RNA synthesized after infection is found in the ribosome fraction, provided that the extraction is carried out in 0·01 *M* magnesium ions[12]. We have confirmed this finding and have studied the distribution of the new RNA among the ribosomal units found in the density gradient. Fig. 4 shows that this RNA, labelled with ^{14}C-uracil, bands in the same position as *B* band ribosomes. There is no peak corresponding to the *A* band. In addition, there is radioactivity at the bottom of the cell. This is free RNA as its density is greater than 1·8, and, moreover, it must have a reasonably high molecular weight to have sedimented in the gradient. Lowering of the magnesium concentration in the gradient, or dialysing the particles against low magnesium, produces a decrease of the *B* band and an increase of the *A* band. At the same time, the radioactive RNA leaves the *B* band to appear at the bottom of the gradient. This shows that the uracil has labelled a species of RNA distinct from that of the bulk of *B* band ribosomes, since the specific activity of the RNA at the bottom of the cell is much higher than that of the *B* band. Fig. 5 shows that this RNA turns over during phage growth. There is a decrease by a factor of four in the specific activity of the *B* band after 16 min. of growth in ^{12}C-uridine. Similar results have been obtained using $^{32}PO_4$ as a label.

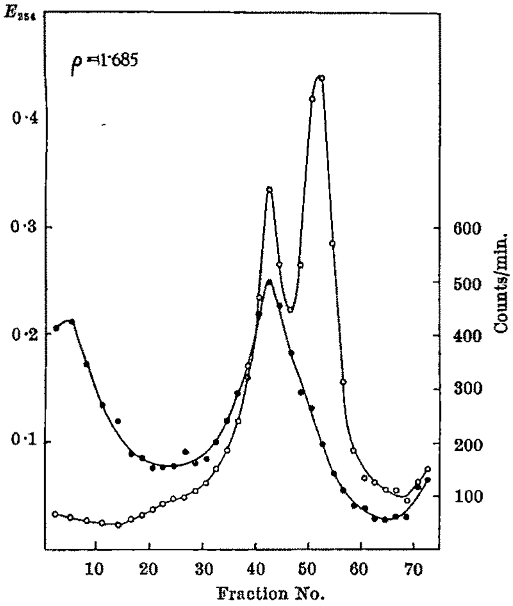

Fig. 6. RNA after isotope transfer in phage-infected cells. *E. coli* B grown in 10 ml. of ^{15}N (99 per cent) and ^{13}C (60 per cent) algal hydrolysate medium were starved in buffer, infected with *T*4 and growth initiated by addition of glucose and dephosphorylated broth ($^{14}N^{12}C$). $^{32}PO_4$ was fed from the second to seventh min. after infection. The culture was mixed with a fifty-fold excess of *E. coli* B grown in nutrient broth, infected in buffer and then grown for 7 min. in dephosphorylated broth medium. 1 mgm. of purified ribosomes was centrifuged for 36 hr. at 37,000 r.p.m. in cæsium chloride containing 0·03 *M* magnesium acetate. Ultra-violet absorption (○) detects the $^{14}N^{12}C^{31}P$ carrier, radioactivity (●) detects the new RNA in the heavy cells transferred to light medium

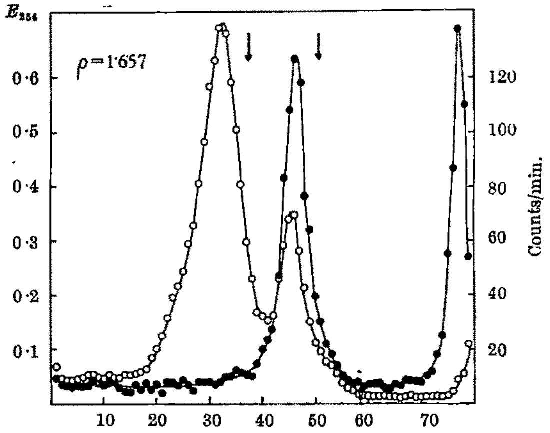

Fig. 7

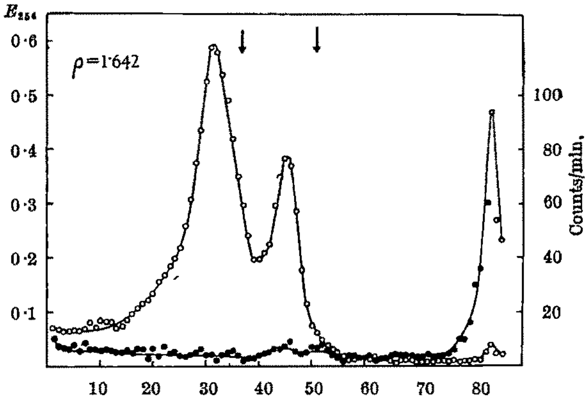

Fig. 8

Figs. 7 and 8. Distribution and turnover of newly synthesized protein in ribosomes of phage-infected cells after transfer. *E. coli* B grown in 600 ml. of a salt glucose medium containing $^{15}NH_4Cl$ (99 per cent) were starved in buffer, infected with *T*4 and transferred to $^{14}NH_4Cl$ medium. $^{35}SO_4$ was fed for the first 2 min. of infection and one half of the culture removed (Fig. 7). The other half received an excess of $^{32}SO_4$ and ^{32}S-methionine and growth was continued for a further 8 min. (Fig. 8). 1 mgm. of purified ribosomes was centrifuged for 39 hr. at 37,000 r.p.m. in cæsium chloride containing 0·05 *M* magnesium acetate. Drops were assayed for ultra-violet absorption (○) and for radioactivity (●). The arrows mark the expected positions for the peaks of ^{14}N *A* and *B* bands. The radioactivity at the top of the gradient is contaminating protein

These results do not distinguish between a messenger fraction and a small proportion of new ribosomes which are fragile in cæsium chloride and which are also metabolically unstable. In order to make the distinction, the experiment was carried out with an isotope transfer, in the following manner: Cells grown in a small volume of $^{15}N^{13}C$ medium were infected with *T* 4, transferred to $^{14}N^{12}C$ medium and fed $^{32}PO_4$ from the second to the seventh minute. They were mixed with a fifty-fold excess of cells grown and infected in $^{14}N^{12}C^{31}P$ medium. Fig. 6 shows that the RNA formed after infection in the heavy cells has a density greater than that of the *B* band of the carrier. Its peak corresponds exactly with the density of the *B* band of $^{15}N^{13}C$ ribosomes (Fig. 2) although it is skewed to lighter density, and its response to changing the magnesium concentration was that of a *B* band. There is no radioactive peak corresponding to the *B* band of the carrier: this means that no wholly new ribosomes are synthesized after phage infection. As already shown, the new RNA does not represent random labelling of *B* band ribosomes; therefore it con-

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stitutes a fraction which is added to pre-existing ribosomes the bulk of the material of which has been assimilated before infection. This result conclusively eliminates model I.

To distinguish between models II and III an experiment was carried out to see whether pre-existing ribosomes participate in protein synthesis after phage infection. Cells were grown in ^{15}N medium, infected with phage, transferred to ^{14}N medium and fed $^{35}SO_4$ for the first 2 min. of phage growth. Fig. 7 shows that only the *B* band of pre-existing ribosomes becomes labelled with ^{35}S and there is no peak corresponding to a ^{14}N *B* band. All this label can be removed by growth in non-radioactive sulphate and methionine (Fig. 8). In this experiment, the incorporation of ^{35}S into the total extract was measured and the amount in the *B* band found to correspond to 10 sec. of protein synthesis. This is probably an over-estimate since it is unlikely that pool equilibration was attained instantaneously. This value corresponds quite closely with the ribosome passage time of 5–7 sec. for the nascent protein in uninfected cells[15]. In addition, electrophoresis of chymotrypsin digests of *B* band ribosomes shows that the radioactivity is already contained in a variety of peptides. It would therefore appear that most, if not all, protein synthesis in the infected cell occurs in ribosomes. The experiment also shows that pre-existing ribosomes are used for synthesis and that no new ribosomes containing stable sulphur-35 are synthesized. This result effectively eliminates model II.

We may summarize our findings as follows: (1) After phage infection no new ribosomes can be detected. (2) A new RNA with a relatively rapid turnover is synthesized after phage infection. This RNA, which has a base composition corresponding to that of the phage DNA, is added to pre-existing ribosomes, from which it can be detached in a cæsium chloride gradient by lowering the magnesium concentration. (3) Most, and perhaps all, protein synthesis in the infected cell occurs in pre-existing ribosomes.

These conclusions are compatible only with model III (Fig. 1), which implies that protein synthesis occurs by a similar mechanism in uninfected cells. This, indeed, appears to be the case: exposure of uninfected cells to a 10-sec. pulse of $^{32}PO_4$ results in labelling of the RNA in the *B* band and not in the *A* band of ribosomes, and this RNA can be detached from the ribosomes by lowering the concentration of magnesium ions. Similarly the nascent protein can be labelled by a short pulse of $^{35}SO_4$; it is located in the *B* band and most of the label is removed by growth in non-radioactive sulphate. In contrast to what was observed in infected cells, residual stable radioactivity is found in both bands, reflecting the synthesis of new ribosomes.

In order to act as an intermediate carrier of information from genes to ribosomes, the messenger has to fulfil certain prerequisites of size, turnover and nucleotide composition. In the accompanying article, Gros *et al.*[16] have analysed the distribution of pulse-labelled RNA in sucrose gradients. They have shown that in uninfected cells there is an RNA fraction which has a rapid turnover and which can become attached reversibly to ribosomes depending on the magnesium concentration. The *T*2 phage-specific RNA shows the same behaviour and both are physically similar, with sedimentation constants of 14–16 *S*. We have carried out similar experiments[17] independently and our results confirm their findings. These suggest that, although the messenger RNA is a minor fraction of the total RNA (not more than 4 per cent), it is not uniformly distributed over all ribosomes, and may be large enough to code for long polypeptide chains. When ribosomes, from phage-infected cells labelled with $^{32}PO_4$ for five min., are separated by centrifugation in a sucrose density gradient[18] containing 0·01 *M* Mg^{++}, most of the messenger is found in 70 and 100 *S* ribosomes, contrary to previous reports[12]. When the magnesium concentration is lowered, the radioactivity is found in three peaks of roughly equal amount: (1) corresponding to a small residual number of 70 *S* ribosomes; (2) corresponding to 30 *S* ribosomes; (3) a peak of very high specific activity at 12 *S*. Separation of the RNA extracted from such ribosomes with detergent shows all the counts to be located in a peak at 12 *S*, skewed towards the heavier side. These results suggest that the messenger is heterogeneous in size and may have a minimum molecular weight of about ¼ to ½ million. Similar results have been obtained in uninfected cells[17].

The undissociable 70 *S* ribosomes are enriched for messenger RNA over the total ribosomes of this type and it has been shown that they are also enriched for the nascent protein[17]. These ribosomes have been called "active 70 *S*" ribosomes, by Tissières *et al.*[19], and they appear to be the only ribosomes which preserve the ability to synthesize protein *in vitro*. This leads one to suspect that there is a series of successive events involved in protein synthesis, and that at any time we investigate a temporal cross-section of the process.

The exact determination of the rate of turnover of the messenger RNA should give information about the process of protein synthesis. This might be stoichiometric, in the sense that each messenger molecule functions only once in information transfer before it is destroyed. Its rate of turnover should then be the same as that of the nascent protein; but experiments to test this idea have been limited by difficulties in pool equilibration with nucleotide precursors.

It is a prediction of the hypothesis that the messenger RNA should be a simple copy of the gene, and its nucleotide composition should therefore correspond to that of the DNA. This appears to be the case in phage-infected cells[11,20], and recently Ycas and Vincent[21] have found a rapidly labelled RNA fraction with this property in yeast cells. If this turns out to be universally true, interesting implications for coding mechanisms will be raised.

One last point deserves emphasis. Although the details of the mechanism of information transfer by messenger are not clear, the experiments with phage-infected cells show unequivocally that information for protein synthesis cannot be encoded in the chemical sequence of the ribosomal RNA. Ribosomes are non-specialized structures which synthesize, at a given time, the protein dictated by the messenger they happen to contain. The function of the ribosomal RNA in this process is unknown and there are also no restrictions on its origin in the cell: it may be synthesized by nuclear genes or by enzymes or it may be endowed with self-replicating ability.

This work was initiated while two of us (S. B. and F. J.) were guest investigators in the Division of

Biology, California Institute of Technology, Pasadena, during June 1960. We would like to thank Profs. G. W Beadle and M. Delbrück for their kind hospitality and financial support.

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UNSTABLE RIBONUCLEIC ACID REVEALED BY PULSE LABELLING OF *ESCHERICHIA COLI*

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WHEN *Escherichia coli* cells are infected with *T* even bacteriophage particles, synthesis of host proteins stops[1], and much if not all new protein synthesis is phage specific[2]. This system thus provides an ideal model for observing the synthesis of new proteins following the introduction of specific DNA. In particular, we should expect the appearance of phage-specific RNA, since it is generally assumed that DNA is not a direct template for protein synthesis but that its genetic information is transmitted to a specific sequence of bases in RNA. It was thus considered paradoxical when it was first noticed[3] that, following infection by the *T* even phages, net RNA synthesis stops even though protein synthesis continues at the rate of the uninfected bacterium. This could mean that DNA sometimes serves as a direct template for protein synthesis. Alternatively, net RNA synthesis may not be necessary so long as there exists the synthesis of a genetically specific RNA that turns over rapidly. This possibility was first suggested by experiments of Hershey[4], who, in 1953, reported that *T*2 infected cells contain a metabolically active RNA fraction comprising about 1 per cent of the total RNA. Several years later, Volkin and Astrachan[5] reported that this metabolic RNA possessed base ratios similar, if not identical (considering uracil formally equivalent to thymine), to those of the infecting *T*2 DNA. By 1958 they[6] extended their observation to *T*7 infected cells, where again the RNA synthesized after phage infection had base ratios similar to those of the phage DNA.

During these years, evidence[7] accumulated that the sites of much, if not all, protein synthesis are the ribosomal particles, and it was thought most likely that ribosomal RNA was genetically specific, with each ribosome possessing a base sequence which coded for a specific amino-acid sequence (one ribosome–one protein hypothesis). Direct verification of this hypothesis was lacking, and its proponents[8] were troubled by the fact that, except for phage-specific RNA, it was impossible to find any correlation within a given organism between the base ratios of DNA and RNA. Moreover, there was no evidence that phage-specific RNA was ribosomal RNA.

Nomura, Hall and Spiegelman[9] have recently discovered that following *T*2 infection there is no synthesis of typical (see below) ribosomal RNA and that the phage-specific RNA sediments at a slower rate (8*s*) than ribosomal RNA (16*s* and 23*s*). The genetic information for the synthesis of phage-specific proteins does not reside in the usual ribosomal RNA. Instead, if we assume that the synthesis of phage-specific proteins also occurs on ribosomes, then the phage-specific RNA might be viewed as a 'messenger' (to use the terminology of Monod and Jacob[10]) which carries the genetic information to the ribosomes. Furthermore, unless we postulate that there exist two different mechanisms for protein synthesis, there should also exist within uninfected normal cells RNA molecules physically similar to the phage-specific RNA and having base ratios similar to its specific DNA.

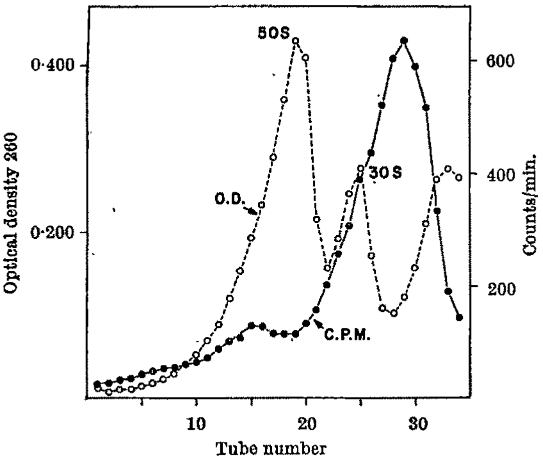

Fig. 1. Sedimentation of pulse-labelled 10^{-4} Mg^{2+} extract. 1 ml. of a crude extract labelled by a 20-sec. pulse of ^{14}C-uracil and made in 10^{-4} *M* Mg^{2+} and 5×10^{-3} *tris* buffer (*p*H 7·4) was carefully layered on top of a 24-ml. sucrose gradient (5–20 per cent) in a Spinco *SW*25 swinging-bucket tube. It was spun 2 hr. 45 min. at 25,000 r.p.m. at 4° C. The tube bottom was then punctured and 10 drop samples taken to measure 2600 Å. absorption (2 drops) and the radioactivity in 5 per cent trichloracetic acid precipitates (8 drops). All points were counted to at least 1,000 counts

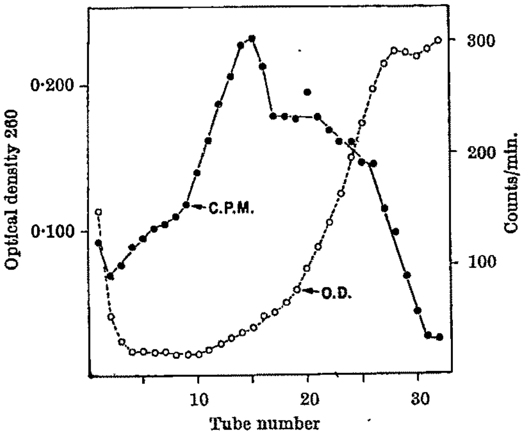

Fig. 2. Sedimentation of pulse-labelled 10^{-4} Mg^{2+} extract, long run. An extract as in Fig. 1 run for 11 hr. at 25,000 r.p.m., 15° C., on a sucrose gradient. Drop collection and radioactivity measurements were made as described in Fig. 1

Here we present evidence that RNA molecules physically similar to phage-specific RNA exist in normal *E. coli* cells and that, under suitable ionic conditions, they are associated with ribosomal particles.

Experimental plan. Most (80–85 per cent) RNA in actively growing *Escherichia coli* cells is found in ribosomal particles composed of 64 per cent RNA and 36 per cent protein[11]. There are two sizes[12] of ribosomal RNA, 16*s* (molecular weight = $5 \cdot 5 \times 10^5$) and 23*s* (molecular weight = $1 \cdot 1 \times 10^6$). The 16*s* RNA is derived from both 30*s* and 50*s* ribosomes, while 23*s* RNA is only found in 50*s* ribosomes. The other principal (10–15 per cent) form of RNA in *E. coli* is soluble RNA (now more appropriately called transfer RNA), which functions in the movement of activated amino-acids to the ribosomes. At least 20 (one for each amino-acid) different transfer RNA molecules exist[13], all of which have molecular weights about 25,000 and sedimentation constants of 4*s*.

Collectively, ribosomal and transfer RNA comprise at least 95 per cent of *E. coli* RNA. Thus messenger RNA, if present, can amount to at most only several per cent of the total RNA. Now if the messenger were stable, only a corresponding fraction of newly synthesized RNA could be messenger RNA; the great majority of new RNA being the metabolically stable ribosomal and transfer RNA's. If, however, messenger RNA is turning over (as is suggested by the original Hershey experiments) then a much larger fraction of newly made RNA must be messenger. For example, if the messenger functions only once for the synthesis of a single protein molecule, its lifetime might be only several seconds. In this event, if we look at the RNA synthesis occurring during a very short interval, then most of the newly synthesized RNA would be messenger even though this fraction may comprise only 1 per cent of the total RNA.

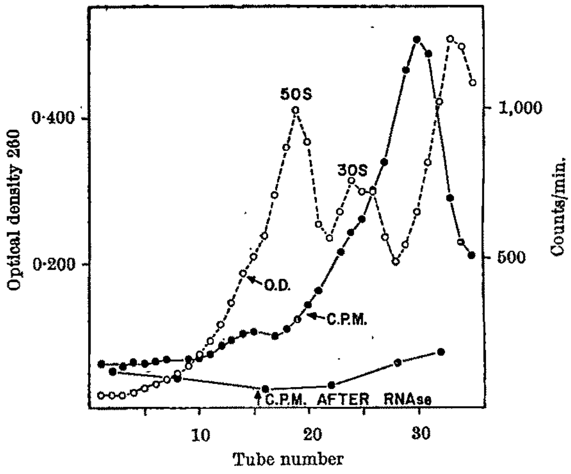

Fig. 3. Sedimentation of *T*2-infected 10^{-4} Mg^{2+} extract. An extract in 10^{-4} Mg^{2+} of cells infected with *T*2 at a multiplicity of 20 and labelled with phosphorus-32 between the second and fifth minutes after infection. Run as in Fig. 1. Several samples were treated with ribonuclease (10 γ/c.c.) to determine the background of label not in RNA

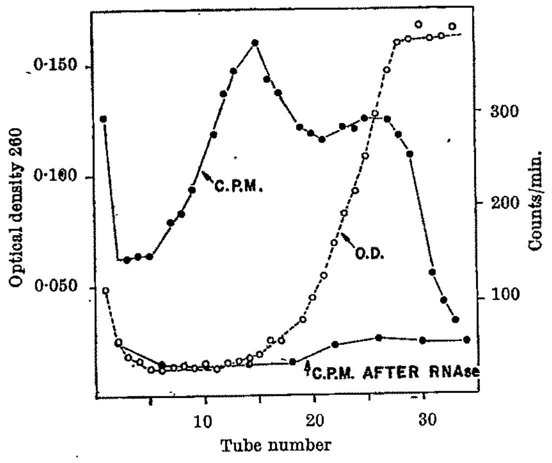

Fig. 4. Sedimentation of *T*2-infected 10^{-4} Mg^{2+} extract, long run. An extract as in Fig. 3 run for 11 hr. at 25,000 r.p.m. 20° C. Measured as in Fig. 1

We have therefore exposed *E. coli* cells to short pulses (10–20 sec. at 25° C. where the time of generation is about 90 min.) of radioactive RNA precursors (^{32}P or ^{14}C-uracil), rapidly chilled the cells with crushed ice and *M*/100 azide, prepared cell-free extracts by alumina grinding, added deoxyribonuclease at 5γ/ml., and examined the newly made RNA using the sucrose-gradient centrifugation technique[14]. In some experiments ^{14}C-uracil was given to cells of a pyrimidine-requiring mutant (*B*148) which was briefly starved (30 min. at 25° C.) for uracil. No difference has been seen between the properties of RNA labelled in these two ways. Starved cells incorporate more radioactivity, and they were used in most of the experiments reported below.

Results. Radioactive uracil (or phosphorus-32) is incorporated in RNA within several seconds after addition of the isotope. The RNA labelled by 10–20 sec. pulses is stable at 4° C. in cell-free extracts where ribonuclease and polynucleotide phosphorylase are not active. Traces (1γ/ml.) of ribonuclease degrade it under conditions where RNA in ribosomes is untouched. This suggests that it exists less protected than bound ribosomal RNA. Similarly, addition of phosphate permits the polynucleotide phosphorylase[15] in the cell extract to degrade preferentially pulse-labelled RNA. Our experiments thus as a matter of routine avoid the use of phosphate buffers.

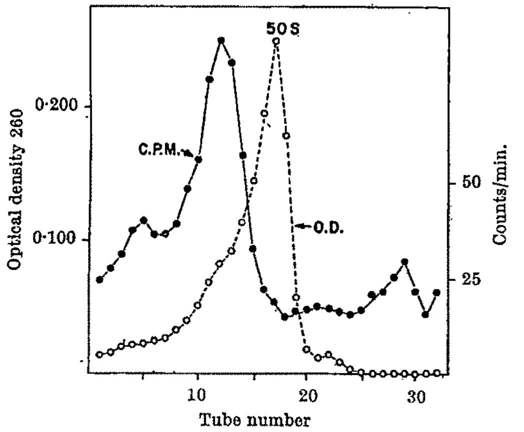

Fig. 5. Pulse-labelled active 70*s* ribosomes in 10^{-4} Mg^{2+}. An extract of cells labelled with a 20-sec. pulse of ^{14}C-uracil, made in 10^{-4} *M* Mg^{2+}, was purified by three 1 hr. centrifugations at 40,000 r.p.m. in 10^{-4} *M* Mg^{2+}, 5×10^{-3} *M tris* (*p*H 7·4). The resuspended pellet was run as for Fig. 1

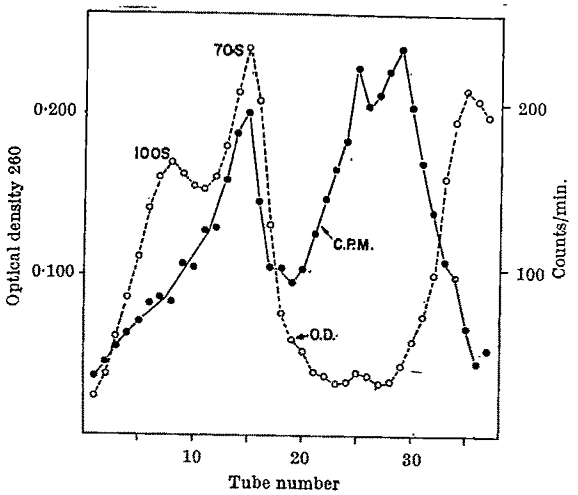

Fig. 6. Sedimentation of pulse-labelled 10^{-4} Mg^{2+} extract in 10^{-2} *M* Mg^{2+}. Magnesium was added to the 10^{-4} Mg^{2+} extract of Fig. 1 to bring it to 10^{-2} *M* Mg^{2+}. The sample was then run on a sucrose gradient in 10^{-2} *M* Mg^{2+}, 5×10^{-3} *M tris*, for 2 hr. 45 min. The measurements were made as described for Fig. 1

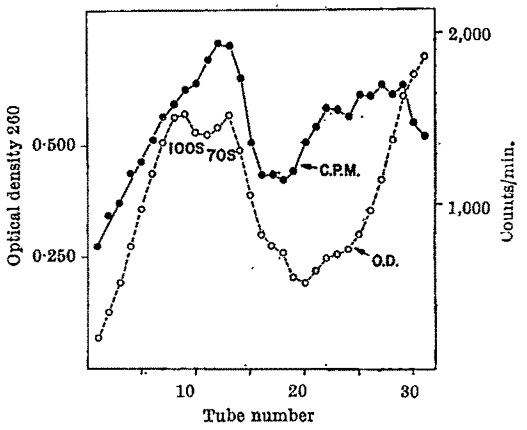

Fig. 7. Sedimentation of *T*2-infected 10^{-2} Mg^{2+} extract. An extract in 10^{-2} *M* Mg^{2+} of cells infected with *T*2 and labelled with phosphorus-32 from the second to the fifth minutes after infection. Run on a sucrose gradient in 10^{-2} *M* Mg^{2+} for 2 hr. 45 min. at 25,000 r.p.m. Drop collection and radioactivity measurements as for Fig. 1

Figs. 1 and 2 show how RNA labelled by a 20-sec. exposure of uninfected cells to ^{14}C-uracil sediments in a sucrose gradient. The cell-free extract contains 10^{-4} *M* magnesium ions in which 30*s* and 50*s* ribosomes predominate. The majority of the radioactivity is not associated with ribosomes but moves with a 14–16*s* peak. There is also RNA which moves more slowly, in addition to a faster forward fraction moving at 70*s*. The slower sedimenting fraction shows up more clearly in the longer centrifugation shown in Fig. 2. Here a sharp 14–16*s* component is seen together with material sedimenting at 4–8*s*. Figs. 3 and 4 illustrate experiments with extracts from *T*2 infected cells exposed to phosphorus-32 from 2 to 5 min. after infection. They are similar, if not identical, to Figs. 1 and 2. Both have a major 14–16*s* component, a slower trailing fraction, and about 10 per cent of the material moving at 70*s*. The 70*s* component can be purified by three 1-hr. centrifugations at 40,000 r.p.m. with 10^{-4} *M* magnesium ions to concentrate the faster-moving ribosomes of Fig. 1. Fig. 5 shows the purified ribosomes consisting largely of 50*s* particles together with a now visible 70*s* component. The radioactivity, however, sediments as 70*s* ribosomes.

Still more label is attached to 70*s* particles when extracts are made in 10^{-2} *M* magnesium ions or when additional magnesium ions are added to 10^{-4} *M* extracts. Figs. 6 and 7 again illustrate the parallel appearance of normal and phage-infected extracts. About 30 per cent of label sediments with the 70*s* and 100*s* ribosomes, with the specific activity of 70*s* ribosomes generally greater than that of 100*s* ribosomes. When slightly lower concentrations of magnesium ions are used to give similar amounts of 30*s*, 50*s* and 70*s* ribosomes, label is specifically associated only with 70*s* particles and no label is in 50*s* ribosomes. The radioactivity sedimenting about

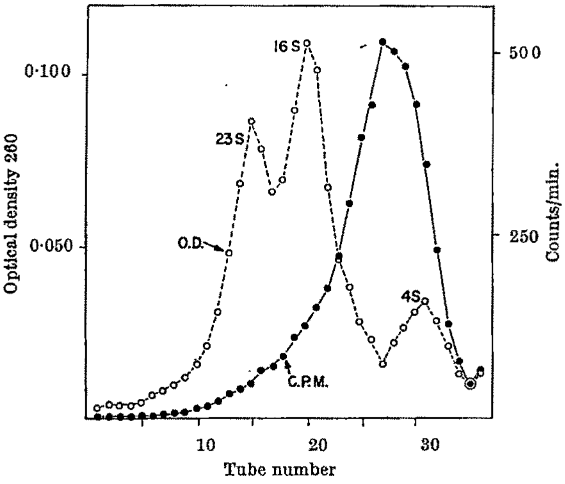

Fig. 8. Sedimentation of pulse-labelled RNA. An extract of cells labelled with a 20-sec. pulse of ^{14}C-uracil was treated with 0·5 per cent sodium lauryl sulphate ('Duponol') and then extracted three times with phenol. The RNA was then precipitated three times with alcohol and finally resuspended in 10^{-2} acetate buffer (*p*H 5·1). The RNA was run on a sucrose gradient, containing 10^{-2} acetate (*p*H 5·1) and M/10 sodium chloride, for 10 hr. at 25,000 r.p.m., 4° C. The drop collection and radioactivity measurements were made as described for Fig. 1

30*s* shows a very broad peak (20–40*s*) and it is probable that high concentrations of magnesium ions cause the 14–16*s* material either to aggregate or to assume a more compact shape.

Purified RNA, prepared by sodium lauryl sulphate ('Duponol') and phenol treatment of 20-sec. pulse extracts, sediments as shown in Fig. 8. Again the pattern is identical to that of labelled RNA from *T*2 infected cells (Fig. 9). Most newly synthesized RNA has a sedimentation constant about 8*s*; there is no appreciable synthesis of typical 16*s* or 23*s* ribosomal RNA. Since much new RNA in crude extracts moves at 14–16*s*, we considered the possibility that pulse-RNA is more sensitive than ribosomal RNA to degradation during the phenol extractions. Sodium lauryl sulphate (0·5 per cent) alone was therefore added to labelled crude extracts. This detergent treatment[12] both separates RNA from protein and completely inhibits action of the latent ribonuclease[16] release upon ribosome breakdown. When such treated extracts were run in sucrose gradients, the results were identical to those with phenol-prepared RNA.

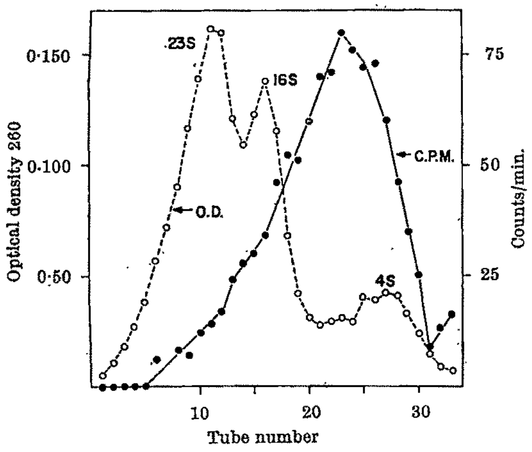

Fig. 9. Sedimentation of *T*2-specific RNA. RNA of cells infected with *T*2 and labelled with phosphorus-32 between the second and fifth minute, prepared and run as described for Fig. 8

The turnover of pulse-RNA is seen in chase experiments in which an excess of cold uracil is added after a 10-sec. ^{14}C-uracil pulse. After 15 min. at 37° C., all the label in crude extracts leaves the 14–16*s* component and is incorporated in the metabolically stable ribosomal and soluble RNA. In our kinetics experiments, the 16*s* RNA molecules always become labelled before appreciable labelling of 23*s* RNA molecules; likewise the 30*s* ribosomes become labelled before the 50*s* ribosomes. Since the base ratios of 16*s* and 23*s* chains are identical[17], it is likely that the 23*s* molecules form from two 16*s* chains.

Discussion. Our pulse experiments show that uninfected cells contain unstable RNA with sedimentation constants and attachment properties similar to those of *T*2-specific RNA. It is tempting to believe that these unstable molecules convey genetic information and are 'messenger' RNA. Complete homology with phage-specific RNA, however, will be demonstrated only if the base ratios of pulse-RNA are those of DNA. Unfortunately, in *E. coli*, the DNA base ratios do not differ greatly from ribosomal RNA, and as yet the base ratios of *E. coli* pulse-RNA are not precisely known. But we do have preliminary results (Hayes, D., and Gros, F., unpublished results) from *Staphylococcus aureus* which indicate an RNA component rapidly turning over and possessing DNA-like base ratios. Moreover, in yeast, there is also reported an unstable RNA resembling DNA in base composition[18]. We thus believe that our current measurements with *E. coli* will extend this fact and rule out the possibility that pulse-RNA is a precursor sub-unit from which ribosomal RNA is built up.

A messenger role for pulse-RNA fits nicely with its specific attachment to 70*s* ribosomes, the sites of protein synthesis[19]. Our experiments reveal two types of attachment. In one, the labelled RNA moves reversibly on or off 70*s* ribosomes depending on the concentration of magnesium ions. The second type of attachment binds pulse-RNA irreversibly to 'active' 70*s* ribosomes. These are 70*s* ribosomes which do not break apart with 10^{-4} M magnesium ions and which Tissières *et al.*[19] have shown to be the principal, if not sole, site of *in vitro* protein synthesis. They can be washed several times with 10^{-4} M magnesium ions without losing their ability to act in protein synthesis, and so they must have their genetic RNA firmly attached. It is reassuring that they can contain pulse-RNA. At least two stages, one reversible, the other not, thus exist in active 70*s* formation.

So far as we can tell, pulse-RNA, when associated with ribosomes, sediments at exactly 70*s* or 100*s*. Many experiments show that the label sediments neither faster nor slower than ordinary ribosomes. We find it hard to believe that the 14–16*s* RNA component which exists in 10^{-4} M magnesium ion extracts can combine with 70*s* or 100*s* ribosomes without altering their rate of sedimentation. Another possibility is that with 10^{-2} M magnesium ions, the 14–16*s* component assumes a more compact configuration similar to that of the RNA in a 30*s* particle. It could then associate with a free 50*s* ribosome to form a 70*s* ribosome which is transformed to an active 70*s* ribosome.

In conclusion, we state our findings: bacteria contain an RNA component turning over rapidly which is physically distinct from ribosomal or soluble (transfer) RNA. This fraction behaves, in its range of sedimentation constants and its attachment to ribosomes in high magnesium ion concentrations, exactly as does the phage-specific RNA made after *T*2 infection. Furthermore, it is associated with the active 70*s* ribosomes, the site of protein synthesis.

Our working hypothesis is that no fundamental difference exists between protein synthesis in phage-infected and uninfected bacteria. In both cases typical ribosomal RNA does not carry genetic information, but has another function, perhaps to provide a stable surface on which transfer RNA's can bring their specific amino-acids to the messenger RNA template.

These experiments were initiated when F. Gros was visiting the Biological Laboratories (May–August 1960). The pyrimidine-requiring strain *B*148 was kindly provided by Dr. Martin Lubin. The financial support of the National Science Foundation and the National Institutes of Health is gratefully appreciated.

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DR. W. H. FITTON, F.R.S.

By JOAN M. EYLES

WILLIAM HENRY FITTON, physician and geologist, died in London on May 13, 1861, at the age of eighty-one. In an obituary notice, Sir Roderick Murchison, director-general of the Geological Survey, described him as "truly one of the British worthies who have raised modern geology to its present advanced position".

Fitton, of English descent, was born and brought up in Dublin and his proficiency in the classics enabled him to gain, in 1798, the Senior Scholarship of Trinity College. Even then he was a fossil collector, a hobby which brought him into the hands of the military on suspicion of being a rebel. In 1808, he went to Edinburgh to study medicine, and there attended the geological lectures of Robert Jameson, who was responsible for stimulating an interest in science and natural history in many young men at that time. Fitton graduated M.D. at Edinburgh in 1810, but continued to study medicine and chemistry in London until 1812. In that year he moved to Northampton, where he was in practice until 1820. Then marriage to a lady of considerable means enabled him to give up his practice and return to London, where his house soon became a regular meeting place for men of science and, in particular, geologists.

Fitton did not become a Fellow of the Geological Society until 1816, but his first geological paper, "On the Geological Structure of the Vicinity of Dublin", appeared in 1811 in the first volume of the Society's *Transactions*. In 1817 he contributed to the *Edinburgh Review* the first of a number of reviews, often lengthy, of geological works, which are noteworthy for the valuable information they contain on the history and progress of geology. These may still be consulted with profit by the historian of geology, for Fitton was widely read, had a clear analytical mind, and wrote with the facility of a classical scholar. Secretary of the Geological Society during 1822–24, he was elected president in 1827, and during his term of office introduced an important innovation, the publication of the *Proceedings* of the Society, of which the first number appeared in April 1827. In this publication the presidential addresses were published, Fitton's being the first to appear, and thus began that valuable annual series in which for many years the progress of geology was reviewed. Fitton was also instrumental in appointing, as curator and librarian, in 1829, William Lonsdale, a gifted palæontologist, who served the Society until 1842.

The geological work for which Fitton is best remembered, however, is his elucidation of the stratigraphy of the beds lying between the Kimmeridge Clay and the Chalk, that is, of the upper part of the Jurassic and the Lower Cretaceous. The succession comprises limestones, clays and sandstones, some marine and some fresh-water. During the course of a prolonged study of these beds in both the south-east of England and north-east France, he was able to establish the correct order of succession, a major advance in British stratigraphy. For these researches he received the Wollaston Medal of the Geological Society in 1852.

Fitton was elected Fellow of the Royal Society in 1815, and was also Fellow of the Linnean, Astronomical and Royal Geographical Societies.

OBITUARIES

Prof. Thomas Alan Stephenson, F.R.S.

Marine biologists in many lands will learn with sorrow of the death on April 3, at the age of sixty-three, of Prof. Thomas Alan Stephenson, for he had travelled widely and visited many ocean shores. Alan Stephenson's first interest was sea anemones, a group of animals which no doubt appealed to his artistic nature, and this culminated in the beautifully illustrated Ray Society monographs on British sea anemones which appeared in 1928 and 1935. He was on the staff of the Department of Zoology at University College, London, during 1922–30, and in 1923 made a short investigation for the States of Guernsey on the biology of the ormer, *Haliotis*, with the view of legislation to check the noticeable decrease in supply.

In 1928 he joined the Great Barrier Reef Expedition under C. M. Yonge and led the shore party concerned with the ecological investigations on the reefs. His knowledge of anemones made him most suitable for this expedition, and he naturally devoted much time to the corals themselves, making also valuable observations on growth. But his wider interest in shore ecology found full scope in a general study of reef ecology, on which one of his joint papers with his wife, G. Tandy and M. Spender, will have lasting value as evidence of the state of Low Isles in that year. It has already formed the basis of two later small-scale surveys.

In 1930, Stephenson was elected to the chair of zoology in the University of Cape Town. It was then that he developed his grand idea of the comparison of shore faunas on a world-wide scale. Our knowledge of the distribution of the intertidal fauna and flora had been restricted to detailed surveys at isolated points either in easy reach of marine laboratories or at places visited by expeditions. Stephenson was the first to organize a continuous survey along a great length of coast and its value lay in the fact that the same people saw the whole length of coast so that their observations were as comparable as possible.

This first great survey, over a period of ten years, extended from Port Nolloth on the west coast of Africa, round the Cape, to Durban on the east coast. It thus covered regions in the south Atlantic, the Southern and Indian Oceans, and extended through a range of climates as well as areas influenced by different ocean currents. The work was done with all his usual exactitude, and the correct identification of species was ensured so far as possible. The great series of papers produced by Stephenson and his colleagues will remain as a lasting memorial to a unique zoologist and as a reference point for all time. It was then that he discerned the underlying simplicity of the zonation patterns on the shore.

In 1940, he was appointed professor of zoology in the University of Wales at Aberystwyth, where he had been a student, and here he continued to inspire research on shore ecology. But every opportunity was still taken to continue his chief aim of seeing for himself as much of the coastline of the world as possible. With his wife Anne, who was always his constant and devoted collaborator, he explored the coasts of North America and of Bermuda. He was to have visited the Mediterranean this summer.

It was Alan's hope to write a book bringing together all his vast knowledge, and it is sad that he cannot now do this. But in fact a great deal has been published and is available to the world and the pioneering has been accomplished. The outburst of interest in marine science is ensuring that many more shores are being surveyed. The stimulus has been given and the standard set by Stephenson and his concept of three universal intertidal zones is being confirmed.

All Stephenson's work was characterized by its extreme finish, and everywhere could be seen his meticulous care for detail. But as well as being a first-class naturalist he was also an artist. His paintings of marine animals and plants are exquisite portrayals and their composition emphasizes his deep artistic appreciation and disclosure of pattern and rhythm, so beautifully exemplified in his little book on *Seashore Life and Pattern*.

The deepest sympathy of their many friends will go out to his wife in her bereavement.

F. S. Russell

Dr. Daphne Atkins

Dr. Daphne Atkins, a familiar figure to workers at the Plymouth Laboratory during the past 35 years, died on March 1. She was born on March 4, 1896, at Oxted, Surrey, the daughter of N. E. W. and Elisa Atkins. She was educated at the Kent County School for Girls, Tunbridge Wells, and from there went on to Bedford College, London, where she took her degree, in zoology and geology, in the early nineteen twenties.

Her first visit to Plymouth was probably in 1922 when I well remember her as a fellow student at the Easter class. After graduation she was soon to return to Plymouth where, supported initially by a series of research grants, she continued to work for much of the rest of her life. Her only notable period of absence was during and for some time after the War when she returned to Oxted to care for her aged parents.

She made major contributions to our knowledge of marine invertebrates. She observed the living animal, she studied its anatomy by dissection and in sections, in exact detail she followed the course of the ciliary currents concerned with respiration, feeding and cleansing in bivalve molluscs, polyzoans and brachiopods. She published her conclusions in admirably presented and most beautifully illustrated papers. These must have numbered at least forty and were largely published in the *Journal of the Marine Biological Association*, the *Quarterly Journal of Microscopical Science* and the *Proceedings of the Zoological Society of London.*

Her earliest research, suggested by J. H. Orton, on the moulting stages of pea-crabs (*Pinnotheres*) was published in 1927. While this led to further work on post-embryonic development, it had very different and far-reaching side issues. These included accounts of a fungal infection and of an entoniscid (Isopod) parasite on *Pinnotheres*, examples of contributions to parasitology which included descriptions of orthonectid, rhabdocœl and copepod parasites in bivalves.

Observing the effects of *Pinnotheres* on the gills of *Mytilus*, she noted that the damage caused led to the appearance of secondary or supernumerary food grooves with an associated reversal of beat in the adjacent frontal cilia. This led to further work on abnormal gills and then on regeneration. These are very valuable papers containing information which could very suitably form the groundwork of further important studies. In the case of their author they led to a series of eight papers "On the Ciliary Mechanisms and Interrelationships of Lamellibranchs" published between 1936 and 1943. In the course of some 400 pages she ranged widely throughout the bivalves describing and figuring in the most precise and beautifully illustrated detail the course of ciliary currents and the different types of cilia, with such matters as cuticular fusion and the nature of ctenidial musculature. These papers are an abiding source of knowledge, one to which I am glad to pay my testimony of gratitude and admiration. They put all workers on the bivalve Mollusca, now and in the future, in her debt.

Another source of interest, again initially stimulated by J. H. Orton, was in the Polyzoa. Early in her research career she published an account of their ciliary feeding mechanisms followed by a detailed systematic and anatomical account of the Loxosomatidea of the Plymouth area. Later she was to describe the structure and feeding mechanisms of cyphonautes larvæ. Her latest interest, made possible by the more extensive collecting range of the post-war research vessel, *Sarsia*, was in Brachiopoda with which her last seven papers, dealing with development of the lophophore, with feeding mechanisms and with systematics, are concerned.

Although of an extremely retiring disposition, Daphne Atkins was far from being devoid of personality. Within the field of her research interests she held most decided views, supported as they were by the results of years of the most careful observation. She has left a magnificant monument in her long series of published papers. As the Plymouth Laboratory made her work possible, this itself is a justification for the existence of that Laboratory.

C. M. YONGE

NEWS and VIEWS

The First American Manned Ballistic Rocket Flight

ON May 5, at 14.34 hr. U.T., a *Redstone* rocket was launched from Cape Canaveral in Florida, carrying Commander Alan Shepard, of the U.S. Navy, in a space capsule. This first United States attempt at manned ballistic rocket flight was entirely successful. The capsule was accelerated to a maximum speed of 5,100 miles per hour, reached a height of 115 miles and travelled 302 miles down-range over the Atlantic. As it re-entered the atmosphere the capsule's heat-shield was seen from the waiting ships to be glowing red. Commander Shepard seems to have suffered no ill-effects as a result of his 15-min. flight, during which he was subjected to a deceleration of 11 *g*. He was in radio communication with the ground throughout, and was able to climb out of the capsule before a helicopter arrived to pick him up. Though this rocket firing was only a first step towards orbital flight, Commander Shepard was able to test orbital techniques by controlling the orientation of the capsule under zero-gravity conditions.

New Carbon Isotope, Carbon-16

A NEW carbon isotope of mass 16 has been discovered by physicists of the Atomic Weapons Research Establishment, Aldermaston, working in collaboration with scientists of the Clarendon Laboratory, Oxford. A carbon-14 target was bombarded with a beam of 6-MeV. tritons (ions of tritium) from the Aldermaston Van de Graaff accelerator. In some cases, the force of the collision was observed to be sufficiently great to split the triton into its constituents of one proton and two neutrons and the two neutrons were captured by the carbon-14 to form a new isotope, carbon-16. By making precise measurements with a large magnetic spectrograph on the emitted protons, the mass of the new isotope was found to be 16·014702 atomic mass units. It was anticipated that carbon-16 would suffer beta-decay like carbon-14 and carbon-15, but with the difference that the product of the decay would still have sufficient energy to emit a neutron. This occurred, and use was made of this property to measure the half-life, which was found to be 0·74 sec. The most abundant isotope of carbon is, of course, carbon-12. Isotopes of mass 13, 14 and 15 are known, of carbon-14 and -15 are radioactive, and the former is the basis for the carbon-14 dating of archæological specimens.

Ministry of Aviation : Mr. W. O. Broughton

MR. W. O. BROUGHTON has been promoted to deputy chief scientific officer and appointed to the post of director of air navigation and reconnaissance research and development in succession to Mr. C. J. Carter, who has joined the defence research staff in Washington. Mr. Broughton started his career in the Civil Service in 1935 at the Royal Aircraft Establishment, where he was engaged on development work connected with automatic pilots, the *Queen Bee* pilotless target aircraft and stabilized automatic bombsights. In 1942 he transferred to Headquarters, Ministry of Aircraft Production, to take charge of a section concerned with the development of automatic pilots, gyroscopes and stabilizing equipment. He returned to the Royal Aircraft Establishment in 1947 as head of the Controls Division of the Instrument and Air Photography Department, remaining in this post until 1954, when he was transferred to Headquarters, Ministry of Supply. In 1955 he was appointed assistant director, being responsible for administration of research and development connected with automatic flight control systems, automatic blind landing, flight instrument systems and aircraft fire control systems. In addition to these systems, Mr. Broughton now assumes responsibility for research and development on air photography, bombsights, flight simulators and non-radio aircraft navigation systems.

Physics at Birmingham: Prof. W. F. Vinen

DR. W. F. VINEN, who has been appointed to a chair of physics in the University of Birmingham at the early age of thirty-one, was educated at Watford Grammar School and Clare College, Cambridge, where he was elected to a research fellowship in 1955. In 1958, he was appointed a University demonstrator in physics and a Fellow of Pembroke College. In close collaboration with his contemporary, Dr. H. E.

Hall, recently appointed to a chair at Manchester (see *Nature*, March 18, p. 877), Dr. Vinen conducted an elegant series of studies on the hydrodynamics of superfluid helium, with particular reference to the development of turbulence and the frictional effects arising from the interactions of the thermally excited 'normal fluid' with the vorticity of the ground-state, the 'superfluid'. The work of these two collaborators, which must ultimately be ranked as a classic investigation, soundly establishing quantum hydrodynamics on an experimental basis, culminated on Dr. Vinen's side with a direct demonstration of the quantization of circulation in the superfluid. This beautiful experiment may well be regarded as a worthy conclusion to the work of more than twenty years in Cambridge on the properties of superfluid helium, which began equally strikingly with the discovery of the fountain effect. In the organization of a new department and the development of new fields of research, Dr. Vinen will have full scope for the inventiveness and versatility which have been such a notable characteristic of his work up to the present.

Director of the National Fishery Institute, Ecuador: Mr. F. Bourgois

MR. FRANÇOIS BOURGOIS has been appointed director of the National Fisheries Institute, Guayaguil (Ecuador), for a period of four years. Mr. Bourgois has been Food and Agriculture Organization fishery officer in Haiti since last July. Prior to that, he was fishery officer and the Organization's representative in Tunisia for three and a half years. While in Tunisia, Mr. Bourgois was concerned with finding and charting new fishing grounds, improving the sardine fisheries and extending Tunisian trawling farther offshore. Together with one of the Organization's master fishermen, he introduced the use of underwater lamps into Tunisian sardine fishing. Mr. Bourgois joined the French Fisheries Service in 1945 as a fisheries expert and administrator. He was previously fisheries adviser to the Viet Nam Government, and fisheries expert with the Supreme Commander for the Allied Powers. His first assignment for the Food and Agriculture Organization was in 1956 in Libya. The Ecuadorian National Fishery Institute is the second major U.N. Special Fund fisheries project, with the Food and Agriculture Organization as executing agency, to be established in Latin America. The project calls for a Government contribution of 663,040 dollars over a four-year period, and a special fund contribution of 633,800 dollars. The purpose of the Ecuadorian Institute is to help the Government foster the country's economic development by a substantial expansion of fishing and ancillary activities. Facilities and maintenance for the Institute's two research vessels, and personnel are to be provided by the Ecuadorian Navy. The Navy will also provide hydrographic and meteorological information on areas not studied by the Institute's vessels and will provide a ship for several months of whale-marking each year in the seas of Ecuador.

Space Research in Britain

REPLYING to a question regarding Great Britain's contribution to a space research programme, Mr. D. Freeth, Parliamentary Secretary for Science, said in the House of Commons on May 2 that Great Britain's programme comprises five main elements. There is the programme of scientific experiments using sounding rockets. Further experiments will be made in satellites to be launched by the United States, with which Britain is also collaborating in testing communications using satellites. Thirdly, Britain is participating actively in the preparatory commission set up to plan a European organization for co-operation in scientific research in space. Great Britain and France have also proposed to other European Governments a co-operative programme for the development of satellite launchers beginning with one based on *Blue Streak* to be fired from Woomera. Finally, Britain is doing much analysis of satellite tracking data, including the operation of one of the three World Data Centres which act as international clearing houses for information on space research. Future plans in Britain will be considerably affected by the results of the discussions now taking place with other countries regarding the third and fourth elements in this programme. Mr. Freeth added that Britain is in very close touch with both the Australian and Canadian Governments, and he strongly disagreed with the suggestion that discouragement of British participation in a space programme is leading to an exodus of the finest brains of the country to the United States and Canada.

In a written answer on May 2, Miss Pile, Assistant Postmaster-General, said that the Post Office is co-operating in a wide-ranging communications satellite research programme which covers orbits, altitudes and frequencies, and the design of the electronic equipment in the communication satellites themselves. The first requirement is a ground station for joint experiments with the Americans in satellite communications across the Atlantic.

In reply to questions on May 4, the Prime Minister said that the Minister for Science, in his speech to the Geological Society on April 26, was re-affirming his acceptance of the view of the Advisory Council on Scientific Policy that a full-scale space programme on a purely national basis, including the development of a large launcher, would not be justified solely on scientific grounds. The wider problem of developing a launcher for heavy satellites is under discussion in Europe, and any further statement of policy must await the outcome of discussions. He hoped that Britain would be able to get the joint scheme, because of the enormous sums involved if the matter were to be pressed forward in all its aspects. In other questions reference was made to the expenditure of only £5 million on scientific research by the National Coal Board, and to the cost of the Russian rocket programme for launching heavy satellites as about equal to the cost of the whole defence programme of Britain. Mr. Macmillan emphasized that it is a matter of determining the right priorities and in this matter the Government looks to the Advisory Council on Scientific Policy for guidance,

Industry, Science and the Chemical Engineer

ON April 25, at the thirty-ninth annual general meeting of the Institution of Chemical Engineers, Mr. W. K. Hutchison, deputy chairman of the Gas Council, delivered the presidential address. He referred to the rapid growth in membership of the Institution. This occurred chiefly because of post-war expansion; chemical industry and chemical plant manufacturers all began to offer opportunities for employment to chemical engineers on a scale not contemplated before the War. The greatest demand is in the field of production, and, in particular, plant operation, where techniques of management are of special importance. The Institution is able to make one of its most useful contributions to the education of engineers by providing the teaching institutions with detailed information on the type of problems encountered by the professional engineer. In time, each chemical engineering course offered by various institutions would develop its own style and character and the employer would remain free to go to whichever seemed best able to produce the type of man he wanted. The final test of the engineer would always be success or failure in carrying out the work entrusted to him. The Institution could do no more than prepare him for such a test.

Mr. Hutchison referred to the concept of 'unit operations' which, for thirty years, has been retained as a basis for instruction in chemical engineering. Behind all unit operations there are fundamental physical or chemical concepts, so related that, if used with the necessary degree of mathematical skill, they might make unnecessary much of the existing content of chemical engineering instruction. Mr. Hutchison also mentioned an interesting situation in continental Western Europe, where only small groups are willing to call themselves chemical engineers or to recognize the coherent set of principles within which a profession can be practised. Many of the functions claimed by chemical engineering are fulfilled by combined teams of chemists, physicists and engineers.

The attraction of chemical engineering to many young men is its obvious connexion with the realities of industrial processes. The attitude is that of the engineer rather than of the scientist; the laboratory and the lecture theatre are tolerated only as stepping-stones to the world outside. If there is a latent conflict between the two aspects of engineering, "the engineer directing the great powers in nature for the use and convenience of man", and the scientist intent in his laboratory on advancing the frontiers of knowledge, the Institution does provide a solution. It is both a corporate body of practising engineers and a meeting ground for scientists. The primary aim of the Institution is the advancement of science —by which is meant all knowledge—through the skill of practising engineers and scientists alike.

The Cotton, Silk and Man-Made Fibres Research Association

In May 1960, the working party that had been set up to consider the possible fusion of the British Cotton Industry Research Association and the British Rayon Research Association recommended that the two Associations should be merged into one research association as from July 1, 1961. It has now been decided that this will be known as the Cotton, Silk and Man-Made Fibres Research Association, and its main responsibilities will be to serve the processing and merchant converting sections of the industries. The officers of the new Association will be as follows: *Director*, Dr. D. W. Hill; *Deputy Director*, L. A. Wiseman; *Assistant Directors*, G. W. Lobb (to be responsible for research administration), L. H. C. Tippett (to be responsible for physical and mechanical research), and Dr. A. R. Urquhart (to be responsible for chemistry and chemical processing). A large part of the research programmes and other work previously undertaken by the original Associations is being continued by the new organization. The main source of the Research Association's industrial income will be a research levy forming part of the levy collected by the Cotton Board. The Association will also receive a grant from the Department of Scientific and Industrial Research. Plans are already well advanced for the provision of a new chemistry and finishing building at the Shirley Institute, Didsbury, Manchester, which, it is hoped, will be completed by the end of 1962 or early 1963.

Science Museum Exhibition of a Fourteenth-Century Astronomical Clock

The Science Museum will be exhibiting during May 16–June 25 the first full astronomical clock to be produced in Britain for 250 years. The clock is an exact reproduction of one designed and constructed nearly 600 years ago by John Dondi, who was, at times, professor of logic, medicine and astronomy in Italy. During the years 1348–64, Dondi constructed his astronomical clock, which came to fame during the next two centuries, but afterwards it seems to have sunk into oblivion, and little was heard of it until the late G. H. Baillie, in the 1930's, translated the Latin manuscript which Dondi left behind giving a full description of just how he made the clock, with working drawings. The clock remained in Italy until 1585 and then went to Spain, where it was destroyed during the Peninsular Wars. Constructed within about fifty years of the invention of the mechanical clock, at a time when the standard clock was a clumsy forged-iron blacksmith's production, Dondi's clock presented an achievement of great refinement. Astronomical clocks showing the motions of the Sun, Moon and nodes began to be less rare in Europe during the late fifteenth and sixteenth centuries; but those embodying the motions of the five planets as well are rare at any period. The names of Immser and Baldewein came into prominence in Central Europe about 1550; in Western Europe little was done until Johann Klein of Prague and Aureliano and Cajetano of Vienna constructed their clocks in the last quarter of the eighteenth century. Later, clocks were built by Schwilgué in 1842 and Jensen in 1956, and in England there was only Samuel Watson in 1680 and Henry Bridges in 1730. It has fallen to Mr. H. Alan Lloyd to take up Baillie's work and complete it. After publishing a full précis of the translation in 1955, he decided to make a reconstruction of the clock. He was able to find collaborators in the managing director, Mr. Geoffrey Buggins, and in his co-director and chief craftsman, Mr. Peter Haward, of Thwaites and Reed, Ltd. Following the exhibition in London, the clock will be taken to the Smithsonian Institution, Washington.

X-ray Protection at High Voltages

The National Committee on Radiation Protection and Measurement of the U.S. National Bureau of Standards has issued a new handbook with information about how to obtain medical protection from X-rays up to 3×10^6 V. (National Committee on Radiation Protection and Measurements. Report No. 26. Pp. viii+52. (Washington, D.C., Government Printing Office, 1961.) 25 cents.) The handbook sets out standards of safety and contains data and recommendations relating to all persons involved, including the manufacturers of the apparatus and designers and builders of the rooms in which it is housed. Basic requirements for safety standards are essentially the same in this handbook as in its immediate predecessor (No. 60). Tables have been revised to conform with the new lower maximum permissible dose adopted by the Committee in 1957, and the differences in the maximum permissible doses allowed in controlled areas and their environs. Recommendations have been changed to cover situations created by recent technical advances in machine design, and by the development of new radiological techniques. The underlying philosophy, however, remains as it was originally expressed by the Committee thirty years ago, namely, that unnecessary radiation exposure should always be avoided and all exposure kept to the minimum compatible with practical clinical requirements.

Field Studies in Scotland

DESPITE the attractiveness of the Study Centre at Garth, the Scottish Field Studies Association may have to discontinue its activities for lack of support. In contrast to the encouraging number of students who are attending Field Study Centres in England, the number of student days spent at Garth fell from 2,972 in 1959 to 2,539 in 1960. Of the 29 courses offered, 7 had to be cancelled for lack of support. The Association is appealing not only for financial help but also for increased support from Scottish and other local education authorities. In her foreword to the annual report, Dr. Margaret Stewart writes that "there is plenty of lip service to the importance of Field Studies in education curricula, but so long as it is left to the initiative of individual teachers the work of the Association cannot be developed on a firm and lasting basis" (Annual Report, 1960. The Importance of Field Studies in Modern Education. By Paul F. Holmes. Garth House as a Centre for Ecological Botany. By Fred N. and Brenda D. Haynes. Pp. 27+4 plates. (Glasgow: Scottish Field Studies Association, 1961.) 2*s.* 6*d.*). Information about the Association can be obtained from 179 West Regent Street, Glasgow 2.

Organization of Sandwich Courses

THE National Advisory Council on Education for Industry and Commerce has started an inquiry on the organization of sandwich courses in technical colleges and other colleges of further education. A sub-committee under the chairmanship of Mr. E. L. Russell, chief education officer for Birmingham, has been set up "to examine the organization of sandwich courses with a view to using college staff, equipment and accommodation and industrial training facilities to the best advantage; and in particular to consider 'end-on' arrangements". The sub-committee is examining the various ways in which practical experience in industry is combined with academic study in technical college courses. These methods include the 'end-on' system by which two quite separate groups of students follow the same course, but use the college alternately, one group being in college while the other is in industry. Any organization or persons wishing to submit evidence to the sub-committee is asked to write to the Secretary, National Advisory Council on Education for Industry and Commerce, Ministry of Education, Curzon Street, London, W.1.

Zoological Society of London

DURING 1960 the Zoological Society of London was the recipient of three munificent bequests which will enable it to extend considerably its activities. The Trustees of the Nuffield Foundation granted the Society £100,000 to found an Institute of Comparative Medicine. Mr. Jack Cotton is providing £250,000 to help the rebuilding programme, and the Ford Foundation gave 550,000 dollars (nearly £200,000) to endow research fellowships in animal breeding. A major part of the new research activities will lie in the field of comparative medicine. The Institute of Comparative Medicine is to be associated with the name of Lord Nuffield. The grant is also intended to allow for the creation of two research fellowships in comparative medicine. The Ford grant makes it possible to undertake a systematic study of the reproductive habits and physiology of those animals in the Society's collections which breed readily in captivity. These studies will be supervised by a committee of well-known scientists distinguished for their contributions to knowledge of reproductive physiology. On the basis of the advice of this committee, the Council has already appointed the first three research Fellows. The Society is now planning new laboratories for their accommodation. Mr. Cotton's gift is the key which sets in motion the plan for reconstruction of the Regent's Park Gardens as devised by Sir Hugh Casson and Mr. Peter Shepheard in consultation with the Society's resident architect, Mr. F. A. Stengelhofen. Details of the bequests and other information about the many forward-looking activities of the Society are set out in its annual report for 1960 (Pp. 54+8 plates. London: Zoological Society of London, 1961). H.R.H. the Duke of Edinburgh has accepted the presidency in succession to Sir Landsborough Thomson.

The Ciba Foundation

THE report for 1960 of the Ciba Foundation for the Promotion of International Co-operation in Medical and Chemical Research, which includes, as usual, details of symposia and their participants, of the study groups and research meetings, and lists of publications held and issued, records that the Foundation provided accommodation during the year for 825 scientists from 37 countries (Pp. 63. London : Ciba Foundation for the Promotion of International Co-operation in Medical and Chemical Research, 1961). Five three-day international conferences were held and membership of these symposia is now limited to about 30. Besides four one-day study groups, the Foundation arranged five evening discussion meetings, five research meetings, a guest symposium on "Aspects of Ciné-Indoscopy and Ciné-Radiology", seven sessions of scientific films, and a new series of dinner meetings known as the Dunant Coterie. Three such meetings were held in 1960 and the twelfth Ciba Foundation Lecture was delivered by Prof. J. Konorski, the fourth annual lecture on ageing by Dr. P. J. Thung, and the Louis Rapkine Lecture by Medicin-Lieutenant M. Flauder. Seven Anglo-French bursaries were awarded by the British selection committee for medical graduates to visit France, and two by the French selection committee for visits to London Medical Schools. Three volumes of the proceedings of symposia, one of a colloquium on endocrinology and two of study groups were published during the year.

Photon Rocket

OPTICAL and mechanical phenomena to be encountered by a space traveller moving with a velocity approaching that of light are discussed by S. M. Rytov (*Priroda*, 4, 64 ; 1960). The greatest danger to such a traveller will be encounters with relativistic cosmic dust and wind. A collision of the space ship with a cosmic particle one milligram in weight will spell complete destruction. In spite of such a warning G. G. Zelkin describes the construction of a purely hypothetical photon rocket (*Priroda*, 11, 69; 1960), many kilometres in length and propelled by fuel, the energy of which is wholly transformed into luminous energy.

A New Elementary Particle

IN a brief note Din Da-Tzao and E. N. Kladnitzkaya (*Priroda*, 8, 17; 1960) give an account of the discovery in the Dubno laboratory of a new elementary particle—an antisigma minus hyperon. This discovery was made by a group of research workers under the leadership of V. I. Weksler and Van Gan-Chan. As shown by the particle track photograph this particle was produced through the collision of a pi-meson with the nucleus of a carbon atom.

French Scientific and Technical Books 1950–60

A COLLECTED catalogue of French scientific and technical books 1950–60, with an introduction by P. Piganiol, comprises a selection of books at present on sale and published between 1950 and 1960 (*Catalogue Collectif des livres Français de Sciences et Techniques*, 1950–1960. Pp. viii+242. Paris: Cercle de la Librairie, 1961). The books are classified by subject in five main sections under science, and eleven under technology, and alphabetically by title within the sub-sections. There are alphabetical subject and author indexes.

The Society for General Microbiology

THE Society for General Microbiology held its annual general meeting on April 10, at the Royal Institution. For the forthcoming year the following officers were elected: *President*, Prof. R. Lovell; *Treasurer*, Prof. K. E. Cooper; *Editors*, Prof. B. C. J. G. Knight and Mr. A. F. B. Standfast; *Meetings Secretary*, Dr. B. W. Lacey; *General Secretary*, Dr. S. T. Cowan. The Marjory Stephenson Memorial Lecture, "The Development of Microbiology", was given by Prof. B. C. J. G. Knight, and a symposium on the Microbial Reaction to Environment was held on April 11 and 12. The subject of the Society's symposium in 1962 will be "Microbial Classification".

Electron Microscopy

THE Electron Microscopy Group of the Institute of Physics and the Physical Society is holding its annual conference at the University of Nottingham during July 10–14. The conference will be concerned mainly with the physics of the electron microscope and its applications. Among the main topics to be discussed are: "Electron Microscopes and Electron Optics"; "Electron Microscopy of Thin Films"; "The Theory and Scope of Electron Diffraction Methods"; "Miscellaneous Techniques and Applications of Electron Microscopy". The conference will also include a symposium on "The Physics of Sectioning and the Embedding Process". Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

Seminars on Nuclear Science

THE European Nuclear Energy Agency of the Organization for European Economic Co-operation has arranged with the Atomic Energy Research Establishment at Harwell and the Centre d'Etudes Nucléaires at Saclay for two international seminars on nuclear energy. Designed for the teaching staffs of universities and higher technical colleges, each seminar will accept about fifty applicants, who will have the opportunity to study the latest advances in nuclear science and techniques, to do certain practical work, and to visit nuclear installations. The Harwell seminar will take place during July 10–21, and that at Saclay, June 26–July 7. The costs of both seminars are being borne by the European Nuclear Energy Agency, and participants will only be required to pay travelling and living expenses. Further information can be obtained from the Organization for European Economic Co-operation, European Nuclear Energy Agency, 38 Boulevard Suchet, Paris 16*e*.

Announcements

PROF. K. E. BULLEN, professor of applied mathematics in the University of Sydney, has been awarded the William Bowie Medal for Unselfish Co-operation in Research by the American Geophysical Union, in recognition of his contributions to knowledge of the interior of the Earth.

DR. K. J. IRVINE is to succeed Mr. W. E. Bardgett, at present research manager for research and development of the United Steel Companies, Ltd., when the latter retires on July 1. Dr. Irvine will take the title of metallurgical research manager.

THE Institute of Fuel is organizing a second conference on "Waste Heat Recovery", which will be held in Bournemouth during May 15–19. Further information can be obtained from the Institute of Fuel, 18 Devonshire Street, Portland Place, London, W.1.

THE theme of the 1961 convention of the British Institute of Radio Engineers is to be "Radio Techniques and Space Research". The Convention is being held in the University of Oxford during July 5–8. Further information can be obtained from the General Secretary, British Institution of Radio Engineers, 9 Bedford Square, London, W.C.1.

THE Royal Meteorological Society is holding a two-day meeting at the University of Exeter during July 12–14. The meeting will include symposia on "Winds and Circulation in the Upper Atmosphere" and "Medium-scale Diffusion in the Atmosphere". Further information can be obtained from the Assistant Secretary, Royal Meteorological Society, 49 Cromwell Road, London, S.W.7.

THE fifth international conference on "Ionization Phenomena in Gases", sponsored by the Association of the German Physical Societies (Plasma and Discharge Physics Group), will be held at Munich during August 28–September 1. Further information can be obtained from Prof. K. H. Höcker, Technische Hochschule Stuttgart, Herdweg 51, Stuttgart.

THE 1961 biennial conference of the Oil and Colour Chemists' Association is to be held at Torquay during May 30–June 3. The general title of the conference will be "Physics in Surface Coatings". Further information can be obtained from the General Secretary, Oil and Colour Chemists' Association, Wax Chandlers' Hall, Gresham Street, London, E.C.2.

ERRATA. IN the note entitled "The Geological Society of London", appearing on p. 492 of May 6 issue of *Nature*, the final sentence refers to newly elected members of the Society's Council. Furthermore, Dr. P. A. Sabine, one of the newly elected secretaries, is the chief petrographer, H.M. Geological Survey.

THE SCALE OF THE SOLAR SYSTEM

By THE STAFF, MILLSTONE RADAR OBSERVATORY, LINCOLN LABORATORY*,
MASSACHUSETTS INSTITUTE OF TECHNOLOGY,
WESTFORD, MASSACHUSETTS

THE close approach of Venus during April 1961 has permitted a re-determination of the scale of the solar system by means of radar distance measurements of this planet. Previously, measurements of this kind have been made by Price *et al.*[1] and Evans and Taylor[2]. These earlier experiments led to a value for the solar parallax of $\pi = 8{\cdot}8022 \pm 0{\cdot}0001$ sec. of arc. This is not in agreement with new measurements made using the Millstone Radar Observatory of the Massachusetts Institute of Technology, Lincoln Laboratory, located at Westford, Massachusetts, in March and April 1961.

In all the experiments the transmitter is operated for a period somewhat longer than the two-way travel time. Following the transmission interval (usually about 5 min.) the received energy is integrated at different range-intervals for a duration corresponding to the echo time. In this way most possible sources of spurious echoes are removed. Allowance is made in the summation process for the gradual drifting of range with time. A display of the results of thirty such 'runs' for April 8 is shown in Fig. 1. Included in the processing is a calibration pulse, chosen to approximate the expected intensity of the Venus echo. The Venus echo may be seen to rise some 23 standard deviations above the mean noise-level. The echo intensity corresponds to a received power-level of 163 db. below 1 mW.

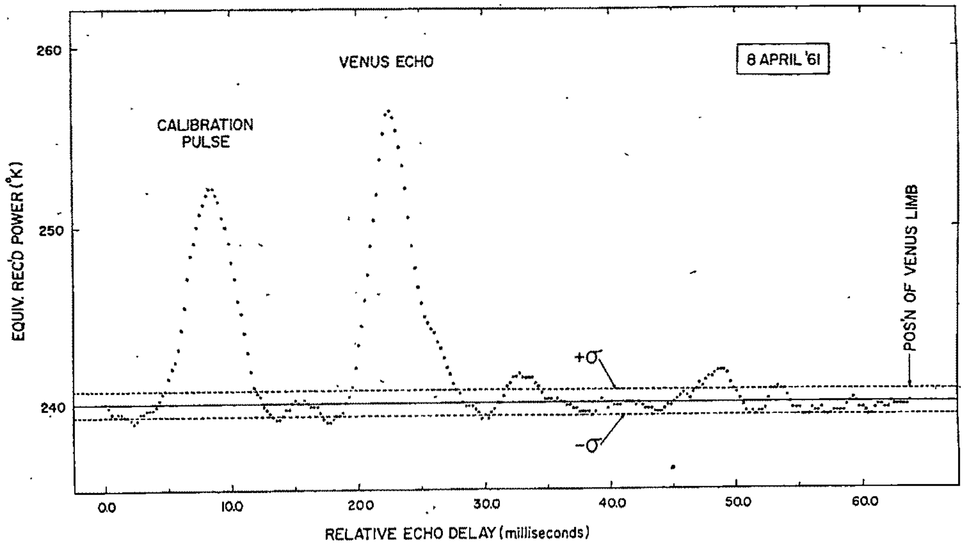

Fig. 1

This equipment operates with the following parameters: frequency, 440 Mc./s.; aerial gain, 37·5 db.; peak transmitter power, 2·5 MW.; receiver noise temperature, 240° K.; transmitter pulse-lengths, 0·5, 2, or 4 msec. The transmitter interpulse period has been varied at approximately daily intervals during the course of experiments which began on March 6. In this way the uncertainty in the total number of interpulse periods in the time for the pulse to travel the two-way distance to and from Venus can be removed. The value for the range of the planet has been converted to a value for the solar parallax by using an ephemeris computed in a manner described by Pettengill and Price[3]. The preliminary value obtained from twenty independent experiments conducted during the period March 6–April 12 is:

$$\pi = 8{\cdot}79450 \pm 0{\cdot}00008 \text{ sec. of arc}$$

which corresponds to a mean solar distance of 149,597,700 ± 1,500 km. The observations are being continued and it is expected that a final analysis of all the measurements will yield a value substantially more accurate than the new value reported here.

From the intensity of the echo we conclude that Venus scatters about 12 per cent of the incident energy. This is not greatly different from the amount scattered by the Moon (7 per cent, ref. 4) and suggests that Venus has a solid surface.

A frequency analysis of the returned signal power discloses very little spectral broadening. Two 4-min. runs analysed to date show that essentially all the returned power is contained within a band-width of less than one cycle per sec. Until some measure of the angular scattering law is obtained it is impossible to determine the planetary rotation. It is interesting, to note, however, that if one assumes an angular distribution similar to the Moon, an apparent rotational period consistent with the synodic period (584 days) is obtained.

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* Operated with support from the U.S. Air Force.

[1] Price, R., Green, P. E., Goblick, T. J., Kingston, R. H., Kraft, jun., L. G., Pettengill, G. H., Silver, R., and Smith, W. B., *Science*, **129**, 751 (1959).

[2] Evans, J. V., and Taylor, G. N., *Nature*, **184**, 1358 (1959).

[3] Pettengill, G. H., and Price, R., *Planetary and Space Science*, **5**, 70 (1961).

[4] Fricker, S. J., Ingalls, R. P., Mason, W. C., Stone, M. L., and Swift, D. W., *J. Research* (*Nat. Bur. Stand.*), **64** D, 455 (1960).

FUNGICIDES IN AGRICULTURE AND HORTICULTURE

FROM time to time estimates are published of the enormous annual loss of crops throughout the world due to pests and diseases. A considerable proportion of these losses, equal to the produce of millions of acres of agricultural land, arises from fungal diseases, and in most cases the use of fungicides provides the most effective remedy. In recent years there has been good progress in the development of new fungicides; crop losses are still great, however, even in Britain, where potato blight, for example, still destroys thousands of tons of potatoes each year. Meetings concerned with research on fungicides do not occur frequently in Britain and considerable importance was therefore attached to the symposium entitled "Fungicides in Agriculture and Horticulture" organized by the Pesticides Group of the Society of Chemical Industry and held in London during March 20–21.

The programme was well balanced to include treatment of fundamental as well as practical aspects of the subject. The three papers delivered on the first morning under the general heading of "Current Usage and Recent Developments" and dealing with fruit, glasshouse crops and agriculture respectively were mainly practical. It is well known that organic fungicides have been used increasingly in crop protection in recent years, but it was interesting to hear from these three speakers that inorganic materials such as elemental sulphur, lime-sulphur and Bordeaux mixture still hold a very important position.

Dr. A. H. M. Kirby dealt briefly with soft and stone fruits and more fully with pome fruits. Scab has been the disease requiring most attention on apple and pear, but of all the chemicals used for its control none is without some defect. For example, captan, which gives excellent control, allows mildew to increase—this, incidentally, is now the most important disease for which a safe, cheap and adequate control is still required. The dangers of phytotoxicity and mammalian toxicity with some of the materials in current use were emphasized, and a particular point made was that the harmonizing of biological and chemical control of pests and diseases is worthy of greater attention in spray programmes.

In glasshouse crops, as W. H. Read pointed out, a system of monoculture is usually practised and the methods and costs of partial soil-sterilization to combat the soil-borne diseases encouraged by this system were given. The disadvantage with chemical soil sterilants is the long period required for the chemical or its breakdown products to disappear from the soil.

Since most glasshouse crops are susceptible to phytotoxicity and mechanical damage, there is usually no definite spray programme in glasshouses, the policy being to wait until disease needs to be controlled. The uses of sprays, aerosols, smokes and vaporization were discussed as methods of dispersing the chemicals employed to control a number of important diseases of glasshouse crops.

Unlike the previous speakers, Dr. F. C. Gayner was not restricted to considering the use of fungicides in Britain. Under the title "Fungicide Usage in World Agriculture" he gave the amount and value of sulphur, copper, mercury and organic fungicides used throughout the world during 1958. Although 12,000 tons of organic fungicides and 300 tons of mercury were used in that year, 275,000 tons of sulphur, used mostly as a dust, and 56,000 tons of copper were sold, the latter mainly as copper sulphate for the manufacture of Bordeaux mixture. Most of this interesting paper was concerned with the use of fungicides to control the eight major diseases on crops of world importance.

The second session of the symposium comprised three fundamental papers on "Theoretical Bases for Fungicides". The first of these, prepared by Dr. A. H. McIntosh, was read by Dr. C. Potter; but in the absence of the author it was inevitable that the discussion suffered some limitations. The results were given for the fungistatic action of nineteen chemicals, some of them well-known fungicides, against *Botrytis fabae* at temperatures between 10° and 25° C. and at intervals of up to 14 days. In general, an increase in temperature was shown to increase the toxicity and speed of action, whereas the apparent toxicity at any one temperature decreased as time passed. It emerged that there is little evidence for the generally held belief that spores are more resistant to fungicides at their optimum temperature for germination and also that the results may be misleading if spore germination tests are assessed before the ED_{50} has been allowed time to reach a steady value. An important point was made here concerning the loss of fungicides by sorption on the glassware used in spore-germination tests. This is particularly true of heavy-metal salts and, in one case cited, using mercuric chloride, some 70 per cent of the original amount was lost from solution in 24 hr.

Physical measurements and biological behaviour also formed an integral part of the succeeding paper by Dr. M. J. Janssen of Utrecht. Work on fungicides in the Netherlands has long been in the forefront of world research, and in recent years a series of papers dealing with the dithiocarbamate fungicides has been published by the team led by Prof. G. J. M. van der Kerk. Dr. Janssen's contribution was concerned with the mode of action of the dithiocarbamates, and he divided the work on this subject into three phases, dealing mainly with the most recent work relating to metal complex formation. He showed that the dithiocarbamates act in much the same way as 8-hydroxyquinoline, for which the importance of metal chelation has been demonstrated by Albert. A quantitative agreement exists between toxicity and concentration of the dithiocarbamate ion itself and its complexes with copper. Thus the different growth pattern in several fungi can be ascribed to their sensitivity not only to the dithiocarbamate ion in the medium but also to the complexes in which one or two dithiocarbamate ions are attached to the bivalent copper ion. Reference was also made in this paper to the current fashion of postulating metal-binding as responsible for the observed phenomena in other biological systems. In the discussion, there was general support for Dr. Janssen's opinion that the theory that chelation is of basic importance in relation to the activity of plant growth-regulating substances is unwarranted.

Dr. E. Somers pointed out that in terms of weight of chemical removed from solution by fungal spores, the fungicides in current use possess low toxicity compared with animal poisons, insecticides or even bactericides. He felt that the common method of determining uptake of fungicide by measuring the amount remaining in solution after fungal spores have been removed from it gives little information on the actual amount which has penetrated the spores. Using captan and a series of ring-substituted derivatives no correlation could be established between lipid solubility on one hand, and uptake or fungitoxicity, in terms of the ED_{50}, on the other. It was shown that thiol-groups within the cell are concerned in the action of captan ; furthermore, the chemical appears to be taken up only by living cells. This is quite different from the results obtained with radioactive copper, the uptake of which was found to be non-specific. Thus, any part of the fungal cell, whether living or dead, will apparently remove copper-64 from solution ; that which accumulates on the outside of the cell therefore probably plays no part in the fungicidal activity of this metal.

The first paper on the second day of the symposium, given by Prof. G. J. M. van der Kerk of Utrecht, was concerned with new developments in organic fungicides. An authority in this field, Prof. van der Kerk first described how fungicide research in Holland is co-ordinated between several institutions. He then went on to forecast that another general group of organic fungicides superior to the dithiocarbamates on grounds of activity, simplicity of molecular structure and cost is unlikely to be found. He considered it more likely that in the future specific materials will be developed to control specific diseases.

In the field of protectant fungicides, the organo-tin compounds have recently been introduced commercially as a result of research which started in 1950. Triphenyltin acetate is already marketed by Germany for use on a wide range of crops, but its phytotoxicity and mammalian toxicity require special attention. The triphenyltin sulphides, on the other hand, are much less toxic towards higher plants and animals but their antifungal spectra also show more specificity.

In the search for systemic fungicides progress has been made along three main lines: by using chemicals which are translocated within the plant and are themselves fungitoxic; by using chemicals which are broken down by the plant or the pathogen to form systemically distributed fungicides; and by using compounds which are neither fungicidal *per se* nor give rise to fungicidal degradation products but interfere with the host's metabolism and render it resistant to attack. Prof. van der Kerk reported on the progress being made in Holland along all three of these lines. Procaine (diethylaminoethylester of *para*-aminobenzoic acid) shows promise as a systemic fungicide *per se*, but none of its derivatives is of interest and it may be significant that procaine has so far been shown to be active only against powdery mildew on cucumber. This disease again showed itself to be useful for the demonstration of systemic fungicidal activity with sodium dimethyldithiocarbamate. This compound, which is an active plant-growth regulator, is degraded within the bean plant and, by means of an elegant bioassay technique involving *Glomerella cingulata*, can be shown chromatographically to form at least three antifungal derivatives. Resistance to *Cladosporium cucumerinum* has been increased by providing cucumber plants with enantiomorphs of naturally occurring amino-acids; likewise, thiourea and phenylthiourea, which are inactive *in vitro*, have been found to confer resistance against certain diseases when provided to plants through their roots.

The activity of fungicides in relation to their chemical structure was considered by several speakers in relation to the particular compounds with which they were interested. Dr. D. Woodcock, in his contribution, dealt specifically with structure-activity correlationships of some nitrogenous antifungal chemicals. The importance of physical properties such as lipophilic character was discussed in relation to mode of action. Chelation was also considered, in particular with regard to its influence on the fungitoxicity of oxine (8-hydroxyquinoline) and related compounds. Other compounds dealt with by Dr. Woodcock included imidazolines, guanidines and triazines.

Prof. R. L. Wain and his colleagues at Wye College have been closely associated with research on systemic fungicides for some years and Prof. Wain gave an account of the progress made in this field. He directed attention to the important fact that most plants possess a wide degree of resistance to fungal attack, and pointed out that in some cases this resistance may depend on the possession by the plant of anti-fungal chemicals within its tissues. The observation that the tissues of *Vicia faba* were fungitoxic provided an opportunity of investigating this system from the point of view of "natural systemic fungicides". The isolation of the anti-fungal compounds present in *Vicia faba* has, in fact, been in progress at Wye for some time. Early in these investigations it had to be shown that the observed effect was due to the presence of fungitoxic chemicals and that the broad bean was the most suitable subject for the investigation. Details of the biological work leading to chemical extraction of the fungal inhibitors and the techniques developed for dealing with large numbers of fractions obtained from the extracts were given by Dr. D. M. Spencer. Dr. C. H. Fawcett then described the successful extraction of the most potent two chemicals : one a keto-yne and the other a sulphur-containing acid with certain properties similar to those of a nucleotide. While somewhat unstable, there is no doubt that these materials are highly fungitoxic and their presence in *Vicia faba* may well prove to be of considerable significance.

The last two contributions dealt with two new fungicides now in use. D. J. Higgons described the research which led to the field trials and ultimate commercial application of 2,6-dichloro-4-nitroaniline, particularly against *Botrytis cinerea* on lettuce, and Dr. W. F. Jepson dealt with the orchard use against scab diseases and the possible mode of action of *n*-dodecylguanidine acetate.

The contributions to this symposium ranged widely within the field of fungicides. The practical aspects of their use were discussed, theories on mode of action were described, research being carried out towards the development of new fungicides was reported and details were given of two new materials now on the market. Perhaps part of its undoubted success can be ascribed to the comparative rarity of meetings such as this and it is to be hoped that others will follow at suitable intervals. The organizers of the symposium are to be congratulated on their choice of subject, and the participants—indeed all those interested in fungicides—look forward to the early publication of the monograph containing the contributions and discussions. D. M. SPENCER

THE NATIONAL PARKS COMMISSION

THE eleventh annual report of the National Parks Commission*, which covers the year ended September 30, 1960, again records disappointment at the non-introduction in Parliament of any Bill to give effect to the amendments to the Act which the Park Planning Authorities and the Commission recommended to the Minister. This was in spite of the fact that at a Conference of Park Planning Authorities at Windermere in May, the Permanent Secretary to the Minister stressed the importance of having suggestions ready in case they were wanted at short notice. Within the limits of its powers, the Commission records a year of quiet progress, particularly in the designation of areas of outstanding natural beauty, for which the Minister has now confirmed 12 orders covering 1,700 square miles, and several local planning authorities have taken advantage of the special powers provided by the Act for actions aimed at preserving and enhancing the natural beauty of these areas. After informal discussions with the chief bodies concerned the Commission is now considering what advice to give to the Government regarding the Broads in the light of the recommendations of the Bowes Committee of inquiry into inland waterways.

Much of the report consists of notes on progress and activities in the individual Parks, which it is impossible to summarize here, but with the excellent illustrations which continue to characterize the report, the Commission's annual report is itself a first-class piece of publicity, and one encouraging general note struck in the report is that it has at last been able to expand such activities and to appoint a small additional staff for the purpose. A conference of park authorities on the subject was arranged in July 1960, at which one topic discussed was the provision of information centres in all the National Parks. This has been limited hitherto primarily by finance which the 1949 Act failed to provide. The Commission regards such information centres as of the utmost importance and has proposed to the Ministry of Housing and Local Government an increased appropriation for the purpose.

A disappointing feature of the Commission's work stressed in the report is the lack of progress with long-distance routes. Although all but one of seven proposals for such routes submitted to the Minister have been approved, after ten years work not a single route has yet been completed on the ground, and there are still major difficulties in regard to the earliest and longest—the Pennine Way. Apart from this, development questions figure largely in the preoccupations of the Commission, the question of electricity distribution lines underground being again prominent. Strong exception is taken to the attitude of the South Wales Electricity Board to a request by the Pembrokeshire County Council for a small amount of underground cable in the vicinity of Whitesands Bay, St. David's, as quite at variance with the assurances given by Lord Mills when Minister of Power in a debate on the Electricity Bill, 1957. Moreover, in spite of further assurances given on the amenity clause in the Act in a debate in the House of Lords, in February, the Commission, while noting the Government's claim in the debate that all officers of the Electricity Boards are fully alive to the provisions of Section 37 of the Act, suggests that their appreciation of its implications could sometimes be improved.

Like difficulties have arisen in electricity supply to Borrowdale and to Martindale, and while regretting the existence of the Ballistic Missile Early Warning Station at Fylingdales in the North York Moors National Park, the Commission records its thanks to the Air Ministry for collaboration in an attempt to minimize the impact on the scenery. Protests was also made against the proposal, now abandoned, to rebuild Princetown Prison in the Dartmoor National Park and the Peak Park Planning Board was supported in its opposition to the proposal of the Sheffield Corporation to construct a refuse tip at Ringinglow, a proposal finally rejected by the Minister on appeal. Much anxiety is also expressed about the multiplicity of proposals for the erection of aerial masts on prominent sites, and despite the existence of Government arrangements, under which all proposals for new masts must be cleared centrally, and anyone proposing a separate site must show strong reasons for not conforming, the Commission continues to watch the position with some concern, although permission to erect a police mast of 150 ft. on Foel Eryr in Mynydd Preseli has recently been refused by the Minister.

* National Parks and Access to the Countryside Act, 1949. Eleventh Report of the National Parks Commission for the year ended September 30, 1960. Pp. vi+71+8 plates. (London: H.M.S.O., 1961.) 6s. net.

THE WORLD HEALTH ORGANIZATION

THE outstanding activity of the World Health Organization in 1960 was the assistance given to enable the Republic of the Congo (Leopoldville) to meet the emergency health situation in which it found itself immediately after attaining independence. This is described in the annual report of the Director-General for 1960*.

Acting on a request from the Secretary-General of the United Nations for assistance with the rapidly deteriorating health conditions in the Congo, the Organization accepted the responsibility of co-ordinating, through the Ministry of Health, the activities of all international personnel concerned with the health of the civilian population. The report outlines the action taken by the World Health Organization during July, August and September, with the assistance of medical teams provided by national Red Cross societies and certain Governments.

The programme for the eradication of malaria from the world is reported as having made significant progress in various fields, although handicapped by a persistent shortage of funds. Sixty-one countries

* Official Records of the World Health Organization, No. 105: the Work of WHO, 1960—Annual Report of the Director-General to the World Health Assembly and to the United Nations. Pp. x+224. (Geneva: World Health Organization; London: H.M.S.O., 1961.) 6 Swiss francs; 10s.; 2 dollars.

are now fully engaged in this programme; in another nineteen, planning has reached an advanced stage, and, in seventeen other areas—mainly in Africa—the Organization is assisting with preliminary work. Some idea of the complexity and scope of these campaigns is conveyed by the variety of the Organization's work on malaria as reflected in this report—a wide range of research, assistance with training, planning and organizing campaigns and, as the programmes advance, with evaluating and assessing their efficacy and results.

The World Health Organization's assistance to Governments for the control of yaws, smallpox and tuberculosis, leprosy and various diseases increased significantly during the year. Medical research was intensified on practically all communicable diseases, the main co-ordinated effort being centred on virus diseases and on bilharziasis.

The year 1960 was the first year of full implementation of the intensified programme of medical research. Although the programme is concerned mostly with communicable diseases, work has begun on cancer, cardiovascular diseases, radiation medicine and human genetics. It is made clear in the report that the Organization's role is primarily to provide services to research and to co-ordinate the work being done in national centres. "It is, I believe", writes the Director-General in his introduction, "a gratifying fact that much of the medical research supported by WHO has grown out of the Organization's current field programme and is being carried out by national institutes and national staffs".

For the World Health Organization, as for the United Nations, the emergence of new independent States brought a marked increase in membership. In planning for the future, account has been taken of the particular needs of these newer countries, so as to ensure the most effective use of their often limited resources in the progressive development of balanced health programmes.

In most countries essential health work is being handicapped by shortage of trained professional and auxiliary personnel, and much of the Organization's work is concerned with education and training in one form or another. Under the Organization's fellowships programme, more than 1,000 fellowships were provided in the period covered by the report. In addition to details of the fellowships awarded for both group training and individual studies, the report gives information on the other aspects of the World Health Organization's work to further the training of professional and auxiliary health workers: courses, study tours, seminars and a variety of other meetings were organized, and experts were provided to serve as visiting teachers or as members of demonstrations and training teams. For example, in the first nine months of 1960, the Organization sent more than 160 nurses to help with nursing and midwifery training in forty-five countries.

In the field of environmental sanitation, emphasis was laid on the training of sanitary engineers and sanitarians, and on providing safe water-supply and waste disposal. In certain regions—Europe, for example—work was concentrated on river, lake and sea pollution and other problems resulting from the rapid growth of cities, such as air pollution. The growing use of atomic energy in power production, and of ionizing radiation in industry and medicine has given rise to several problems, and many Governments are becoming increasingly aware of their responsibilities in radiation health. The Organization's programme is orientated mainly towards assisting health authorities to strengthen their competence in radiation questions in order to meet these responsibilities.

MEASURING INDIGESTION

INVESTIGATORS in the food research group and analytical chemistry laboratories of the Stanford Research Institute have developed a method to measure the degree of digestibility of any food for a particular individual. Basically, one reason for the discomfort caused by certain foods is related to the bacteria flora that inhabit the intestines of all mammals. When a person eats a food he can digest only partially it is attacked, when it reaches the lower part of the small intestine, by organisms which digest the remaining material. These bacteria form hydrogen gas as a by-product in the process, and resulting discomfort depends on the amount of gas; this, in turn, depends on the constitution of the flora of that individual and the quantity of undigested food.

The project team reasoned that if gas pressure is present, the hydrogen should pass through the semi-permeable intestine wall into the blood stream. Because hydrogen is inert and therefore unchanged by body chemistry, the gas should eventually pass into the lungs and be expelled in the breath. They modified a gas chromatograph so that, if a bag is blown up by the subject, the hydrogen in the breath can be measured. By making the test after feedings of separate foods, they could determine what foods and how much are causing indigestion.

Some aspects of the investigation were sponsored by the U.S. Department of Agriculture and the Idaho Bean Commission. A unique application of the method was utilized by the latter organization and is outlined in a recent issue of *Research* (13, No. 1; January–February 1961). Beans in many individuals produce hydrogen and methane, which also are detectable by chromatography. A chemical and leaching process was developed by the food research group to make beans more generally digestible and greatly reduce gas formation, while not altering their taste. The breath-analysis technique was a useful tool in proving the bean treatment.

The individual digestive idiosyncrasies of animals, which cannot announce the fact that they have 'heartburn', may be detected by the chromatograph method. Various medical applications have also been suggested. For example, because intestinal bacteria populations increase rapidly by feeding on foods undigested in the stomach, the chances for future digestion problems are increased. With individual analysis, it may become possible to control the number of undesirable bacteria through selection of easily digested foods which will 'starve them out'.

A RADIO WAVE MECHANISM TO ACCOUNT FOR THE KNOWN DISTRIBUTION OF VAN ALLEN BELTS ABOUT THE EARTH

By JOSEPH M. BOYER

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A NUMBER of recent theories[1,2] related to the entrapment of charged particles in the Van Allen belts about the Earth appeal solely to the magnetostatic field of the Earth or Sun for the 'bottle' or trap mechanism. It may be of some value, conversely, to ascertain if the Van Allen belt phenomenon can be predicted in some detail by a simple theory which deliberately omits any recourse to the magnetostatic flux of the Sun or Earth as a charge-trapping means.

Due to the work of Mie[3] and Debye[4] it may be shown that upon the closed surface of a spherical body of matter a system of resonant modes of current will be established by illumination of the body by incident electromagnetic waves vibrating at frequencies within a certain portion of the spectrum called the 'critical band'. Excitation of such resonant surface current modes on the spherical body will, in turn, produce maxima in the scattered electromagnetic fields in the spherical directions φ, θ, of the space surrounding the body; such field distributions will differ from those established by the same body when illuminated by waves having vibrational frequencies lying outside the 'critical band' spectrum region. Here, the electrical constant of the matter comprising the spherical body is denoted k_1, and the electrical constant k_2 of the immersion medium about the body is first assumed to be that of a vacuum. We have: $k_1e = e_1/e_0$; $k_1\mu = \mu_1/\mu_0$; $k_2e = e_0 = 8{\cdot}854 \times 10^{-12}$ farads/metre (M.K.S.) ; $k_2\mu = \mu_0 = 1{\cdot}257 \times 10^{-6}$ henries/metre (M.K.S.) ; and k_1/k_2 is other than unity in terms of the critical band frequency spectrum considered.

Now, let the medium k_2 contain a continuous spectrum of electromagnetic energy emitted by a source so distant that wave-fronts are essentially plane in the vicinity of the sphere. By the spherical vector wave-functions, the resultant electromagnetic field will be:

$$\overline{E} = -\sum_n (a_n\overline{M}_n + b_n\overline{N}_n), \quad \overline{H} = -\sum_n \frac{k}{i\omega\mu}(a_n\overline{N}_n\overline{M}_n) \quad (1)$$

The particular solution of equation (1) for the sphere is well known; therefore the reader is referred to the excellent treatment of the series by J. A. Stratton[5] for details. By means of equation (1) we can secure the relationship of wave-length λ to sphere radius a for the first three significant resonance modes. These are:

No. 1. $\alpha = 2\pi/\lambda_a = 1{\cdot}0$
$\lambda = 6{\cdot}28a$
No. 2. $\alpha = 2\pi/\lambda_a = 2{\cdot}30$
$\lambda = 2{\cdot}73a$
No. 3. $\alpha = 2\pi/\lambda_a = 3{\cdot}45$
$\lambda = 1{\cdot}82a$

using the wave-number $2\pi/\lambda$ in standard notation.

Permit the Earth, stripped of its atmosphere, to be considered the spherical matter zone immersed in space. A line x is drawn from the centre of the Earth along the ecliptic plane to terminate at a point o. The length of the line x is $149{\cdot}5 \times 10^6$ km. The source of incident waves is located at point o. For the Earth sphere with a mean radius equal to 6,371·23 km., the first three resonant modes may now be ascertained:

No. 1. $\lambda = 40{\cdot}01 \times 10^6$ m. = 6·28 Earth radii. Frequency = 7·49 cycles/sec.

No. 2. $\lambda = 17{\cdot}39 \times 10^6$ m. = 2·73 Earth radii. Frequency = 17·25 cycles/sec.

No. 3. $\lambda = 11{\cdot}59 \times 10^6$ m. = 1·82 Earth radii. Frequency = 25·88 cycles/sec.

Effects which take place along the line x as the result of electromagnetic resonances of the illuminated Earth are of first interest. For the case of Mie scattering of the Earth at modes 1, 2 and 3, we can determine the magnitude of the secondary field established in the direction of o, compared with a fictitious isotropic scattering body which maintains a constant field $\overline{E^s}$ in all spherical directions φ, θ, when radiating a total power $\overline{P^s}$ isotropic. The scatter field magnitude in the direction o is called backscatter[6] cross-section. Fig. 1 shows the backscatter cross-section σ of a highly conducting sphere (solid line) and that of a water sphere (dotted line) normalized with respect to πa^2.

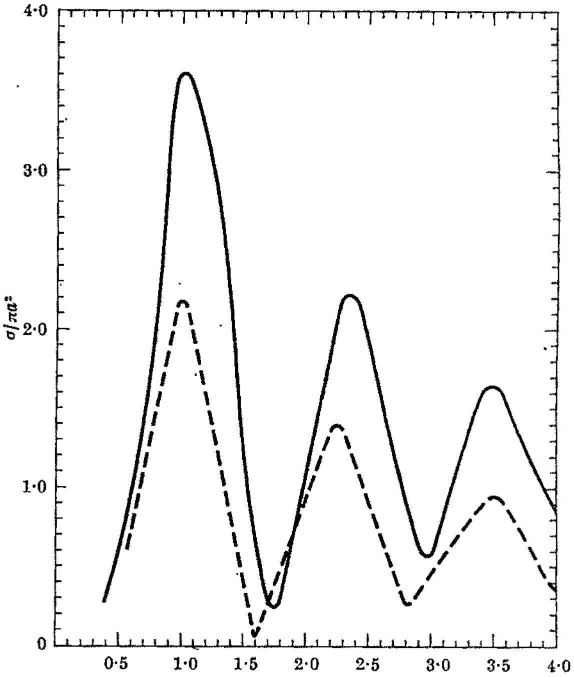

Fig. 1. Backscattering from spheres (from Aden)

a, radius of sphere; $\alpha = Ka = \frac{2\pi}{\lambda} a$

———, Metal sphere; — — —, water sphere

It will be noted that the backscatter cross-section of a sphere illuminated with energy vibrating at frequencies within a critical bandwidth, approximately $\alpha = 0{\cdot}8$ to $\alpha = 6{\cdot}0$, differs markedly from the case of either Rayleigh or specular scatter. Within the critical band, it is seen that peaks and minima are evident in the magnitude of the backscatter cross-section. Again, Fig. 1 discloses that although the electrical constant of the matter making up the spherical body may affect the magnitude of backscatter cross-section it does not sensibly disturb the radius/wave-length relationship of the electromagnetic resonances. The energy scattered back in the direction o will establish electromagnetic standing waves[7] along this axis due to phase interference with the incident field. The intensity of the maxima (loops) of such standing waves will be greater for those wave-lengths within the critical band producing resonance conditions with the Earth sphere, although all finite magnitudes of backscatter field from the Earth will introduce some standing wave phenomenon for frequencies within the illumination spectrum. Emphasis is placed, however, on the very lowest frequency resonances as a decelerating mechanism for high-energy particles.

A. L. Aden[8] has demonstrated the existence of electromagnetic backscatter standing waves produced by water and metal spheres in experiments conducted at a wave-length of 16·23 cm. As the scattered field from the Earth is a spherical wave in the vicinity of the planet, the standing wave which results is damped by radiation. Its configuration, for a single frequency, is therefore a damped sinusoidal wave[9] along the line x, the position of the loops and nodes stationary, with an amplitude variation at a period ω. It must be admitted that employment of an Earth stripped of its atmosphere envelope introduces some grounds for objection to the realism of the model offered so far. In this cursory survey of the theory, only an approximation of the true radius of the Earth scattering shell will be made, for it is clearly evident that rigorous treatment will display a frequency selective term for the extremely low-frequency scatter radius, with complicated entrapped wave effects occurring at the high-frequency end of the spectrum.

The Lyman–Spitzer analysis[10] of the planetary atmosphere shell is selected as a model. The Spitzer atmosphere acts like a gas up to about 600 km., with lighter gases predominating in the atmosphere population above about 300 km. Although free electron densities quoted by authorities differ to some degree, sufficient accuracy here will be attained by use of a density curve with peaks at about 300 km.[11], then decreases at higher altitudes. A mean scattering shell altitude of 300 km. will be selected, producing a total sphere radius of 6,671·23 km. The atmosphere 'corrected' Earth will then introduce resonance modes in the illuminating spectrum at the following wave-lengths:

No. 1. $\lambda = 41{\cdot}89 \times 10^6$ m. = 6·57 Earth radii. Frequency = 7·16 cycles/sec.

No. 2. $\lambda = 18{\cdot}21 \times 10^6$ m. = 2·86 Earth radii. Frequency = 16·47 cycles/sec.

No. 3. $\lambda = 12{\cdot}14 \times 10^6$ m. = 1·9 Earth radii. Frequency = 24·71 cycles/sec.

I am confining attention in this article to the electromagnetic standing waves along line x generated by scatter interference only at wave-lengths Nos. 1, 2 and 3. The position radially from the Earth for each standing wave distribution may be computed or taken directly from the Aden measurements given in the reference. There will be no interaction between such individual frequency fields existing along x if we assume initially that the space k_2 surrounding the planet is free of all charges. This pure field or fictitious state will be labelled the particle-free standing wave case. Under this condition, examination of the polarization characteristics of the incident and scattered wave shows that the conditions for maximum interference between the two fields can exist only within a narrow, solid angle cone pointing toward the point o, with the magnitude of the induced standing wave distribution falling off in other radial directions φ, θ.

Now, let the radiant source at o not only generate electromagnetic waves but also high-energy charged particles, some of which approach the Earth along the incident axis x. J. R. Coleman[12] has examined my matter-induced electromagnetic standing-wave theory in terms of the electron capture mechanism involved in the radio-frequency case. L. M. Kovrizhnykh and V. H. Veksler[13] have studied a similar situation involving capture oscillations of both charged and neutral particles in a radio standing-wave type cyclotron. These analyses emphasize that for a fixed loop field strength of an electromagnetic standing wave the force F exerted on the particle is proportional to wave-length. In terms of decelerator action, Coleman notes that even in the case of a 'solar wind' electron moving at a velocity of 10^6 m./sec. capture will occur when such particle is injected into a radio standing wave the fundamental wave-length of which lies between 10×10^6 and 60×10^6 m. with a loop field-strength of 0·17 V./m. Such extreme particle velocity, of course, is not required in the study of the Van Allen belts found near the Earth, but assumes importance in terms of wave-length/field strength requirements for much larger planets such as Jupiter or Saturn, the first-mode resonance wave-length of which is at least 439×10^6 m. Coleman also makes a preliminary extension of the equivalent black-body temperature of the Sun into the Earth critical spectrum band with encouraging results.

Briefly, then, we have a planetary mass under illumination from a radiant source of large size such as a star, inducing electromagnetic standing waves along the incident direction. Here, individual frequency components of such standing waves do not interact field-wise but do act on the injected charges in such a manner that an integrated force obtains, with the longest wave components of the stationary wave producing first-order effects. Particles injected into such standing-wave envelope region will either be captured if the mass/velocity relationship to wave-length/field strength is critical or be deflected as if by an electromagnetic lens and escape. The requirement for a mechanism to establish the radial Van Allen belts observed about the Earth is still not satisfied, however, by this model, for appreciable magnitude 'capture' fields in the form of large intensity standing waves do not exist at other than the incident axis direction.

However, now let an injected particle, say, an electron, be captured by the incident axis electromagnetic standing wave. Once captured, the electron enters into a steady-state condition which I shall term the particle-wave case. Such particle, as

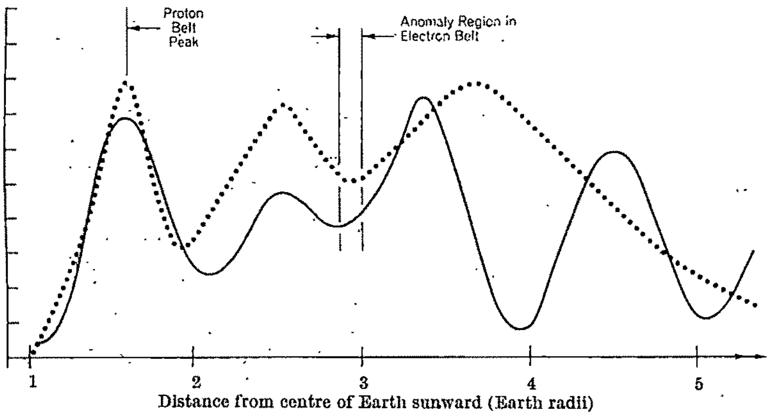

Fig. 2. Distribution of known Van Allen belts compared with calculated wave due to Earth dipole resonance in solar elf electromagnetic illumination
. . . ., Van Allen belt distribution (after Simpson *et al.*); ——, calculated Earth electromagnetic standing wave envelope for modes 1, 2 and 3

Coleman shows, will oscillate along a path concentric with the incident axis x. Studying the Coleman analysis, I realized that by appealing to electromagnetic theory, such an oscillating charged particle becomes a source of current in space. Neglecting the ramifications of the relativistic mass of such oscillating particle in this article, it is clear that the particle becomes a new source of low-frequency radiation in the Earth capture model. Due to the extremely low frequency of the entrapping wave, the particle space current constitutes an extended line source; the Poynting vector of the particle-generated wave must, therefore, lie normal to the incident axis x. Radiation of electromagnetic energy in the direction normal to axis x will introduce increased magnitudes of interference with the sphere scattered wave in a new direction φ, θ displaced off the incident axis. The effect of this interference is to increase the width of the conical standing-wave region pointing to the source. The process is now cumulative in that newly injected particles can be entrapped or else migrate into the widened solid angle zone of standing wave to oscillate along new radial directions. Ultimately, the planet will be surrounded by a set of anular zones of standing waves with loops and nodes distributed radially.

Fig. 2 shows the mathematical average envelope obtained by superimposition of the standing waves resulting solely from resonance modes 1, 2 and 3 of the Earth exosphere scatter model traced as a solid line. The radial distribution of the closest Van Allen belts after Simpson *et al.*[14] is given as a dotted line. It will be seen that a maximum in envelope intensity (loop) exists close to the 1·5 Earth radii position of the known proton belt, and the anomaly in the electron belt at 2·85–3·0 Earth radii is evident without recourse to the Cape Town variation in the Earth magnetostatic field. Beyond 4 Earth radii, the computed field envelope falls off more rapidly than the distribution given by Simpson, with a new maximum produced at about 4·4 Earth radii. It is felt that a model based on the addition of a larger number of critical band frequency components, as well as an extension of the radial distance scale, will fit the known belt distribution more satisfactorily.

Calculations by me of the standing wave distribution in a plane normal to the ecliptic (θ) shows that when the first three resonance modes are superimposed, a deep null or electromagnetic 'pole' occurs at 93 degrees above the radio 'equator' or ecliptic plane of the Earth, permitting deep penetration of particles in the high latitudes.

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OBSERVATION OF FAR-INFRA-RED RADIATION FROM SHOCK WAVES

By M. F. KIMMITT, A. C. PRIOR and P. G. SMITH
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INFRA-RED radiation in the wave-length range 0·1–0·6 mm. has been observed in the emission from a plasma generated by a shock wave in argon. This work follows a successful joint experiment with the Atomic Energy Research Establishment, Harwell, in which the emission in the range of wave-lengths 0·1–1·6 mm. was observed from plasmas produced in *Zeta*. The apparatus used was designed for the latter experiment, the details of which are being published elsewhere[1]. The spectra obtained from the observations on the shock tube described here, as from those on *Zeta*, are characteristic of bremsstrahlung, but show regions where the emission is reduced by the black-body limitation.

Under the conditions of these experiments, and in the wave-length region examined, line and band spectra arising from transitions between atomic energy-levels, or between energy-levels of molecules present as impurities, should be absent or insignificant. The observed emission should therefore be due entirely to free-free electron transitions (bremsstrahlung) which, according to the calculations of Gaunt[2]

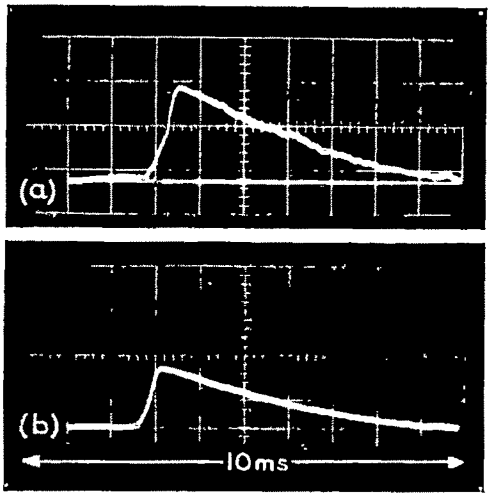

Fig. 1. Oscilloscope traces obtained without spectral resolution. (*a*) Heavily absorbing plasma produced from shock into 1 torr argon. (*b*) Relatively transparent plasma produced from shock into 0·25 torr argon

and Cilliė[3], result in a surface brightness $P_{\text{eff.}}$ given, when $h\nu c \ll kT$, by :

$$P_{\text{eff.}}\ d\nu = 1{\cdot}63 \times 10^{-35}\ Z^2\ g\ T^{-1/2}\ n_e n_i\ d\ d\nu \text{ watts/cm.}^2\text{/steradian/cm.}^{-1} \quad (1)$$

where Z is the ionic charge, T the absolute temperature, n_e and n_i the electron and ion densities, d the plasma thickness, ν the wave number, and g the Gaunt correction factor given by :

$$g = (\sqrt{3}/\pi)\ \ln\ (3kT/2h\nu c)$$

If we take $n_e = n_i = n$, $Z = 1$, and a mean value of 3 for g, these being appropriate to the conditions of the experiment, (1) reduces to :

$$P_{\text{eff.}}\ d\nu = 5 \times 10^{-35}\ n^2\ T^{-1/2}\ d\ d\nu \text{ watts/cm.}^2\text{/steradian/cm.}^{-1} \quad (2)$$

This expression neglects self-absorption, which will prevent the emission from exceeding the black-body value for the appropriate temperature, T; it also neglects plasma resonance effects, and applies only at frequencies sufficiently above that for plasma resonance.

The radiation emerged through a crystal quartz window of diameter 1·5 cm. in the side of the shock tube of 5 cm. diameter, and passed into the spectrometer, which has been briefly described by Roberts[4]; a cone of radiation of half-angle 12° was accepted through the window. The detector was a carbon bolometer similar to that described by Boyle and Rodgers[5], and had a time constant of about 4 msec., the amplified output being displayed on an oscilloscope. The pass band of the spectrometer is normally about 10 per cent of the wave-length to which it is set, but where a greater signal was required in order to examine pulse shapes, the whole spectrum from 0·1 to 1·5 mm. wave-length could be accepted. The energy sensitivity of the system was calibrated by comparison with a black body of known temperature.

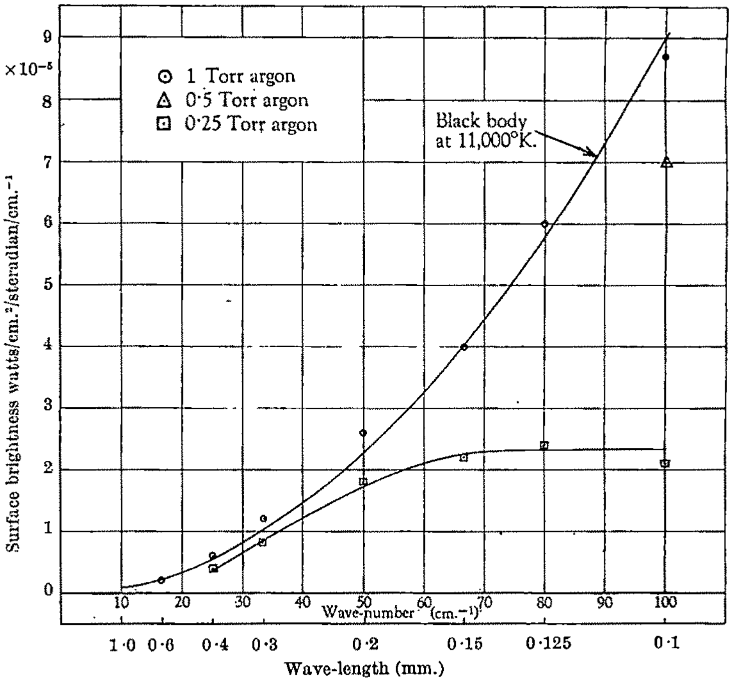

Fig. 2. Observed radiation

The length of heated gas behind the shock front was about 50 cm. as it passed the window, and its velocity about 5×10^6 cm./sec. ; the corresponding transit time of 100 μsec. was much less than the time constant of the detector, which therefore gave an approximately integrated signal. The shock-tube walls are silvered and would be expected to have a reflectivity of 98–99 per cent. For a heavily absorbing plasma, the signal received from the plasma covering the window and filling the spectrometer aperture is preceded and followed by a signal of about half the value, only half the spectrometer aperture being then filled with radiation. This is demonstrated by Fig. 1(*a*), where the steep central portion of the integrated signal is preceded and followed by portions of lower slope. The transit time of the hot gas as deduced from Fig. 1(*a*) agrees with that obtained independently from measurements by resistance thermometer. For a plasma having negligible absorption, and with perfectly reflecting walls, the radiation received should be independent of the position of the shock wave in the tube, since each part of the plasma will fill one or other half of the spectrometer aperture, for all positions of the plasma in the tube. This is demonstrated in Fig. 1(*b*), where no abrupt changes of slope occur but only gradual changes due to imperfect reflectivity.

In principle, the instantaneous radiation intensity can be deduced by measuring the slopes on photographs such as Fig. 1. In practice, the signal was generally too small for this, and instead the peak amplitude was measured and related to the slope by comparison with traces obtained without spectral resolution, where the slopes could be measured ; the results are plotted in Fig. 2. The shock-wave Mach number was measured by means of resistance thermometers spaced either side of the spectrometer aperture at approximately 1 metre apart. At an initial pressure of 1 torr of argon, a Mach number of 15 was measured. From the theoretical calculations of Frood (private communication), these shock-wave conditions should give a temperature of $\sim$ 11,000°K. and an electron density of $\sim 3\times10^{16}$ cm.$^{-3}$, assuming equilibrium to be established in the plasma behind the shock front. For this temperature and density, equation (2) would, for a wave-length of 0·1 mm., give a value of brightness equal to that from a black body if d were put equal to about 1 mm. Thus a black-body spectrum is to be expected, and the experimental points do in fact fit the 11,000°K. black-body curve better than would be anticipated from consideration of likely calibration errors of the spectrometer. Since the available thickness of the plasma, allowing for reflexion from the walls, is $\sim$ 150 cm., any density n greater than $\sim 10^{15}$ cm.$^{-3}$ would be adequate to account for the black-body shape of the spectrum. However, the abrupt changes of gradient in Fig. 1(a) already discussed show that the radiation penetrates only a small portion of the plasma, and this raises the lower density limit to about 2–3×10^{15} cm.$^{-3}$.

For an initial pressure of 0·25 torr of argon, the values of temperature and density calculated from the shock-wave parameters are $T \sim$ 11,000°K. and $n \sim 1\times10^{16}$ cm.$^{-3}$. The approximately flat part of the spectrum indicates that the absorption for the short wave-lengths must be small, as confirmed by the pulse shape (Fig. 1(b)), so that for this region equation (2) should apply; using this with $d = 100$ cm. (shock length $= 35$ cm.) a value of $n \sim 6\times10^{14}$ is obtained. The observations at the longer wave-lengths give good agreement with the calculated temperature. The single observation with an argon pressure of 0·5 torr being slightly below the black-body curve indicates a value of $n \sim 10^{15}$ cm.$^{-3}$ as compared with 2×10^{16} cm.$^{-3}$ deduced from the shock-wave parameters.

All the measurements, both of shock-wave parameters and of radiation, are rather crude time averages for shocks which were probably decaying at a significant rate. Under these conditions, the agreement of the temperature deductions is remarkably good, and the lower densities deduced from the radiation measurements are reasonable, since the calculated densities are for thermal equilibrium, which may well not be reached.

These exploratory measurements have indicated the potential value of far-infra-red spectroscopy for shock-wave plasma diagnostics. Investigations of plasmas with electron densities greater than $\sim 5\times10^{14}$ cm.$^{-3}$, which are beyond the range of standard microwave methods, should be possible, and with a faster detector[6] now being actively developed, much more detailed information should be obtainable than in the present experiments.

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PHOTOPRODUCTION OF HYDROGEN, PHOTOFIXATION OF NITROGEN AND A UNIFIED CONCEPT OF PHOTOSYNTHESIS

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BY fractionating isolated chloroplasts, it has been demonstrated experimentally that the overall process of photosynthesis can be separated into a light and a dark phase[1]. The dark phase includes a series of reactions, driven by adenosine triphosphate and reduced triphosphopyridine nucleotide, that convert carbon dioxide into carbohydrate[1-4]. Since the same series of dark reactions is now known to occur also in non-chlorophyllous cells[5,6], it is appropriate to regard carbon dioxide assimilation as another example of cellular activity which is essentially similar in photosynthetic and non-photosynthetic cells. Consequently, carbon dioxide assimilation must be regarded as lying essentially outside the domain of photosynthesis proper[3]. (This conclusion is based on the fact that grana, as contrasted with whole chloroplasts, cannot assimilate carbon dioxide to the level of carbohydrates but retain a capacity for photochemical oxygen evolution and photosynthetic phosphorylation[41]. Our concept would not exclude a *catalytic* participation of carbon dioxide in the mechanism of oxygen evolution as has recently been proposed by Warburg *et al.*[43] and supported by Stern and Vennesland[44].)

What is peculiar to photosynthesis is its light phase. For green plants this comprises the light reactions that produce adenosine triphosphate and reduced pyridine nucleotide[2,7-9]: cyclic photophosphorylation (equation 1) and non-cyclic photophosphorylation (equation 2). The terms 'cyclic' and 'non-cyclic' denote the respective electron-flow mechanisms[10] that we have postulated to explain the experimental findings which are summarized by reactions 1 and 2.

$$n\cdot\text{ADP} + n\cdot\text{H}_3\text{PO}_4 \xrightarrow{\text{light}} n\cdot\text{ATP} \qquad (1)$$

$$2\,\text{TPN} + 2\,\text{ADP} + 2\,\text{H}_3\text{PO}_4 + 2\,\text{H}_2\text{O} \rightarrow 2\,\text{TPNH}_2 + 2\,\text{ATP} + \text{O}_2 \qquad (2)$$

Cyclic photophosphorylation (reaction 1), a reaction that produces only adenosine triphosphate, has been found in every photosynthetic organism tested so far: higher green plants[11-20], algae[21-22] and photosynthetic bacteria[23-28]. This is in agreement with the view presented elsewhere[10,29,30] that cyclic photophosphorylation (reaction 1) is the common denominator of all photosyntheses in plants and in bacteria. By contrast, non-cyclic photophosphorylation, as represented by reaction 2, has thus far been found only in chloroplasts.

The assimilation of carbon dioxide to the level of carbohydrate requires, in addition to adenosine triphosphate, reduced pyridine nucleotide. In isolated chloroplasts, these joint requirements for assimilation of carbon dioxide are satisfied by a combination of cyclic and non-cyclic photophosphorylation[2] (reactions 1 and 2). In photosynthetic bacteria, we have previously investigated only the case in which the electron donor was hydrogen gas. The contribution of light was then limited to the formation of adenosine triphosphate by cyclic photophosphorylation (reaction 1); reduced pyridine nucleotide was formed in a 'dark' reaction, at the expense of hydrogen gas[29]. We had no experimental evidence previously for a non-cyclic photophosphorylation mechanism in photosynthetic bacteria which would be analogous to non-cyclic photophosphorylation in chloroplasts.

The purpose of this article is to present a unified concept of photosynthesis that includes both cyclic and non-cyclic electron-flow mechanisms in photosynthetic bacteria and green plants. Evidence for the operation of a special non-cyclic electron-flow mechanism (without oxygen evolution) in photosynthetic bacteria was obtained by measuring not only photoreduction of pyridine nucleotides[31,32], but also photoproduction of hydrogen and photofixation of nitrogen gas.

Non-cyclic Electron-flow in Bacteria

(*a*) *Photoproduction of hydrogen gas from thiosulphate.* Photoproduction of hydrogen gas by photosynthetic cells was first observed by Gaffron and Rubin in the green alga *Scenedesmus*[33] and by Gest and Kamen in photosynthetic bacteria[34]. In algae, the photoproduction of hydrogen seemed to depend on internal electron donors of metabolic origin[33], whereas photoproduction of hydrogen by photosynthetic bacteria appeared to depend on the presence of exogenous organic acids and carbon dioxide[34-40]. The hydrogen evolved has been thought to arise from a photodecomposition of water[33,40] or from a decomposition of α-ketoglutaric acid[38].

From the point of view of the electron-flow theory of photosynthesis[10,41], which does not involve a photolysis of water, the photoproduction of hydrogen could be viewed as a reduction of H^+ by a hydrogenase with the aid of electrons expelled from excited chlorophyll molecules[41,42]. In accordance with that theory, electrons expelled from chlorophyll would be replaced by electrons donated by cytochromes, which would then become oxidized. The oxidized cytochromes would in turn become reduced by the external electron donors. The cytochrome system in photosynthetic bacteria would thus be a gateway for the entry of electrons and for their transfer to chlorophyll, where they would be raised, at the expense of the energy of absorbed light, to a potential sufficient to reduce H^+ (cf. refs. 41 and 42).

If this interpretation were correct, then photoproduction of hydrogen by photosynthetic bacteria should occur at the expense of simple, physiologically suitable, inorganic electron donors—a possibility for which there was no experimental evidence[37]. We therefore undertook to investigate the photoproduction of hydrogen from thiosulphate by *Chromatium* cells.

Fig. 1 shows that *Chromatium* cytochromes are reduced by thiosulphate, and can serve, therefore, as a point of entry for electrons from that source. Fig. 2 shows that in the light, but not in the dark, gas was evolved by *Chromatium* cells in the presence of thiosulphate. No carbon dioxide was supplied and potassium hydroxide was present in the centre well of the reaction vessel. Evolution of gas ceased when the light was turned off and was resumed when light was turned on again. The gas evolved was identified as hydrogen by adsorption on palladium asbestos[42]. The photoproduction of hydrogen gas was dependent on the concentration of thiosulphate, and was abolished by heating the cells (Fig. 3). The photoproduction of hydrogen gas approached the stoichiometry of reaction 3, that is, for one molecule of thiosulphate used, two electrons and two protons were combined with the aid of hydrogenase and evolved as hydrogen gas.

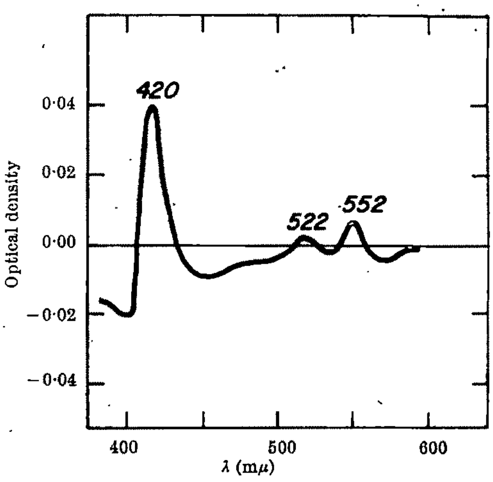


Fig. 1. Reduction of *Chromatium* cytochromes by thiosulphate in a cell-free system. Reaction mixture included, in a final volume of 3·0 ml., 0·1 *M tris* buffer, *p*H 7·8; chromatophores containing 0·1 mgm. bacteriochlorophyll and supernatant fluid corresponding to 0·3 mgm. bacteriochlorophyll. 20 μmoles of thiosulphate were added to one of a pair of Thunberg-type cuvettes and the resulting difference spectrum was measured in a Cary spectrophotometer after 20 min. at room temperature. Gas phase, argon.

The evolution of hydrogen by illuminated *Chromatium* cells showed a marked dependence on *p*H (ref.

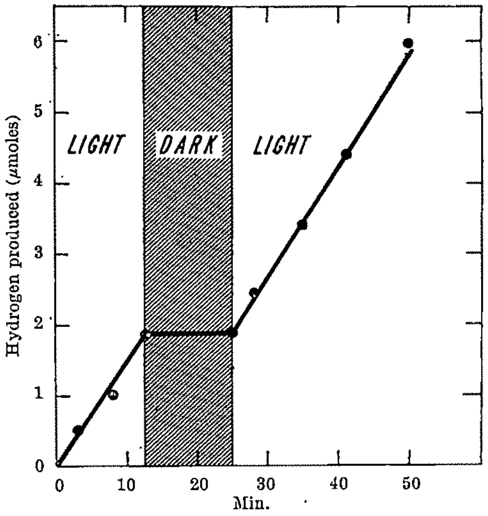


Fig. 2. Light-dependent evolution of hydrogen gas from thiosulphate by *Chromatium* cells. The reaction mixture included 0·1 gm. of washed cells, suspended in 2·6 ml. of a modified nutrient solution from which nitrogen compounds were omitted, 0·3 ml. of 0·5 *M tris* buffer, *p*H 7·2, and 0·1 ml. of 0·2 *M* sodium thiosulphate. 0·1 ml. of 20 per cent potassium hydroxide was put in the centre well of the Warburg manometer flasks. The reaction was run at 30° C. Gas phase, argon. Illumination 50,000 lux

42). The reaction was very vigorous at pH 6·5, but little hydrogen was evolved at pH 8·0.

Photoproduction of hydrogen from thiosulphate was inhibited by nitrogen gas and ammonium ions[42], as is the evolution of hydrogen in the presence of organic electron donors[34,38]. However, neither ammonia nor nitrogen gas inhibited fixation of carbon dioxide by illuminated *Chromatium* cells when thiosulphate was the electron donor. This indicated that, although under the conditions of our experiments ammonia and nitrogen inhibited the flow of electrons to H^+, they did not inhibit the flow of electrons that is required for assimilation of carbon dioxide by way of pyridine nucleotide.

Growing *Chromatium* cells were found, by analysis, to have oxidized in four days 27 millimoles of added thiosulphate to 54 millimoles of sulphate. During this period the appearance of the culture indicated a transitional formation of elemental sulphur. The results are in agreement with the following sequence of reactions, in which thiosulphate is the donor of electrons that are activated by light and used either for the assimilation of carbon and nitrogen into cellular substance or for the photoproduction of hydrogen gas:

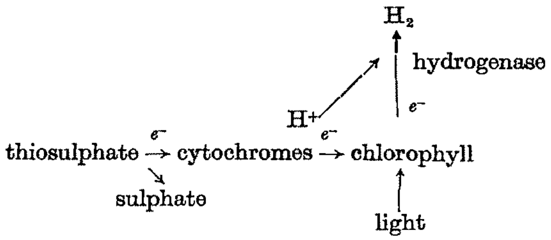
$$S_2O_3^= + 2\,OH^- \xrightarrow{\text{light}} S + SO_4^= + 2\,e^- + H_2O \quad (3)$$

$$S + 8\,OH^- \xrightarrow{\text{light}} SO_4^= + 6\,e^- + 4\,H_2O \quad (4)$$

$$10\,H_2O \xrightarrow{\text{dark}} 10\,H^+ + 10\,OH^- \quad (5)$$

$$\text{Sum: } S_2O_3^= + 5\,H_2O \xrightarrow{\text{light}} 2\,SO_4^= + 8\,e^- + 10\,H^+ \quad (6)$$

The proposed electron-flow mechanism[42] for the photoproduction of hydrogen from thiosulphate envisages the following electron transfer:



The results with thiosulphate provided the first experimental evidence for hydrogen evolution dependent on light, from an 'inorganic' electron donor by a photosynthetic organism. Similar results were obtained when thiosulphate was replaced by succinate as electron donor[41].

It appears, therefore, that *Chromatium* uses light energy to raise electrons, supplied by thiosulphate to chlorophyll, via cytochromes, to a reducing potential at least equal to that of molecular hydrogen. We have also observed evolution of hydrogen gas in the *dark* by cell-free *Chromatium* preparations when the electron donor was dithionite, a substance that provides electrons capable of reducing protons without requiring additional energy (a catalytic amount of benzyl viologen was used as an electron carrier in this dark reaction).

(*b*) *Photofixation of nitrogen gas.* Nitrogen fixation by photosynthetic organisms (refs. 45, 35, 38, 46–49) may also be viewed as resulting from a non-cyclic electron-flow in which electrons pass from an external electron donor, via cytochromes, to chlorophyll excited by light, and thence to molecular nitrogen.

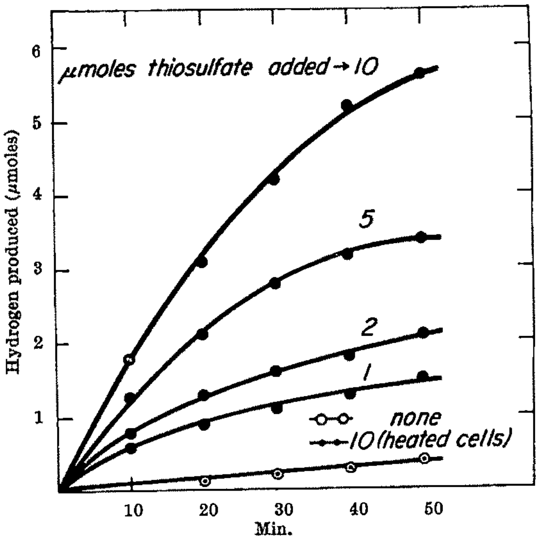


Fig. 3. Photoproduction of hydrogen gas by *Chromatium* cells as a function of added thiosulphate. The control vessel contained cells that had been heated at 100° C. for 10 min. Other experimental conditions are as described for Fig. 2

This interpretation was substantiated by using thiosulphate and succinate as electron donors for fixation of nitrogen gas by illuminated *Chromatium* cells[50]. Fig. 4 shows the result of photofixation of nitrogen gas with thiosulphate as the electron donor. Similar photofixation of nitrogen was obtained with succinate as the electron donor. In both cases, *Chromatium* cells fixed nitrogen only in light. Fixation ceased when the light was turned off and was resumed when the light was turned on again. The dependence of nitrogen fixation on light and an external electron donor was confirmed by the use of nitrogen-15 isotope (Table 1). We are indebted to Dr. C. C. Delwiche for the determinations of nitrogen-15.

In the case of thiosulphate, photofixation of nitrogen was greatly increased by the addition of oxalacetate (Fig. 4), which probably acted as an amino-group acceptor. Succinate served both as electron

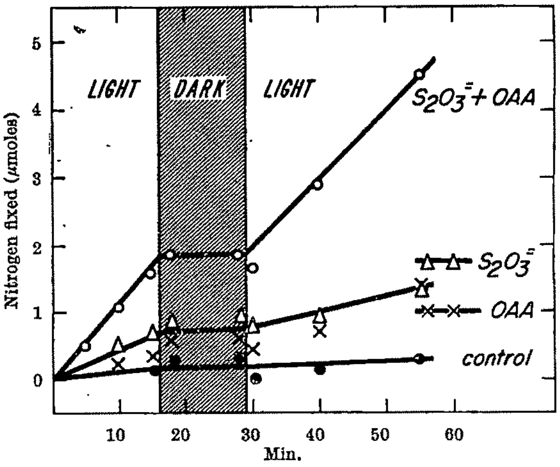


Fig. 4. Effect of thiosulphate and oxalacetate on photofixation of nitrogen gas by *Chromatium* cells. The reaction mixture included in a final volume of 2·9 ml., 0·1 gm. of washed cells, suspended in a modified nutrient solution, pH 7·2 (from which nitrogen compounds were omitted). 20 micromoles each of thiosulphate and oxalacetate were added as indicated. 0·1 ml. of 20 per cent potassium hydroxide was put in the centre well of the Warburg manometer flasks. The reaction was run at 30° C. Gas phase, nitrogen. Illumination 50,000 lux

Table 1. EFFECT OF LIGHT AND ELECTRON DONORS ON FIXATION OF NITROGEN GAS BY *Chromatium* CELLS

Treatment	Nitrogen-15 atom per cent excess
Dark, succinate	0·004
Dark, thiosulphate + oxalacetate	0·003
Light	0·288
Light, succinate	1·049
Light, thiosulphate + oxalacetate	1·467

40 micromoles each of thiosulphate, oxalacetate and succinate were added as indicated. Other experimental conditions as in Fig. 4, except that no potassium hydroxide was included, and the gas atmosphere was 90 per cent argon and 10 per cent nitrogen containing 30 atom per cent excess nitrogen-15. Reaction time was 2 hr.

donor and as a source of the carbon skeleton that is needed for accepting an amino-group.

In preliminary experiments, when cell-free *Chromatium* preparations were supplied in the 'dark' with a mixture of hydrogen and nitrogen gas, they absorbed more gas than in the control treatment in which the gas phase consisted solely of hydrogen. This seems to indicate that cell-free *Chromatium* preparations can fix nitrogen, with the aid of hydrogen gas as the electron donor—an interpretation that was in agreement with experiments using nitrogen-15 (Table 2). Photofixation of nitrogen-15 by cell-free *Chromatium* preparations, although low by comparison with that by whole cells (Table 1) was above the level of significance of isotopic identification with the mass spectrometer used.

Table 2. NITROGEN-15 FIXATION IN THE DARK BY CELL-FREE EXTRACTS OF *Chromatium*

No.	Reductant added	Nitrogen-15 atom per cent excess Experiment *A*	Experiment *B*
1	None	0·0255	0·0274
2	Reduced diphosphopyridine nucleotide	0·0480	
3	Hydrogen gas	0·0362	0·0430

The reaction mixture contained in a final volume of 3 ml.: cell-free extract, containing 0·3 mgm. bacteriochlorophyll, and the following in micromoles: *tris* buffer, *p*H 7·8, 100; magnesium chloride, 5; benzyl viologen, 0·2. Vessels 1 and 3 received 0·5 μmole diphosphopyridine, and vessel 2, 1 μmole reduced diphosphopyridine nucleotide. All vessels received 0·5 atmosphere nitrogen containing 96 atom per cent excess nitrogen-15. Treatment 3 received in addition 0·5 atmosphere hydrogen gas. The experiment was run at 25° C. for 2 hr. in the dark.

These findings appear to support the view that the role of light in photofixation of nitrogen by *Chromatium* is to generate electrons with a reducing potential that is at least equal to that of hydrogen. When exogenous hydrogen was supplied in the gas phase in the dark, light was no longer necessary for the fixation of nitrogen. Pyridine nucleotides seemed to mediate the reduction of nitrogen. However, the experiments on nitrogen fixation by cell-free *Chromatium* preparations are at an early stage and it would be premature to draw final conclusions.

(*c*) *Photoreduction of pyridine nucleotides.* The photoreduction of diphosphopyridine nucleotide was observed by Frenkel[31] and Vernon and Ash[32] in *Rhodospirillum rubrum*, and by Ogata *et al.*[51] in *Chromatium*. In more recent experiments we have found that in the presence of succinate and light, unwashed chromatophores from *R. rubrum*, unaided by enzymes from chloroplasts (cf. ref. 32), reduce both di- and tri-phosphopyridine nucleotide.

We have previously emphasized[10,29] that *Chromatium* is capable of reducing pyridine nucleotide in the dark, when supplied with exogenous electron donors, such as hydrogen gas, which yield electrons at the redox-level of pyridine nucleotide. The reduction of pyridine nucleotide is dependent on light when photosynthetic bacteria are supplied with exogenous electron donors, such as succinate, which yield electrons at a redox-level insufficient for reducing pyridine nucleotide without an additional input of energy. It should be noted, however, that in the case of succinate, the relatively low redox potential characterizes only the first oxidation step, that to fumarate. As fumarate is transformed to malate, *Chromatium* can use it to reduce pyridine nucleotide in the dark[29].

Experiments with cell-free systems of *R. rubrum*, to be reported elsewhere (Nozaki, M., Tagawa, K., and Arnon, D. I.), indicate that the non-cyclic electron transport in photosynthetic bacteria that results in pyridine nucleotide reduction is coupled with formation of adenosine triphosphate, thus providing a parallel to non-cyclic photophosphorylation in chloroplasts[7-9]. Experiments are now under way to determine whether the formation of adenosine triphosphate is also coupled with the non-cyclic electron transport that results in the photoproduction of hydrogen or photofixation of nitrogen gas.

To recapitulate, the reduction of pyridine nucleotides, the fixation of nitrogen and the evolution of hydrogen from such electron donors as thiosulphate or succinate are reactions that in photosynthetic bacteria have been found to require an input of light-energy. These photochemical reactions suggest the existence of a non-cyclic electron-flow mechanism in photosynthetic bacteria as represented diagrammatically in Fig. 5.

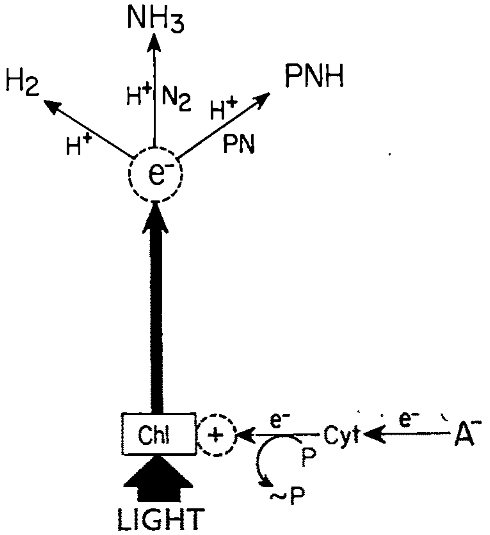


Fig. 5. Scheme for non-cyclic electron flow in *Chromatium*. Details in the text

Non-cyclic Electron Flow and the Evolution of Photosynthesis

Until recently, cyclic photophosphorylation was the only experimentally demonstrable common denominator of the mechanism of photosynthesis in plants and bacteria. Now it has become clear that photosynthetic bacteria may also use part of the absorbed light-energy for a non-cyclic photophosphorylation that is analogous in some respects to that found in chloroplasts.

The most characteristic difference between the non-cyclic electron-flow mechanism of chloroplasts (reaction 2) and bacteria (Fig. 5) is in the electron donor system. In chloroplasts the electron donor is water (that is, OH-), whereas bacteria cannot use water but employ inorganic or organic electron donors such as thiosulphate or succinate[52,53].

This interpretation of the difference between the non-cyclic electron-flow mechanism in chloroplasts and in photosynthetic bacteria is supported by recent evidence, presented in the succeeding article by M. Losada, F. R. Whatley and D. I. Arnon, that it is experimentally possible to replace water as the electron donor in non-cyclic photophosphorylation by chloroplasts and to demonstrate in this manner a bacterial type of non-cyclic electron flow in green plants: a reduction of pyridine nucleotide coupled with the oxidation of an exogenous electron donor and a simultaneous photophosphorylation but without evolution of oxygen.

These findings permit us to interpret with somewhat enhanced confidence, certain aspects of the biochemical evolution of photosynthesis which we have already discussed elsewhere[7,29].

The beginning of photosynthesis may be viewed as an emergence of a porphyrin that gave rise to chlorophyll and permitted the cell to use for metabolic purposes the energy of sunlight. In this primitive photosynthesis, the contribution of light-energy was probably limited to anaerobic cyclic photophosphorylation. No photochemically formed reductant would have been required for the photoassimilation of, say, acetate or for the assimilation of carbon dioxide so long as hydrogen gas was present in the atmosphere[29]. Oparin[54] and Miller and Urey[55] have summarized the evidence that in the early periods of the evolution of life the environment contained simple carbon compounds such as acetate and also hydrogen gas. Photosynthetic bacteria still show this primitive type of photosynthesis. *Chromatium*, for example, when supplied with hydrogen gas, uses it to reduce pyridine nucleotide in the dark, and seems then, as in the case of photoassimilation of acetate, to limit the use of light to the formation of adenosine triphosphate[29] (cf. ref. 30).

As hydrogen gas vanished from the primitive atmosphere, the photosynthetic cell probably became dependent on the photosynthetic apparatus for generating photochemically a strong reductant from such electron donors as succinate or thiosulphate. Light-energy now would have served a dual purpose: it would supply adenosine triphosphate by cyclic photophosphorylation and would also, by photooxidizing the electron donor, provide electrons for reducing pyridine nucleotides by a non-cyclic electron-flow mechanism. In organisms which contain, or can form adaptively, hydrogenase or nitrogenase (photosynthetic bacteria and algae), this phase of photosynthesis can be observed to-day as a photoproduction of molecular hydrogen or a photofixation of nitrogen gas.

The non-cyclic electron-flow mechanism in photosynthetic bacteria operates with such electron donors as reduced sulphur compounds or organic acids. Water (OH^-) became an electron donor only with the emergence of plant photosynthesis. As will be discussed in the following article, it now appears that this event was made possible because plants have evolved an auxiliary reaction dependent on light, in which the electrons from water (OH^-) are first raised to a potential similar to that of the electron donors in bacterial photosynthesis, and are then used in a second photochemical reaction—the non-cyclic electron flow common to plants and bacteria—in which pyridine nucleotide reduction is coupled with the formation of adenosine triphosphate.

Oxygen evolution is peculiar to green plants because it is the product of an auxiliary photochemical reaction, the oxidation of water (OH^-), that is found only in green plants. Water as an electron donor is therefore not involved in the two main events of non-cyclic photophosphorylation in plants and bacteria, the photoreduction of pyridine nucleotide and the coupled formation of adenosine triphosphate.

In both bacterial and plant photosynthesis the photosynthetic events proper are limited to the formation of adenosine triphosphate and reduced pyridine nucleotide by cyclic and non-cyclic photophosphorylation. This transformation of light into the common currency of cellular energy is fundamentally independent of carbon dioxide assimilation. There is no particular reason why adenosine triphosphate or a photochemically generated reductant could not be used for driving endergonic cellular processes other than carbon dioxide assimilation.

In summary, the differences between bacterial and plant photosynthesis seem to centre on the electron donors that are consumed in the reduction of pyridine nucleotide. With some electron donors, as, for example, hydrogen gas, the reduction of pyridine nucleotide by photosynthetic bacteria is a dark reaction. With other electron donors, such as thiosulphate or succinate, an input of light-energy, in a non-cyclic electron-flow mechanism, becomes necessary for the oxidation of the electron donor and the reduction of pyridine nucleotide. Thus, in bacterial photosynthesis, the reduction of pyridine nucleotide may occur with or without an input of light energy depending on the particular electron donor used. By contrast, in photosynthesis of green plants, the reduction of pyridine nucleotide invariably requires an input of light energy because the physiological electron donor is water (OH^-).

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SEPARATION OF TWO LIGHT REACTIONS IN NONCYCLIC PHOTO-PHOSPHORYLATION OF GREEN PLANTS

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NONCYCLIC photo-phosphorylation[1-3] is a photochemical reaction in isolated chloroplasts which yields simultaneously three products: reduced pyridine nucleotide, adenosine triphosphate and oxygen, in accordance with reaction 1 (where P means orthophosphate):

$$\mathrm{ADP} + \mathrm{P} + \mathrm{TPN} + \mathrm{H_2O} \rightarrow \mathrm{ATP} + \mathrm{TPNH_2} + \tfrac{1}{2}\,\mathrm{O_2} \qquad (1)$$

Of the three products of reaction 1, only reduced pyridine nucleotide and adenosine triphosphate are required by chloroplasts in the conversion of carbon dioxide to carbohydrate[4-7]. Oxygen is an excreted by-product, incidental to the use of water ($\mathrm{OH^-}$) as the electron donor in noncyclic photo-phosphorylation by chloroplasts[8]. Photosynthetic bacteria cannot use water as an electron donor, and here the key events of noncyclic photo-phosphorylation—the reduction of pyridine nucleotide and the formation of adenosine triphosphate—also occur, but are not accompanied by an evolution of oxygen (ref. 9 and unpublished work by Nozaki, M., Tagawa, K., and Arnon, D. I.).

Vernon and Zaugg[10], extending the earlier work of Vernon and Kamen[11], have shown that chloroplasts which have lost, through ageing or by poisoning, the capacity to evolve oxygen photochemically, are nevertheless able to reduce triphosphopyridine nucleotide photochemically with electrons donated by a reduced dye, 2,6-dichlorophenol indophenol. (In practice, the dye is supplied in catalytic amounts and kept in a reduced form by substrate amounts of added ascorbate.) Krasnovsky has found that chlorophyll solutions in pyridine can bring about a photochemical electron transfer from ascorbate or other electron donors to pyridine nucleotides or other electron acceptors[12].

We now wish to report that noncyclic photo-phosphorylation in chloroplasts has been experimentally separated into two distinct photochemical reactions: (*a*) a photo-oxidation of water ($\mathrm{OH^-}$) that yields oxygen (reaction 2) and (*b*) a noncyclic photo-phosphorylation of the bacterial type, that is, a photo-reduction of triphosphopyridine nucleotide that is coupled with the formation of adenosine triphosphate (reaction 3). The two reactions have been separated by using reduced (A^-) and oxidized (A) indophenol dyes as summarized here:

$$\mathrm{H_2O} + 2A \xrightarrow{\text{light}} \tfrac{1}{2}\,\mathrm{O_2} + 2A^- + 2\mathrm{H^+} \qquad (2)$$

$$2A^- + \mathrm{TPN} + 2\mathrm{H^+} + \mathrm{ADP} + \mathrm{P} \xrightarrow{\text{light}} \mathrm{TPNH_2} + \mathrm{ATP} + 2A \qquad (3)$$

$$\text{Sum: } \mathrm{TPN} + \mathrm{ADP} + \mathrm{P} + \mathrm{H_2O} \xrightarrow{\text{light}} \mathrm{ATP} + \mathrm{TPNH_2} + \tfrac{1}{2}\,\mathrm{O_2} \qquad (1)$$

Bacterial Type of Noncyclic Electron Flow in Chloroplasts

Table 1 shows that when catalytic amounts of 2,6-dichlorophenol indophenol[10] replaced water ($\mathrm{OH^-}$) as the electron donor (treatment 3), illuminated chloroplasts formed adenosine triphosphate and reduced triphosphopyridine nucleotide without evolving oxygen. Similar results were obtained when reduced 2,3′,6-trichlorophenol indophenol[13] was used instead of 2,6-dichlorophenol indophenol. The participation of water as an electron donor in these reactions was blocked by the addition of *p*-chlorophenyl dimethylurea[10] to, and the omission of chloride[14] from, the reaction mixture. When the participation of water as an electron donor was not blocked (Table 1, treatment 1), the reduction of triphosphopyridine nucleotide and the coupled phosphorylation were accompanied by evolution of

Table 1. NONCYCLIC PHOTO-PHOSPHORYLATION OF THE BACTERIAL TYPE BY CHLOROPLASTS

No.	Addenda	Effective electron donor	Oxygen evolved (μatoms)	Triphosphopyridine nucleotide reduced (μmoles)	Adenosine triphosphate formed (μmoles)
1	None	Water	3·0	3·4	2·4
2	Chlorophenyl dimethyl urea	None	0	0·5	0·2
3	Dye, ascorbate, chlorophenyl dimethyl urea	Reduced dye (ascorbate)	0	3·2	3·4

Vessel 1 contained in a total volume of 3 ml.: washed chloroplast fragments (*P*1*S*2), prepared in the absence of chloride, containing 0·5 mgm. chlorophyll; 0·05 ml. purified spinach phosphopyridine nucleotide reductase, and the following, in micromoles: *tris*/acetate buffer, *p*H 8·0, 40; $MgSO_4$, 5; $K_2H^{32}PO_4$, 10; ADP, 10; TPN, 4; and KCl, 10. In vessel 2, KCl was omitted and 2×10^{-5} *M* *p*-chlorophenyl dimethylurea was added. Treatment 3 was the same as treatment 2 except that 20 μmoles ascorbate and 0·2 micromole dye (dichlorophenol indophenol) were added. The experiment was run for 20 min. at 15° (at a light intensity of 2,000 ft. candles). Oxygen was measured manometrically and triphosphopyridine nucleotide spectrophotometrically at 340 mμ.

The omission of chloride and the addition of chlorophenyl dimethyl urea, a powerful inhibitor of oxygen evolution, blocked the use of water as an electron donor in the chloroplast system. Catalytic amounts of dichlorophenol indophenol served as an electron carrier between ascorbate and the chloroplast system.

oxygen. When the participation of water was blocked but no substitute electron donor was supplied (treatment 2), no significant reaction occurred at all.

The 'bacterial' type of noncyclic electron-flow mechanism that is envisaged as operating in chloroplasts, when the participation of water as the electron donor is blocked (Table 1, treatment 3), is illustrated diagrammatically in Fig. 1. The electron donor, A^-, represents the reduced indophenol dye, and the electron acceptor, *B*, represents triphosphopyridine nucleotide (cf. reaction 3). In accordance with previous formulations, we envisage the formation of adenosine triphosphate as occurring during the electron transport between cytochromes and chlorophyll[15].

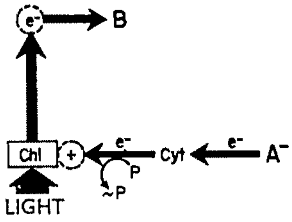

Fig. 1. Scheme for noncyclic photo-phosphorylation of the bacterial type. Details in the text

Photo-oxidation of Water as a Separate Reaction

In the experiments just described, catalytic amounts of phenol indophenol dye mediated the transfer of electrons from ascorbate to triphosphopyridine nucleotide. The dye was *reduced in the dark* by ascorbate and *oxidized in the light* by triphosphopyridine nucleotide. This electron transport was coupled with the formation of adenosine triphosphate (reaction 3).

We will now describe experiments in which catalytic amounts of phenol indophenol dye mediated the transfer of electrons from water (OH^-) to ferricyanide. The dye was *reduced in the light* by water (OH^-) (resulting in an evolution of oxygen) and was *oxidized in the dark* by ferricyanide. This photo-oxidation of water did not result in the formation of adenosine triphosphate (reaction 2).

Indophenol dyes have long been known to be reduced photochemically by chloroplasts[13,16] in a Hill reaction; but unlike such Hill reagents as ferricyanide[1–3], the photo-reduction of 2,3′,6-trichlorophenol indophenol is not linked with photo-phosphorylation[13,17]. Krogmann and Jagendorf[13] drew from this "the inference . . . that reduction of indophenol dye is primarily by means of uncoupled electron transport", possibly by a " 'short circuit' of that part of the electron transport system which is coupled to phosphorylation".

We have examined the effect of catalytic amounts of indophenol dyes on a variant of noncyclic photophosphorylation in chloroplasts where ferricyanide replaces triphosphopyridine nucleotide (reaction 4).

$$2Fe^{3+}Cy + H_2O + ADP + P \xrightarrow{\text{light}} 2Fe^{2+}Cy + \tfrac{1}{2}\,O_2 + ATP + 2H^+ \quad (4)$$

The results are shown in Table 2. The addition of catalytic amounts of indophenol dye to the ferricyanide system has almost completely suppressed the formation of adenosine triphosphate without impairing oxygen evolution (treatments 2 and 4). The addition of catalytic amounts of indophenol dye has thus converted the noncyclic photo-phosphorylation reaction with ferricyanide into a more limited reaction, a photo-oxidation of water that is not accompanied by photo-phosphorylation.

Table 2. SEPARATION OF PHOTO-PRODUCTION OF OXYGEN AND PHOTO-PHOSPHORYLATION BY CHLOROPLASTS

No.	Treatment	Effective electron acceptor	Oxygen produced (μatoms)	Adenosine triphosphate formed (μmoles)
1	Ferricyanide	Ferricyanide	4·5	4·7
2	Ferricyanide Dye A +	Oxidized dye (Ferricyanide)	5·1	1·3
3	Ferricyanide	Ferricyanide	3·3	3·5
4	Ferricyanide Dye B +	Oxidized dye (Ferricyanide)	3·5	0·8

The reaction mixture contained, in a final volume of 3 ml., unwashed chloroplast fragments containing 0·2 mgm. chlorophyll, and the following in micromoles: *tris*/HCl buffer, *p*H 8, 100; $MgSO_4$, 5; $K_2H^{32}PO_4$, 10; adenosine diphosphate, 10; potassium ferricyanide, 10. Dye *A* (0·2 μmole 2,6-dichlorophenol indophenol) or dye *B* (0·05 μmole 2,3′,6-trichlorophenol indophenol) was added where indicated. 10 per cent potassium hydroxide was present in the centre well of the manometer vessels. The reaction was run for 12 min. at 15° C. in the light (2,000 ft. candles). The gas phase was nitrogen.

In the experiments illustrated in Table 2, it was possible to measure the oxygen evolution that accompanies the photo-reduction of indophenol dyes because they were supplied in catalytic amounts and were kept in the oxidized state by the presence of substrate amounts of ferricyanide. A direct measurement of oxygen evolution that accompanies the photo-reduction of 2,6-dichlorophenol—or 2,3′,6-trichlorophenol indophenol—is difficult to make because these dyes, when used in concentrations that are required for manometric measurements of evolved oxygen, absorb too much light. This reduces the effective light intensity to such an extent that chloroplasts cease to evolve oxygen[16]. Indophenol dyes are therefore generally used as Hill reagents in low concentrations, and their photo-reduction is measured not manometrically by oxygen evolution but optically, by their decolorization[16,18].

The observed effect of indophenol dyes could not be attributed to inhibiting, uncoupling, 'shortcircuiting' or by-passing a phosphorylation step in reaction 4. As shown in Table 3, the addition of indophenol dyes to the noncyclic photo-phosphorylation system with triphosphopyridine nucleotide (reaction 1) did not suppress photo-phosphorylation and has, in fact, slightly increased it. As in the ferricyanide system (Table 2), the addition of the dye to the triphosphopyridine nucleotide system did not affect the oxygen evolution.

Table 3. EFFECT OF INDOPHENOL DYES ON NONCYCLIC PHOTO-PHOSPHORYLATION BY CHLOROPLASTS IN THE PRESENCE OF TRIPHOSPHOPYRIDINE NUCLEOTIDE (TPN)

No.	Treatment	Effective electron acceptor	Oxygen produced (μatoms)	Adenosine triphosphate formed (μmoles)
1	TPN	TPN	3·3	3·2
2	TPN Dye *A* +	TPN	3·1	4·2
3	TPN	TPN	3·0	3·3
4	TPN Dye *B* +	TPN	3·1	3·6

Experimental conditions were the same as for Table 2, except that the ferricyanide was omitted. 4 μmoles TPN and 0·1 ml. of a purified preparation of triphosphopyridine nucleotide reductase from spinach were added in each treatment.

The photo-oxidation of water is diagrammatically represented in Fig. 2, where *A* stands for oxidized dye. The reaction requires chloride. We regard the photo-oxidation of water by chloroplasts as an auxiliary reaction to supply electrons at an elevated reducing potential for a second photochemical reaction, the bacterial type of noncyclic photo-phosphorylation, during which a phosphorylation, coupled with the reduction of pyridine nucleotide, actually occurs (Fig. 1).

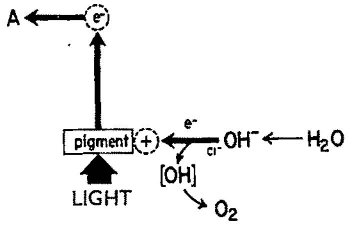

Fig. 2. Scheme for photo-oxidation of water by chloroplasts. Details in the text.

It appears that noncyclic photo-phosphorylation in green plants combines the two component photochemical reactions: (*a*) the auxiliary reaction for photo-oxidation of water (Fig. 2) and (*b*) the bacterial type of noncyclic photo-phosphorylation (Fig. 1) that is used both by photosynthetic bacteria and by green plants for photo-reduction of pyridine nucleotide and a coupled phosphorylation.

Fig. 3 depicts the combined scheme for noncyclic photo-phosphorylation in green plants that we now envisage. *B* is the electron acceptor, triphosphopyridine nucleotide under physiological conditions, but it can be replaced in cell-free systems by ferricyanide. Under special conditions, molecular oxygen can also serve as the electron acceptor *B* in cell-free systems, and this would result in an "oxidative photosynthetic phosphorylation"[17] (compare Fig. 1) or "oxygen-catalysed cyclic photo-phosphorylation"[19] (compare Fig. 3). In the latter case an oxygen exchange reaction would result. The consumption of oxygen at one end (*B* in Fig. 3) would balance the production of oxygen at the other (bottom of Fig. 3).

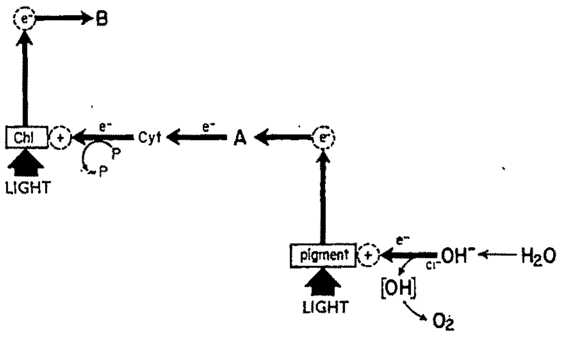

Fig. 3. Scheme for noncyclic photo-phosphorylation in green plants. Details in the text

The intermediate *A* in Fig. 3 is both the electron acceptor for the first, and the electron donor for the second, light reaction (compare Figs. 1 and 2). We have used indophenol dye as an experimental device to separate the two photochemical reactions. The natural intermediates with which the dyes have interacted have not been identified. They may include cytochromes or a quinone[20].

According to the scheme shown in Fig. 3, electron acceptors in chloroplast reactions may be divided into those which react in position *B* and those which react in position *A*. Only the first group, exemplified by triphosphopyridine nucleotide, would give a coupled photo-phosphorylation. 2,3′,6-Trichlorophenol indophenol[13,17] is an example of the second group. However, certain compounds, although reacting preferentially in position *A*, may also be capable of reacting in position *B*.

Since indophenol dyes are reduced by illuminated chloroplasts much more rapidly than ferricyanide[13,21], a catalytic amount of the dye can convert noncyclic photo-phosphorylation with ferricyanide into a photo-oxidation of water reaction (Table 2). In the absence of the dye, ferricyanide acts as a terminal electron acceptor *B* in noncyclic photo-phosphorylation, and oxygen evolution is accompanied by formation of adenosine triphosphate (cf. Fig. 3 and Table 2, treatments 1 and 3). When the dye is present, it is repeatedly reduced during the photo-oxidation of water and re-oxidized by ferricyanide in the dark (cf. Fig. 2 and Table 2, treatments 2 and 4). Here oxygen is produced without the formation of adenosine triphosphate (Fig. 2). However, indophenol dyes do not convert noncyclic photophosphorylation with triphosphopyridine nucleotide into a photo-oxidation of water reaction (Table 3). The dark re-oxidation of the reduced dye by triphosphopyridine nucleotide is precluded by the difference in the respective normal redox potentials (0·217 V. for 2,6-dichlorophenol indophenol and −0·320 V. for triphosphopyridine nucleotide).

Triphosphopyridine Nucleotide Reductase from *Chromatium*

The separation in chloroplasts of the photo-phosphorylation reaction from the photo-oxidation of water underlines the similarities and the differences between noncyclic electron-flow mechanisms in photosynthetic bacteria and in green plants. The difference is most pronounced at the electron donor end, where green plants, but not photosynthetic bacteria, can use water (OH⁻) as the electron donor.

Unlike photosynthetic bacteria, chloroplasts contain neither nitrogen-fixing enzymes nor hydrogenase.

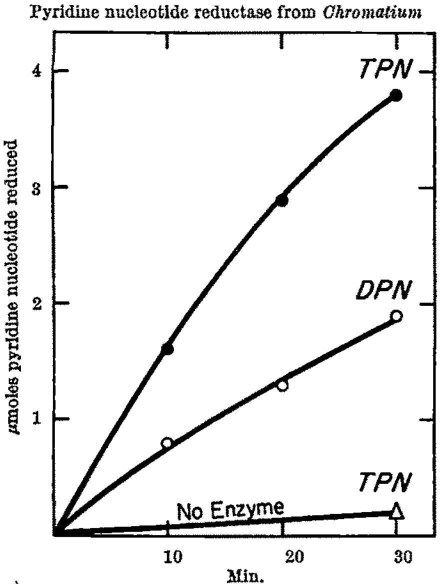

Fig. 4. Pyridine nucleotide reductase from *Chromatium*. Experimental conditions as in Table 4, except that $K_2H^{32}PO_4$ and ADP were omitted. 4 μmoles DPN or TPN were added as indicated

As a consequence, the electron acceptor end of the noncyclic electron-flow mechanism in chloroplasts can be coupled neither to photofixation of nitrogen nor to photoproduction of hydrogen gas, but only to reduction of pyridine nucleotide. However, certain species of green and blue-green algæ constitute exceptions among plants. They can fix nitrogen photosynthetically or they can become adapted to using hydrogen gas as a reductant in photosynthesis (literature cited in ref. 9).

Despite the differences in enzymic composition that govern nitrogen fixation and hydrogen utilization, the noncyclic electron-flow mechanisms in bacteria and chloroplasts are basically similar at the electron acceptor end. This view is strengthened by the recent isolation of a photosynthetic pyridine nucleotide reductase from *Chromatium* and *R. rubrum*. As shown in Fig. 4, the bacterial enzyme catalysed the photochemical reduction of pyridine nucleotides by chloroplasts that have been deprived of their own pyridine nucleotide reductase. When tested by adding to washed chloroplasts, the bacterial pyridine nucleotide reductase was similar to the chloroplast pyridine nucleotide reductase in reducing preferentially triphosphopyridine nucleotide over diphosphopyridine nucleotide[22,23].

The reduction of triphosphopyridine nucleotide was coupled with oxygen evolution when the bacterial enzyme was added to a chloroplast preparation that by itself could not reduce triphosphopyridine nucleotide and thereby evolve oxygen (Table 4). These findings again support the conclusion that reduction of triphosphopyridine nucleotide and oxygen evolution are basically separate phenomena. The bacterial pyridine nucleotide reductase cannot bring about a coupling of pyridine nucleotide reduction with oxygen evolution in a bacterial system.

Table 4. PHOTOCHEMICAL OXYGEN EVOLUTION CATALYSED BY PYRIDINE NUCLEOTIDE REDUCTASE FROM *Chromatium*

Min.	Oxygen evolved (μatoms)	Triphosphopyridine nucleotide reduced (μmoles)
5	0·75	0·76
10	1·60	1·40
20	2·78	2·39
30	3·97	3·18

The reaction mixture contained, in a final volume of 3 ml.: washed chloroplast fragments containing 0·3 mgm. chlorophyll; and the following in micromoles: *tris* buffer, *p*H 7·8, 100; $MgCl_2$, 5; ADP, 10; $K_2H^{32}PO_4$, 10; TPN, 6; and a purified pyridine nucleotide reductase preparation from *Chromatium*. The reaction was run at 15° in the light.

Concluding Remarks

The concept of photosynthesis presented now is a further elaboration, in the light of new experimental findings, of that presented earlier[15,8]. It is now possible to envisage, in more detail, the electron transfer reactions from water (OH^-) that result in oxygen evolution. We had first suggested that this may be a dark reaction between water (OH^-) and a cytochrome component peculiar to green plants[15]. Such a reaction, although it appeared thermodynamically possible[15], could not be described in terms of the properties of known cytochrome constituents of green cells[24]. We next suggested that the interaction between OH^- and cytochromes may require an additional input of energy[19], and now it appears that this energy is supplied by an auxiliary light reaction. This auxiliary light reaction is found in green plants but not in photosynthetic bacteria, and explains why photosynthesis is accompanied by evolution of oxygen only in green plants.

The separation of photo-oxidation of water from the photo-phosphorylation reaction is consistent with the view that photosynthesis in green plants involves the participation of more than one light reaction. Warburg *et al.*[25] found in *Chlorella* an enhancement of photosynthesis under red light by the addition of catalytic amounts of blue light and attributed this effect to a photoactivation of a photosynthetic enzyme ('Luminoferment'). The maximum effectiveness of the catalytic light was at 460 mμ. Another light effect, which seems more directly related to our results, was reported by Emerson *et al.*[26], who observed that enhancement of photosynthesis in *Chlorella* under red light was produced by light of shorter wave-length; in their experiments the supplementary light was not catalytic but of an intensity "sufficient to give measurable photosynthesis". Emerson[27] found the maximum effectiveness of the supplementary light in *Chlorella* at 480 mμ, and a lower peak of effectiveness at about 655 mμ, coinciding approximately with the maximum absorption peaks of chlorophyll *b*.

Emerson[27] suggested from these and similar observations with other algæ that, at the long wavelengths of light absorbed only by chlorophyll *a*, full efficiency of photosynthesis depends on the absorption of light of shorter wave-length by an accessory photosynthetic pigment: chlorophyll *b* in *Chlorella*, or phycobilins in other algæ. The supplementary light in this view is not used as a catalyst but is consumed as a substrate.

The enhancement effect of supplementary light has been confirmed by other investigators[28-30]. Of special relevance to our concept is the conclusion of Myers and French[28] that their results favour "the idea that the interaction [of the light absorbed by chlorophyll *a* and *b*] takes place between chemical products of two separate reactions initiated by light" and the report of Govindjee *et al.*[30] that the enhancement effect is associated with the oxygen-liberating mechanism rather than with reduction of carbon dioxide.

That two light reactions, with the participation of water, are involved in the photosynthetic cytochrome system of both green plants and photosynthetic bacteria has been postulated by Hill and Bendall[24]. However, our findings support the conclusion that green plants possess a special photochemical reaction, photo-oxidation of water, that is absent in photosynthetic bacteria. We have not identified the 'pigment' (Fig. 2) that is involved in the photo-oxidation of water. This pigment is more likely to be chlorophyll *b* or an accessory pigment that is found only in higher plants and algæ, rather than chlorophyll *a* (or the closely related bacteriochlorophyll) found in all photosynthetic cells*.

* *Note added in proof.* Additional evidence that oxygen evolution in chloroplasts is catalysed by a photosynthetic pigment different from that involved in the photoreduction of triphosphopyridine nucleotide and photophosphorylation was recently obtained in this Laboratory in experiments with monochromatic light. In the red region of the spectrum, the photo-oxidation of water was most effective around 644 mμ (a maximum absorption peak for chlorophyll *b*), whereas the effectiveness of red light for photoreduction of triphosphopyridine nucleotide and photophosphorylation showed markedly different spectral characteristics (cf. Arnon, D. I., "Photosynthetic Phosphorylation and a Unified Concept of Photosynthesis", Photosynthesis Symp., Fifth Int. Congr. Biochem., Moscow, 1961).

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INTRACELLULAR STRUCTURES IN TUMOURS INDUCED BY POLYOMA

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CERTAIN animal viruses have been described as forming crystals in the nucleus or cytoplasm of the infected cells (for literature, see ref. 1). Light microscopy of histochemically stained specimens and electron microscopy of similar specimens have indicated the nature and significance of these structures in virus development. The tumour-inducing virus, polyoma, has also been observed to form crystalline-like arrangements of characteristic particles (270 Å.) in the nuclei of mouse embryo cells and of kidneys of hamsters infected with this agent[2]. These particles, however, could not be found in the cells of tumours induced by polyoma in the kidneys of hamsters[2]. An electron microscope study of kidneys of mice, rats, and hamsters infected by polyoma has revealed characteristic particles (270 Å.) in the nuclei of cells, two or three weeks following infection with the virus[3]. Fewer particles have been found in the cells of proliferative lesions in the kidneys of infected animals of the three species, and even smaller numbers in the cells of tumours induced by polyoma in the kidneys of rats and hamsters[4].

In this article the results of extensive electron microscope studies of tumours induced in the kidneys of mice, rats (obtained through the courtesy of Dr. Bernice E. Eddy) and hamsters by tissue culture preparations of polyoma virus will be presented. Only material from kidneys showing gross nodular tumours confirmed by histological diagnosis has been used in the present work. Tissue specimens were fixed in 1 per cent osmium tetroxide buffered with veronal-acetate containing 1 per cent sucrose, dehydrated in ethyl alcohol of graded dilutions, embedded in *n*-butyl methacrylate, and sectioned with glass knives. To enhance the contrast, the sections mounted on grids were floated on a saturated solution of uranyl acetate with the specimen facing down for 30 min.–24 hr. Alternate

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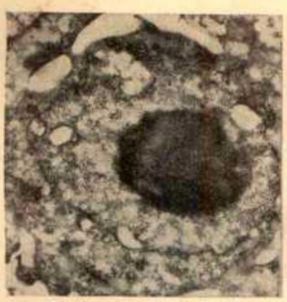
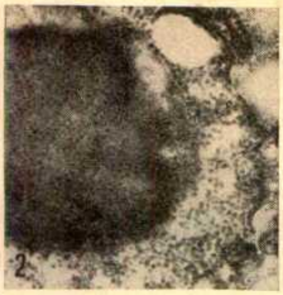
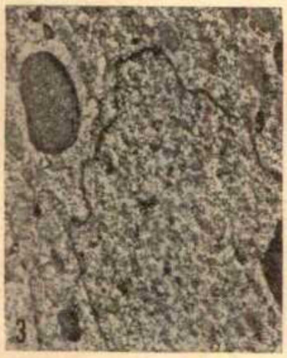






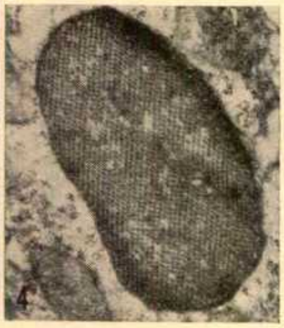


Fig. 1. A cell in polyoma-induced kidney tumour of a hamster with characteristic particles scattered in the nucleus and an inclusion body. (× 10,000)

Fig. 2. Part of Fig. 1 at higher magnification, showing the particles in the inclusion body of the nucleus. (× 21,000)

Fig. 3. Inclusion in the cytoplasm of a cell of a hamster kidney tumour. (× 5,000)

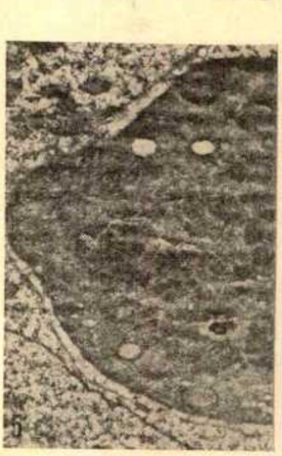
Fig. 4. Cytoplasmic inclusion showing a regular array of particles. (× 22,500)

thin (300 Å.) and thick sections (2μ) were cut. The thick sections were studied by the light microscope, following staining with the Feulgen method. The thin sections were examined in an RCA Type *EMU 3E* electron microscope.

A number of non-specific changes, such as shrinkage of the cells, pyknosis of the nucleus, condensation and vacuolization of the cytoplasm, and alterations in the structure of mitochondria have been observed in the cells of kidney tumours of mice, rats and hamsters. Changes which could be regarded as specific have also been observed in some of the cells in scattered areas of kidney tumours of animals of all three species. Particles, similar to those already described in polyoma-infected cells *in vivo*[5] and *in vitro*[5], have been found scattered throughout the nucleus as shown in Fig. 1, and with more detail in Fig. 2. Vacuolization of the nucleus may also be found (Fig. 1) and the nuclear membrane may be replaced by a tightly packed row of particles. The same nucleus may contain an inclusion body (Fig. 1) filled with characteristic particles in a regular arrangement, as shown at higher magnification in Fig. 2. Occasionally, cells are encountered with enlarged nuclei containing particles scattered at random (Fig. 3) and with cytoplasm showing mitochondria in various stages of degeneration and inclusions, varying from 0·5μ to more than 2μ (Fig. 3). Some of the inclusions are filled with the characteristic particles arranged in regular pattern, as in the largest inclusion shown in the top left-hand corner of Fig. 3, and at higher magnification in Fig. 4. In spite of crystalline-like arrangement of the particles, the structure must not be considered as a crystal until examined by methods such as electron diffraction. In the corresponding thicker (2μ) sections of the same specimen examined by the light microscope, the intranuclear inclusions containing the characteristic particles are positive to Feulgen staining. Cells of tissue cultures of polyoma-infected mouse embryo, examined in the light microscope at various intervals of time after infection, have shown the gradual formation of Feulgen-positive inclusions in the nuclei followed by the appearance of similarly staining inclusions in the cytoplasm[6]. On examination in the electron microscope, the characteristic particles have been found within these inclusions[6]. These inclusions stain as DNA, following acridine orange treatment[7], and the stained inclusions are not removed by digestion with DNA-ase[6]. However, pre-treatment of the cells with pepsin, followed by digestion with DNA-ase, removes the inclusions[6]. This is further indication that the inclusions are of viral nature, as shown in tissues infected by other viruses[7].

Cells have also been observed with most of the cytoplasm filled with strongly osmiophilic material (Fig. 5) in which numerous regular arrays of particles may be seen among mitochondria and vacuoles (Fig. 6). Measurements have been made of 20 particles which appear elliptical in section (Fig. 6). They measure an average of 330 Å. in length and 260 Å. in width. The average distance from centre to centre of the particles is 390 Å. Assuming the particles in the section to be distorted spheres, the diameter of the equivalent circular particles is calculated as 290 Å. In view of the possibility that a peripheral zone of the particles has not been made visible by osmium tetroxide and uranyl acetate, the diameter of the particles, based on the measured centre-to-centre distance and the equivalent circular diameter of the particle, is approximately 390 Å. This measurement of the particle size approximates to that reported for polyoma particles in dried and shadow-cast preparations from infected tissue cultures (440 Å., ref. 8), and in concentrated or partially purified suspensions stained by the negative-contrast method (453 Å.) with potassium phosphotungstate[9].

The particles, which at lower magnifications appear uniformly dense without an apparent membrane or capsid[9], appear to have an internal structure in stained sections examined at higher magnifications (Fig. 7). Similar structure has been seen in the particles present in the sections of cells of infected mouse embryos stained with uranyl nitrate[10]. The structure has been interpreted as reticular and as



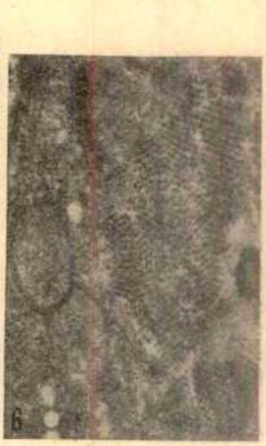


Fig. 5. Cytoplasm of hamster kidney tumour cell showing mitochondria and regular formations of particles. (× 5,000)

Fig. 6. Part of Fig. 5, showing three mitochondria and the characteristic particles. (× 21,000)

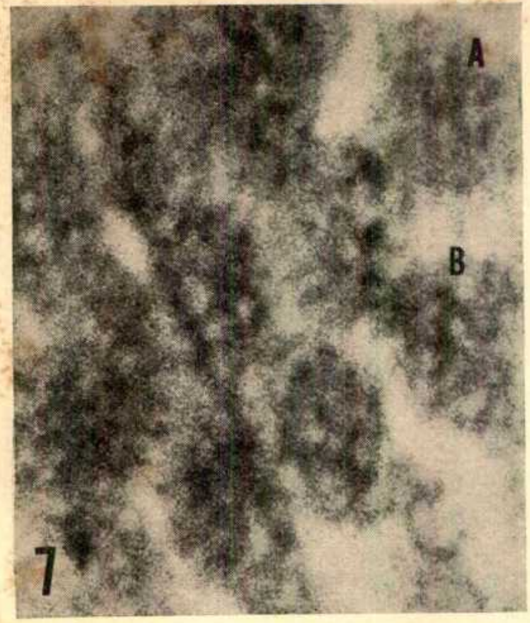


Fig. 7. Some of the particles with outlines of internal structure. (× 540,000)

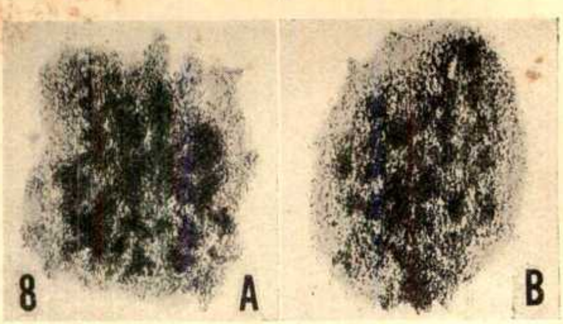


Fig. 8. Schematic drawing of the appearance of two particles shown in Fig. 7

containing an outer coat of lower contrast[10]. A comparison of the appearance of some of the particles (Figs. 7 and 8) with that of the particles stained with potassium phosphotungstate[9] reveals a certain similarity in structure. A detailed description of the structure of the particles present in sections of the kidney tumours will be published.

In conclusion, it may be stated that the characteristic particles in polyoma-infected tissue culture cells and in the kidney cells of infected mice, rats and hamsters are also present in smaller numbers in the cells of kidney tumours of animals of the three species. The observation of particles of similar size and shape in different types of cells infected with polyoma and in tumours induced by this virus in different species gives further support to the identification of these particles as polyoma virus particles.

These investigations have been supported by Grant *C*-4140 and Grant *CRT*-5047 from the National Cancer Institute, National Institutes of Health, U.S.P.H.S.; and by the American Cancer Society Grant *E*-94*B*.

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AN ELECTRON MICROSCOPE STUDY OF THE 'CHLORIDE-SECRETORY CELL' OF *SALMO SALAR* L., WITH REFERENCE TO PLASMA-ELECTROLYTE REGULATION

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THE teleostean kidney is unable to secrete a hypertonic urine. Because of this, the marine teleost must rely on extra-renal electrolyte excretory processes to reduce the salt load incurred in drinking sea-water to replace body-water lost by the process of osmotic dehydration. Smith[1] suggested that the gills might be the site of the extra-renal excretory mechanism, while Keys[2], through the use of a heart–gill preparation, was able to demonstrate the ability of the gill of the eel to transport chloride against a concentration gradient. Afterwards, Keys and Willmer[3] described a type of cell in the gill of this and other species which they suggested might be the site of an active transport system, and tentatively termed it the 'chloride-secretory cell'.

Although similar cells have been described in a variety of marine, euryhaline and anadromous teleosts[4–10,14,15] under varying experimental conditions, their function in electrolyte excretion has not as yet been firmly established. Nevertheless, the adaptive responses observed after transfer between fresh and salt water[5,6], histochemical demonstrations of high cellular chloride content in fish adapted to sea-water[5], correlations between ability to tolerate sea-water and the occurrence of these cells[4,7], and finally the close correlation between their ontogenetical appearance in the migratory salmonids[8-10] and the development of tolerance to sea-water in these species[10-12] provide strong indirect evidence favouring the hypothesis.

On the other hand, several authors have felt that the 'chloride-cell' was not, in fact, associated with electrolyte metabolism. Thus Bevelander[13], on the basis of extensive comparative studies, concluded that the only specialized cells in the branchial epithelium were mucous cells. However, as Copeland[5] has pointed out, at least some of the cell types described by Bevelander as being 'mucous-secretory' had most of the cytological characteristics commonly attributed to 'chloride cells'.

Burden[14] has disclosed a puzzling situation in the course of work on the osmoregulatory ability of hypophysectomized *Fundulus*. Death in fresh-water following the destruction of the pituitary was accompanied by æsthenia, massive decreases in levels of plasma chloride and by atrophy of branchial mucous cells. No obvious variations in 'chloride cell' structure were observed. Copeland[5] has suggested that such cells in *Fundulus* may have dual polarity, excreting electrolytes in sea-water, and functioning in an absorptive role in fresh-water. The situation described by Burden may possibly be accounted for as a failure of the electrolyte absorptive process to cope with increased outward diffusion of electrolytes through surfaces no longer protected by a mucous layer.

Finally, Parry, Holliday and Blaxter[15] have objected to the secretory hypothesis on the grounds that a similar type of cell is found in the pseudobranch in large numbers, where by virtue of blood supply and position, a secretory function was not considered likely; that bilaterally pseudobranchectomized animals were able to survive in sea-water over long periods of time, and that under such conditions no adaptive increases in branchial 'chloride cell' numbers occurred.

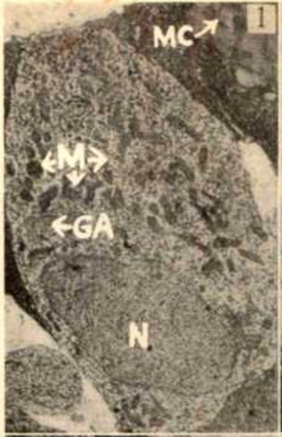
In view of the conflicting evidence on this theoretically important aspect of teleostean osmoregulation, we decided to include an electron microscope examination of the branchial epithelium in an investigation of the processes of parr–smolt transformation and adaptation to sea-water in hatchery-reared yearling Atlantic salmon. A part of this work has already been published in *Nature*[16].

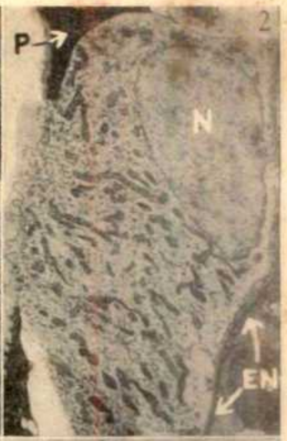
Gill structure was examined in parr adapted to fresh-water, and for purposes of comparison in a two-year-old trout (*Salvelinus fontinalis*), in smolt held for 72 hr. in sea-water (400–420 m.moles chloride/litre), and in smolt acclimatized to sea-water for 10 days. Reference to Figs. 1 and 2 of ref. 16 indicates that under these conditions parr displayed some loss of regulatory ability associated with the early stages of parr–smolt transformation, smolt adapted for 72 hr. to sea-water exhibited maximum deviations from the chloride-levels of fresh-water plasma, while fish held in sea-water for 10 days were considered to have completed the process of adaptation.

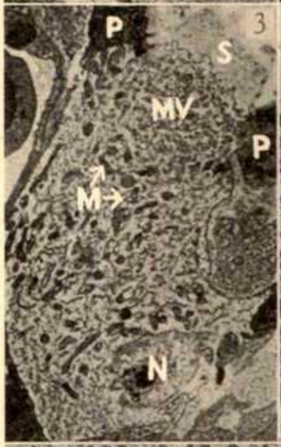
Gill filaments from the first gill arch were fixed in Dalton's fixative at pH 7·4 and embedded in methacrylate. Sections were cut on a Porter–Blum microtome with a diamond knife and photographed at × 2,000 to × 8,000 on a Phillips *E.M.* 75.

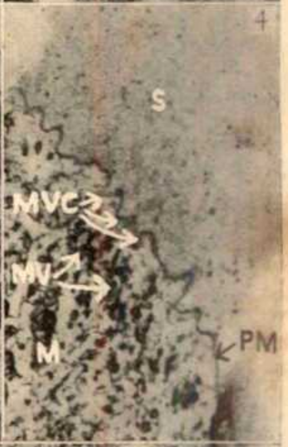
Salvelinus fontinalis (Fig. 1). In this species the 'chloride-secretory cells' were usually columnar, with basally situated ovoid nuclei. There were numerous mitochondria, the majority of which lay between the nucleus and the free pole of the cell. A relatively small number lay basal to the nucleus. Few mitochondria were present in the zone immediately below the apical plasma membrane. Individual mitochondria tended to be elongate with their long axes parallel to that of the cell.

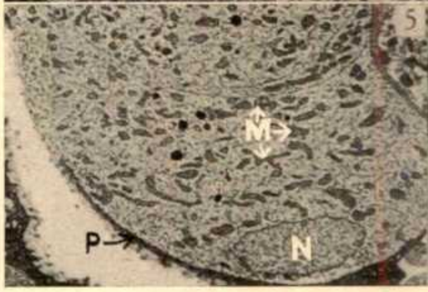
In the mitochondria-free apical zone there were numerous dense micro-vesicles, some of which were either in contact with mitochondria or with the plasma membrane.











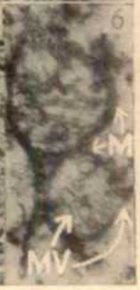


Fig. 1. *Salvelinus fontinalis*. *M*, mitochondria; *G.A.*, Golgi apparatus; *N*, nucleus; *M.C.*, mucous cell. (× 3,750)

Fig. 2. *Salmo salar*. Parr. *E.N.*, endothelium of blood vessel. *P.*, platelet cell. (× 4,500)

Fig. 3. *S. salar*. Smolt. 72 hr. adapted. *S.*, secretory vesicle; *M.V.*, micro-vesicles. (× 3,375)

Fig. 4. *S. salar*. Smolt, 72 hr. adapted. *P.M.*, plasma membrane; *M.V.C.*, micro-vesicles contacting the plasma membrane and discharging. (× 9,000)

Fig. 5. *S. salar*. Smolt. 10-day adapted. (× 3,000)

Fig. 6. *S. salar*. Smolt. 72 hr. adapted. Mitochondria with associated micro-vesicles. (× 45,000)

A variable number of juxta-nuclear Golgi bodies and short lengths of endoplasmic reticulum were also present in the cytoplasm.

The apical plasma membrane was overlain to a variable extent by adjacent electron-dense platelet cells, but in fully developed secretory cells the free membrane was usually the full width of the cell and bulged outward beyond the outer limits of the platelet cells. The membrane of the chloride cell was characterized by the presence of minor undulations, whereas that of the platelet cell usually projected, forming a number of microvilli. External to the cell membrane, there was in most instances a narrow amorphous layer, presumably a secretion product. This suggested a low level of secretory activity in these cells.

Salmo salar (Fig. 2). The 'chloride cells' of the Atlantic salmon parr were in general similar to those described for the trout. Rectangular rather than columnar cells were numerous. Minor differences were found in: (1) a more open cytoplasm with fewer mitochondria, (2) crenated nuclei, and (3) the presence of pale bodies resembling greatly swollen mitochondria. The cells gave the appearance of secretory exhaustion in some instances.

Salmo salar, 72-hr. smolt (Figs. 3, 4 and 6). In the 72-hr. smolt, the 'chloride cells' were either columnar or rectangular. In several features they were clearly distinct from those seen in parr. Mitochondria were often extensively vacuolated and had irregularly arranged cristæ. In some instances the mitochondria had ruptured and tubular cristæ were in the process of being shed into the cytoplasm (Fig. 6).

There was marked evidence of secretory activity with many cells having a mass of amorphous material containing scattered dense granules lying immediately beyond the free apical border. Such cells usually lay in a 'pit' formed by the adjacent platelet cells (Fig. 3).

In cells characterized by a high degree of secretory activity, the apical region was crowded with micro-vesicles of variable size resembling the cristæ shed from the more central mitochondria. With the production of a large secretory mass, the apical region lacked micro-vesicles and the mitochondria-free zone was larger (Fig. 4).

The 'chloride cells' of the 72-hr. smolt were, then, characterized by intense secretory activity of the merocrine type, and apparently related to mitochondrial activity.

Salmo salar, 10-day smolt (Fig. 5). In contrast to the cells just described, those of the 10-day smolt resembled the 'chloride cells' of the parr and trout. They varied from columnar to rectangular in shape, and contained numerous, clearly delineated mitochondria. Few apical vesicles were present and the free surface of the apical cell membrane was reduced. The mass of the secretory product was much less than that seen in the 72-hr. fish, and in many instances no secretory product could be distinguished. In general, a picture of reduced activity characterized the 'chloride cell' of the 10-day smolt.

The 'chloride cell' as described here is comparable to that demonstrated in earlier cytological studies[4–10,14,15] in distribution, overall shape and relative size, in the occurrence of a predominantly basal nucleus, high mitochondrial content, and in the presence of a mitochondria-free apical zone. We have therefore concluded that the type of cell described here represents that investigated in the investigations already cited, and as such is clearly distinct from the typical mucous cell.

The great similarity of these cells in the fresh-water adapted trout, in the parr salmon and in the 10-day smolt is strikingly correlated with chloride-levels in the plasma, which in each instance were apparently regulated within specific limits. It is perhaps notable that among this group some signs of secretory exhaustion were apparent in the cells of the parr, as evidence of an electrolyte imbalance is seen in these fish during the early stages of parr–smolt transformation[16].

In the occurrence of vacuolated mitochondria and an apical zone crowded with micro-vesicles, and in the presence of a large extracellular secretory mass, the cells of the 72-hr. smolt differed markedly from those encountered in animals in dynamic equilibrium with their medium. Such cells gave every indication of heightened secretory activity, which was correlated with sharp increases in electrolyte-levels in the body fluids. The process of secretion appeared to involve formation of large numbers of micro-vesicles by, or in close association with, mitochondria. These vesicles fused with the plasma membrane and were discharged, or released, to form the extracellular secretory mass.

This formation of micro-vesicles in close association with the apical mitochondria and seemingly related to the formation of the extracellular secretory mass is of particular interest in view of Copeland's demonstration by histochemical techniques of a marked concentration of chloride in this area in *Fundulus* adapted to sea-water[5]. It seems reasonable to conclude, then, that the extracellular mass described here is at least in part composed of inorganic electrolytes, possibly excreted in an organic matrix.

The present work corroborates earlier conclusions regarding adaptive changes resulting from transfer of fish from fresh-water into sea-water. Evidence for a secretory function has been presented. In view of Copeland's work already cited[5] and the correlation of enhanced secretory activity and structural changes with maximum increases in electrolyte-levels in plasma, we concluded that this work provides additional support for the hypothesis that the branchial 'chloride-secretory cell' is associated with extra-renal electrolyte excretion in teleosts adapted to the marine environment.

We acknowledge the financial support of the National Research Council of Canada and the Nova Scotia Research Foundation. A full report of this investigation, carried out in the Department of Anatomy and Zoological Laboratory, Dalhousie University, Halifax, Canada, will be published elsewhere.

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LETTERS TO THE EDITORS

SPACE SCIENCE

Satellite Orbit Perturbations in Vector Form

THE first-order perturbation theory of satellite orbits can be formulated simply and elegantly in terms of constants of the unperturbed motion using vector methods. For let the equation of motion be:

$$\ddot{\mathbf{r}} = -\frac{\mu}{r^3}\mathbf{r} + \mathbf{F} \tag{1}$$

where $\mu = GM$ (M = mass of the earth), and $\mathbf{F}$ is a general perturbing force (per unit mass). The energy E and angular momentum $\mathbf{h}$ (both per unit mass) defined as:

$$E = \frac{1}{2}\dot{\mathbf{r}}^2 - \frac{\mu}{r}; \quad \mathbf{h} = \mathbf{r} \times \dot{\mathbf{r}} \tag{2}$$

are constants of the unperturbed motion. Hamilton's integral, which may be written in the form[1]:

$$\mathbf{k} = \frac{\mu}{r}\mathbf{r} + \mathbf{h} \times \dot{\mathbf{r}} \tag{3}$$

provides a further constant of the unperturbed motion under an inverse square law force. Taking scalar products with $\mathbf{h}$ and $\mathbf{r}$, $\mathbf{k}$ is seen to be a vector of length μe lying along the major axis in the direction of apogee. The orbit is over-determined by E, $\mathbf{h}$ and $\mathbf{k}$ which give seven scalar equations.

In the presence of the perturbing force, E, $\mathbf{h}$ and $\mathbf{k}$ are no longer constant, but from (1) satisfy:

$$\begin{aligned} \dot{E} &= \dot{\mathbf{r}} \cdot \mathbf{F} \\ \dot{\mathbf{h}} &= \mathbf{r} \times \mathbf{F} \\ \dot{\mathbf{k}} &= (\mathbf{h} \times \mathbf{F}) + (\mathbf{r} \times \mathbf{F}) \times \dot{\mathbf{r}} \end{aligned} \tag{4}$$

When $\mathbf{r}$, $\dot{\mathbf{r}}$ and $\mathbf{h}$ are taken to refer to the unperturbed orbit, these are the equations of first order in the perturbing force.

It is best to discard two components of $\dot{\mathbf{k}}$ since this has the most complicated equation. Then $\dot{E}$ gives the change in the semi-major axis ($E = -\mu/2a$), $\dot{\mathbf{h}}$ the rotation of the plane of the orbit and the change in eccentricity [$h^2 = \mu a(1-e^2)$], while the rotation of the major axis in the plane is given by the component of $\dot{\mathbf{k}}$ parallel to the minor axis.

The quantities E, $\mathbf{h}$ and $\mathbf{k}$ will give the osculating elements at any time. However, for most purposes the interesting effects are those of period longer than the satellite in its orbit. These are given by the mean rates of change obtained by integrating (4) over a complete orbit, using either the true or the eccentric anomaly, as appropriate, as the independent variable.

The results can often be expressed partly in vector form. For the simple case of a constant force $\mathbf{F}$, corresponding, for example, to radiation pressure on a uniform sphere the orbit of which is entirely in sunlight, the effects are given by:

$$\dot{E} = 0, \; \dot{\mathbf{h}} = \frac{3}{2}\frac{a}{\mu}\mathbf{k} \times \mathbf{F}, \; \dot{\mathbf{k}} = \frac{3}{2}\mathbf{h} \times \mathbf{F} \tag{5}$$

This method has been applied to solar and lunar perturbations and to the effect of radiation pressure when part of the orbit is in darkness. It has also been applied to the second harmonic term in the Earth's gravitational field giving results in agreement with previous work[2].

A treatment essentially in terms of E, $\mathbf{h}$ and $\mathbf{k}$ appears to have been proposed first by Herrick[3], and Musen[4] has applied it to the case of a constant perturbing force giving effectively the results of (5) above.

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The Cosmic-Ray Observations of December 4, 1957

A RECENT communication from Legrand and Helary[1] reports an increase in the total component of cosmic radiation at Paris on December 4, 1957. The increase amounted to about 18 per cent and extended over three hours with the maximum occurring at 2245 U.T. A small increase in the nucleonic component at Thule preceded the Paris event by about three hours. Optical and radio data indicate a flare of importance 1 on the western hemisphere of the Sun at 1657 U.T. (ref. 2).

Intensity enhancements at the surface of the Earth are comparatively rare, only eleven cases having been observed. However, these cosmic ray flare events appear to have certain well-defined properties in common. These include correlation in time with a visible or inferred solar flare, a steep integral rigidity spectrum and approximate simultaneity of occurrence at all points on the Earth's surface accessible to the radiation. The steep rigidity spectrum is indicated by the relative rarity of events seen in ground-level neutron monitors as compared to detectors flown in balloons and the small magnitude (or absence) of the increases measured in the meson component compared to the nucleonic enhancements.

Collins and Jelly[3] have reported that ionospheric conditions were normal throughout the period in which the increase in cosmic radiation occurred and have also pointed out that solar activity was low. Since an undisturbed ionosphere at the time of a cosmic-ray flare is an unlikely event, it was thought worth while to examine the intensity of cosmic radiation monitored at the Sulphur Mountain Laboratory at this time. The Laboratory is on a mountain near Banff, Alberta, Canada (lat. 51·2° N., long. 115·6° W.), at 7,500 ft., and houses both neutron and meson monitors. The neutron monitor, consisting of three boron trifluoride counters in a paraffin – lead pile, has a counting-rate of 100,000/hr., and a cubical Geiger counter telescope containing 10 cm. lead

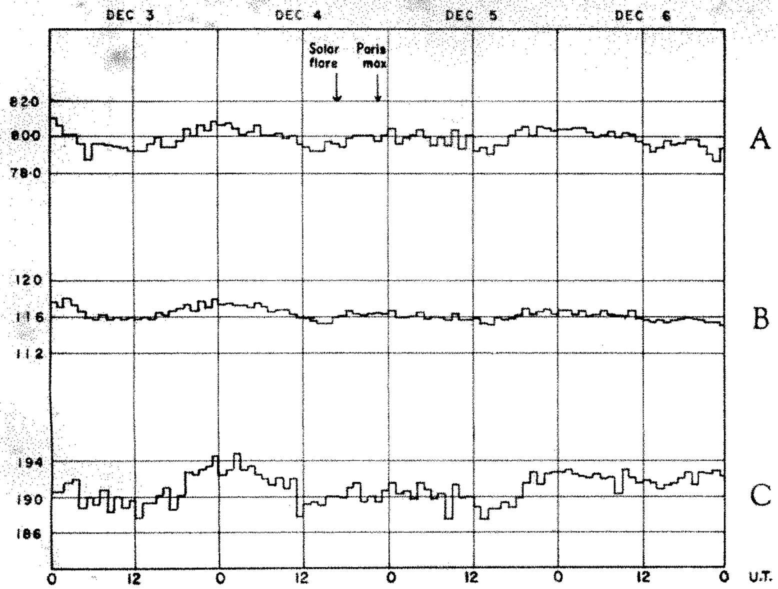

Fig. 1. Rates per hr. of hard, total and nucleonic components : *A*, hard component, *S.F.*, 1,024 ; *B*, total ionizing component, *S.F.*, 2,048 ; *C*, nucleonic component, *S.F.*, 512

measures the intensities of the total ionizing component and the meson component. The counting-rates are respectively 240,000 and 80,000/hr.

The results, corrected for pressure, are plotted in Fig. 1 for the period December 3–6, 1957. It is clear that all three components show no unusual variations during the period of the reported event ; this result is in agreement with other collected data[4] from a large number of stations. Since the data from Paris and Thule are not supported in the records of neighbouring stations, and since the times of the two maxima are well separated from each other and from the time of the optical flare, the events reported are difficult to reconcile with the emerging pattern of the development of cosmic-ray flares. In the absence of corroborative evidence of cosmic ray enhancements or of subsequent Forbush decreases at other stations, and with the negative results of the ionosonde data, it appears reasonable to assume that the reported increase was not associated with an injection of solar protons.

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Cosmic Noise Measurements from 1960 η1 at 3·8 Mc./s.

WE wish to report the results of an experiment to measure cosmic noise at 3·8 Mc./s. from an Earth satellite.

Solid-state radiometers, for the measurement of cosmic noise above the ionosphere, have been designed and built at the Defence Research Board Telecommunications Establishment in Ottawa. A single frequency unit, of a type already described[1], was part of the 1960 η1 (*Transit* II*A*) payload and operated satisfactorily for the life of the battery pack.

The satellite telemetry transmitter was turned on and off via a command link, and since command decisions were dependent on the payload battery condition, on some passes there was no telemetry. For the period June 24–27, 1960, the orbital parameters for η1 were as follows: inclination angle $(i) = 66·7°$, apogee altitude, 1,070 km.; perigee altitude, 625 km. The perigee and apogee occurred in the southern and northern hemispheres, respectively. Telemetry stations were located at Washington, D.C.; Fort Myers, Florida; Antigua, British West Indies; Santiago, Chile; Woomera, Australia; and Ottawa, Canada.

The equipment aboard *Transit* II*A* consisted of a superheterodyne receiver with a 40 kc./s. pass-band centred on 3·8 Mc./s., and to reduce errors arising from changes in gain and band-width, the receiver was switched continuously between the antenna and a resistive reference noise source. Temperature tests from $-30°$ C. to $+30°$ C. showed no measurable change in receiver sensitivity. The absolute value of the sensitivity was limited by the efficiency of the antenna since the main payload requirements did not permit the optimum antenna geometry and weight.

For the purposes of calibration, a single-turn terminated loop was permanently attached to each antenna. The loop was calibrated for a known field-strength, and was then used to obtain the input–output characteristics of the radiometer. Antenna pattern measurements indicated negligible departure from that of the ideal magnetic dipole.

For a terminated antenna in thermal equilibrium, the sky temperature is given by :

$$T = \frac{PA}{KB} \qquad (1)$$

where A = effective antenna aperture in m.^{2}; P = power flux in watts m.$^{-2}$ c.s.$^{-1}$; K = Boltzmann's constant; T = temperature in deg. K.; B = band-width c.p.s.; also

$$P = \frac{E^2}{Z} \qquad (2)$$

where Z = the impedance of the medium in ohms ; E = electric field strength of the incident energy in μV./m. for a noise band-width of 40 kc./s.

Table 1 is given in terms of the equivalent linearly polarized free-space field-strength, for a noise band-width of 40 kc./s. in a plane perpendicular to the major axes of the antennæ. To determine P, a correction factor of 1·5 must be made to the value of field strength, E_m, given in Table 1. This correction assumes an omni-directional noise field, whereas the calibrating field was linearly polarized. The expression for P must therefore be rewritten as :

$$P = 1·5\, E_m{}^2/Z \qquad (3)$$

Where the results were not affected by the radiometer sensitivity, the accuracy was dependent on antenna calibration and the stability of the telemetry link. At low signal-levels, the accuracy was limited by the noise signals generated in other apparatus

Table 1. Centre Frequency 3·8 Mc./s., Noise Band-width 40 kc./s.

June 24, 1960				June 25, 1960				June 26, 1960				June 27, 1960			
Time (G.M.T.)	Field strength (μV./m.)	Height (km.)	Comments	Time (G.M.T.)	Field strength (μV./m.)	Height (km.)	Comments	Time (G.M.T.)	Field strength (μV./m.)	Height (km.)	Comments	Time (G.M.T.)	Field strength (μV./m.)	Height (km.)	Comments
Blossom Point, Maryland, U.S.A. and Ottawa, Canada															
2010	0·8	1,055	Good record	0321	0·8	920	Good record	0303	0·65	880	Earth breakthrough, excellent record	0248	0·6	845	Good record
0338	0·8	880	Good record	0504	0·65	860	Good record	0448	0·8	860	Earth breakthrough, excellent record	0431	0·6	860	Good record, breakthrough
1827	0·7	1,045	Good record												
Fort Myers, Florida, U.S.A.															
2005	0·8	1,035	Good record	No records				No records				0247	0·6	830	Good record
												0433	0·6	810	Breakthrough
Santiago, Chile															
No records				No records				No records				0635	0·85	640	Poor record
Woomera, Australia															
0433	1·50	860	Fair record	1718	1·96	640	Fair record	0400	1·50	860	Good record	No records			
				0412	1·40	790	Poor record								
				1535	2·04	655	Good record								
Pantig (Antigua) British West Indies															
No records				1805	0·90	1,030	Poor record	0310	0·80	780	Poor record	0255	0·65	780	Poor record
												0438	0·65	760	Breakthrough

within the satellite and to a lesser extent by the noise figure of the receiver. From considerations of these factors it is estimated that, for all stations except Woomera, the probable error was +1, −5 db., and for Woomera, the probable error was ±1 db.

Certain features of the results are worthy of further discussion. On the night of June 25, 1960, high-level fluctuating signals were recorded when the satellite was approximately 45° N. latitude in the vicinity of Ottawa, Canada, moving on a north-to-south pass. The fluctuations occurred at the spin-rate of the satellite, which had its spin axis inclined at an angle of approximately 20° to an Earth tangent. At the time of the pass, the f_0F2 at Ottawa, Canada, was 3·5 Mc./s. and it is believed that the fluctuations were due to radiation from the Earth reaching the radiometer. This phenomenon occurred on two successive passes.

Records taken in the northern hemisphere (Ottawa, Canada and Blossom Pt., Maryland) showed no variation in noise power between day and night, whereas those taken at Woomera, Australia, showed considerable variation. Table 1 shows that the local night readings at Woomera were about 2·5 db. higher than the local day readings which were, in turn, 3 db. higher than those for the northern hemisphere.

The one usable record from Santiago, Chile, does not agree with the Woomera recordings even though both stations are at approximately the same latitude. The quality of the Santiago recordings is poor, due to telemetry station difficulties, whereas the Woomera recordings are very good. No ground ionospheric data, which might have resolved this discrepancy, are as yet available from either loc .tion.

The support and co-operation of the Applied Physics Laboratory of the Johns Hopkins University are gratefully acknowledged. Thanks are also due to the National Aeronautics and Space Administration of the United States for recording telemetry data and to the Naval Ordnance Test Station of the U.S. Navy for supplying payload battery power. The contributions of Dr. D. F. Page, of the Defence Research Telecommunications Establishment, in the receiver design and of E. D. R. Shearman, of the Department of Scientific and Industrial Research, Slough, England, in the interpretation of the data are also acknowledged.

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GEOPHYSICS

Odd Harmonics of the Earth's Gravitational Field

VARIATIONS in the perigee radius have been observed for three satellites so far. The first of these is *Vanguard I* (1958 beta), which was used by O'Keefe *et al.*[1] in calculating the third and fifth axial harmonics of the Earth's field. The other two are *Transit IB* (1960 gamma) and *Transit IIA* (1960 eta). Nominal values of the pertinent orbit parameters of these three satellites are listed in Table 1. The data

Table 1. ORBITAL PARAMETERS

Satellite	a, Earth radii	e	i, radians	C_p
Vanguard I	1·3615	0·1903	0·5977	−0·00102
Transit IB	1·0871	0·0278	0·8949	−0·00115
Transit II*A*	1·1312	0·0309	1·1639	−0·00026

on *Vanguard* are from O'Keefe *et al.*[1], those on *Transit* I*B* are given by Guier[2], and those on *Transit* II*A* have not been published before.

For our purposes, it is most convenient to express the perigee radius as a function of θ_p, the latitude of perigee, according to:

$$r_p = r_{po} + C_p \sin\theta_p \qquad (1)$$

Values of C_p are listed in Table 1. The probable errors in the values of C_p are believed to be about 7×10^{-5} for *Vanguard* and about 6×10^{-5} for the two *Transits*.

The dependence of C_p upon the orbit parameters is given by:

$$C_p = (J_3/2J_2) - (5J_5/4J_2P^2)\left(1 + \frac{3}{4}e^2\right)\left[\left(1 - \frac{7}{2}f + \frac{21}{8}f^2\right)/\left(1 - \frac{5}{4}f\right)\right] + (35J_7 16J_2P^4)\left(1 + \frac{5}{2}e^2 + \frac{5}{8}e^4\right)\left[\left(1 - \frac{27}{4}f + \frac{99}{8}f^2 - \frac{429}{64}f^3\right) \div \left(1 - \frac{5}{4}f\right)\right] \qquad (2)$$

In this, $f = \sin^2 i$ and $P = a(1 - e^2)$. The terms involving J_3 and J_5 were apparently first derived by O'Keefe *et al.*[1]. The convention that we are using in defining the coefficients J_2, J_3, etc., is specified by writing the gravitational potential as:

$$U = (1/r) - \sum_{n=2}^{\infty} (J_n P_n(\sin\theta)/r^n + 1) \qquad (3)$$

where $P_n(x)$ is the nth Legendre polynomial, normalized to equal unity when $x = 1$. The value of J_2 is approximately $1{\cdot}083 \times 10^{-3}$.

Substituting the data from Table 1, and the estimated errors, into equation 2 yields:

$$J_3 = (-2{\cdot}36 \pm 0{\cdot}14) \times 10^{-6}$$
$$J_5 = (-0{\cdot}19 \pm 0{\cdot}10) \times 10^{-6}$$
$$J_7 = (-0{\cdot}28 \pm 0{\cdot}11) \times 10^{-6}$$

These should be compared with the values:

$$J_3 = (-2{\cdot}4 \pm 0{\cdot}3) \times 10^{-6}$$
$$J_5 = (-0{\cdot}1 \pm 0{\cdot}1) \times 10^{-6}$$

that O'Keefe *et al.* obtained from *Vanguard* I data alone, neglecting J_7.

It is interesting that the value of J_7 obtained here is larger than the value of J_5, although the difference is less than the probable error. It may be that the values of the harmonics beginning with the fifth will be determined principally by the topography, and will thus tend to decrease slowly but erratically with increasing order.

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Measurements of Air Radioactivity in Italy and their Relation to the First Sahara Atomic Explosion

ON the occasion of the French atomic explosion of February 13, 1960, in the Sahara, a control system of radioactivity in the air was set up covering the whole of Italy. The controlling stations of the system were equipped to collect samples of atmospheric dust by means of suction through a filter. The β-activity of the samples was measured 120 hr. after they had been collected.

The stations were established throughout Italy (from north to south) as follows, the heights being in metres:

	Place	Height above sea-level (m.)		Place	Height above sea-level (m.)
1	Pian Rosà	3,488	15	Bari	4
2	Pallanza	212	16	Napoli	2
3	Ispra	251	17	Resina	610
4	Trieste	8	18	Brindisi	8
5	Milano	123	19	Alghero	21
6	Bologna	64	20	Monte Scuro	1,716
7	Genova	364	21	Elmas (Cagliari)	18
8	Monte Cimone	2,173	22	Messina	54
9	Pisa	3	23	Palermo	30
10	Livorno	3	24	Chinisia (Trapani)	74
11	M. Terminillo	1,875	25	Pantelleria	185
12	Casaccia (Rome)	154	26	Cozzo Spadaro (Syracuse)	40
13	Vigna di Valle	270			
14	M. S. Angelo	844			

Two intervals showing increased activity were observed which, although very weak, may be considered as a consequence of the passage of the tropospheric atomic dust cloud originated by the explosion of February 13, 1960, after it had rounded the Earth following an eastward course along the parallels of latitude. The first interval occurred between February 27 and March 2; the second interval between March 7 and March 15.

The two intervals were probably due to the passage of the radioactive dust cloud after it had completed the first passage around the Earth, divided in two parts driven along different itineraries by winds at different altitudes.

In the first interval, higher values were registered, all corresponding to the stations located at greater heights:

Place	Height above sea-level (m.)	Counts
Pian Rosà	3,488	15·00 pc./m.³
Monte Cimone	2,173	8·00 ,,
M. Terminillo	1,875	0·75 ,,
M. Scuro	1,716	1·8 ,,
M. S. Angelo	844	0·6 ,,
Resina	610	0·22 ,,
Vigna di Valle	270	0·8 ,,
Livorno	3	0·15 ,,

During the second interval, stations located on mountains were particularly involved by the passage of the radioactive cloud. In fact, measurements of radioactivity gave the following values:

Place	Height above sea-level (m.)	Counts
Pian Rosà	3,488	2·20 pc./m.³
Monte Cimone	2,173	1·0 ,,
M. Terminillo	1,875	0·5 ,,
M. Scuro	1,716	3·4 ,,
M. S. Angelo	844	0·6 ,,
Vigna di Valle	270	0·24 ,,
Livorno	3	0·12 ,,

The highest activity observed at these stations during the second half of March 1960 was:

Place	Counts
Pian Rosà	0·4 pc./m.^{3}
Monte Cimone	0·2 ,,
M. Terminillo	0·2 ,,
M. Scuro	0·2 ,,
M. S. Angelo	0·2 ,,
Vigna di Valle	0·2 ,,
Livorno	0·09 ,,

The activities registered were always markedly lower than the highest recorded in previous years. In 1958 Naples and Pisa registered the following highest values, namely, 67 pc./m.^{3} and 43 pc./m.^{3} respectively; in 1959 the highest value at Pian Rosà was 22·5 pc./m.^{3}.

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PHYSICS

Spin-Lattice Relaxation of Pairs of Chromium Ions in Ruby

The electron paramagnetic resonance spectrum of ruby contains many weak lines as well as those of single chromium ions. It has been established from the dependence of their intensity on the chromium concentration that most of these arise from pairs of ions. An example of the 'pair' spectrum is given in Fig. 1. A complete analysis is not available, but Rimai *et al.* have identified the lines due to nearest-neighbour pairs, and find an antiferromagnetic exchange interaction $JS_1.S_2$, with $J=250$ cm.$^{-1}$, to be present[1]. These pairs are not observed at low temperatures, being then almost all in their singlet ground-state. Most of the lines, however, come from more widely separated ions, and many persist even at 1·3° K. The variation of their intensities with temperature indicates that levels separated from the ground-state by 0–15 cm.$^{-1}$ are present.

The spin-lattice relaxation of the ion-pairs in a crystal containing 0·12 per cent chromium has been measured from the recovery after saturation of some of the more prominent lines in the X-band 'pair' spectrum. The recovery was usually adequately described by a single time-constant. The measured times are shown in Figs. 2(*A*–*E*). In part of the range the times vary more or less inversely as the temperature T, suggesting relaxation through single phonons with energies less than kT. At higher temperatures the relaxation times vary more rapidly, presumably as multiple-phonon processes assume importance. At the lowest temperatures the relaxation of some of the lines becomes independent of temperature. This may be evidence that phonons with energies equivalent to several deg. K. are concerned in the relaxation: a variation with $\{1-\exp(-E/kT)\}$ is to be expected in the relaxation between two excited levels if this takes place through single phonon processes to and from a third level the energy of which is lower by an amount E. Relaxation via an upper level, with times varying as $\{\exp(E/kT)-1\}$, has been observed in cerium[2]. Further experiments are necessary to decide between this explanation and the alternative that at the lowest temperatures the measurements were merely of the rate of transfer of spin energy from the pairs to the single ions by temperature-independent spin-spin relaxation.

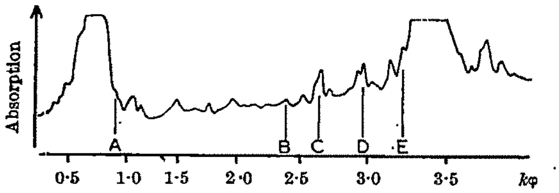

Fig. 1. Paramagnetic resonance of chromium pairs in ruby (room temperature spectrum, radio-frequency 9,400 Mc./s., magnetic field parallel to crystal axis). Single-ion lines occur at fields of 750 and 3,350 φ

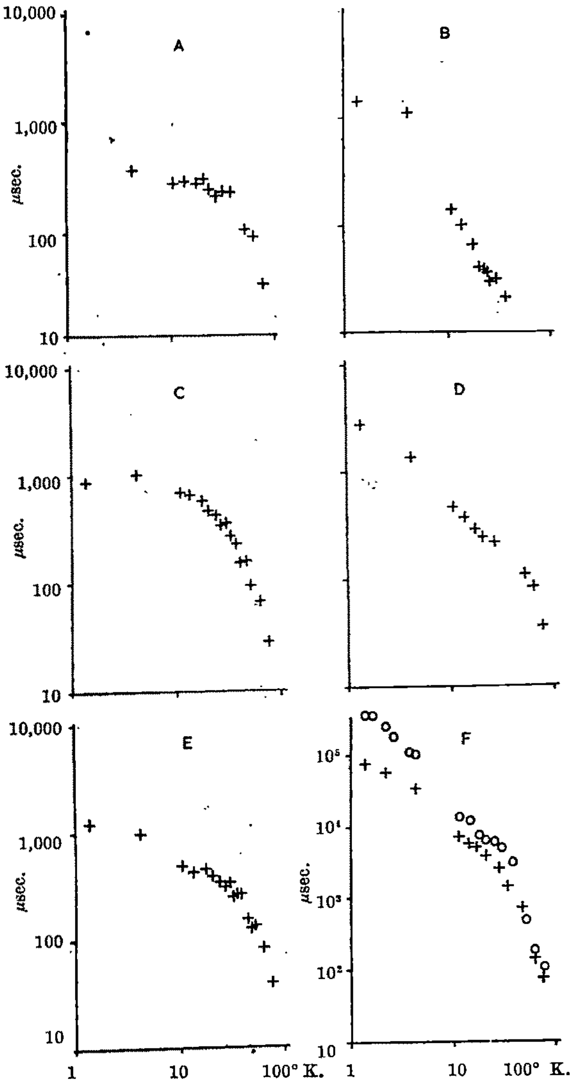

Fig. 2. Relaxation in a ruby containing 0·12 per cent chromium. *A*–*E*, Relaxation times measured from 'pair' lines *A*–*E* in Fig. 1; *F*, relaxation times of 750-φ line (circles) and of 3350-φ line (crosses) in single-ion spectrum

The relaxation of two of the single-ion lines has also been examined. The relaxation times were measured from the last part of the recovery, to reduce the effects of spin-spin relaxation[3]. The measured times are shown in Fig. 2*F*.

The relaxation of the pairs is 10–300 times faster than that of the single ions in the same crystal. Fast relaxation is to be expected in pairs of ions with spins greater than ½ if strong exchange interactions are present, as transitions involving energies of the order of the exchange energy are possible[3]. Since this energy may be much greater than that of the single-ion transitions, far more lattice modes may be available to bring about the relaxation of a pair than of a single ion. Possible means whereby the lattice vibrations might induce the transitions include the 'Van Vleck' modulation of the electric fields of diamagnetic neighbours, and also modulation of anisotropic exchange interactions, or of isotropic interactions in the presence of some other anisotropy.

The relaxation of the pairs seems sufficiently rapid to account for much of the relaxation of the single ions. For example, let us assume that the pairs relax on the average 100 times more rapidly than the single ions, and that each single-ion line is effectively coupled by spin-spin processes to ten 'pair' lines. Then if the populations of the 'pair' levels are 0·1 per cent of those of the single-ion levels, we find that the energy reaching the lattice from the pairs accounts for the entire loss from the single ions. This may possibly provide an explanation of the concentration-dependent relaxation times commonly observed in ruby[4].

A more detailed account of this work will be published on its completion. I should like to thank Dr. R. J. Elliott and Prof. J. H. Van Vleck for helpful discussions, and Drs. C. B. P. Finn, R. Orbach and W. P. Wolf for letting me have details of their work in advance of publication.

Note added in proof. Since this was written, an analysis of the 'pair' spectrum has been reported (Weber, M. J., Rimai, L., Statz, H., and de Mars, G., *Bull. Amer. Phys. Soc.*, 6, 141; 1961). The authors present indirect evidence for fast 'pair' relaxation, and point out the possible connexion with concentration-dependent single-ion times.

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'Memory' of Initial Remanent Magnetization and Number of Repeating of Heat Treatments in Low-temperature Behaviour of Hæmatite

It has recently been reported that the remanent magnetization of hæmatite (αFe_2O_3) indicates a marked thermal hysteresis phenomenon during a cooling–heating cycle to interpose the transition temperature of about 250° K., where the rotation of spin axis occurs: on cooling below 250° K. and then re-heating to room temperature, the direction of the remanent magnetization is still memorized in the specimen, although its intensity does not recover its original value before the cooling–heating cycle[1]. A satisfactory explanation of such behaviour has not so far been given, though much work has been carried out on this problem[2,3]. In this communication, new information on the 'memory' phenomenon will be reported.

The thermo-remanent magnetization and the isothermal remanent magnetization of the synthetic hæmatite powders were subjected to the repeated cooling–heating cycles between liquid nitrogen temperature and room temperature. In both cases, change in the intensity of remanent magnetization after the temperature cycling was measured by an astatic magnetometer at the room temperature. Recoveries relative to the initial remanent magnetizations, which were acquired in various magnetic fields, after the repeated cooling–heating cycles, are shown in Fig. 1 in reference to the numbers of cycle, where the thermo-remanent magnetization is indicated by circles and the isothermal remanent magnetization by dots.

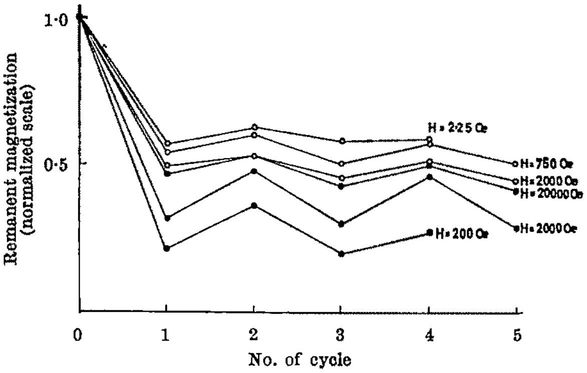

Fig. 1. Remanent magnetization of synthetic hæmatite powders at room temperature after the repeated cooling–heating cycles. ○, Thermo-remanent magnetization; ●, isothermal remanent magnetization

It is found in the figure that the recovery of the intensity of remanent magnetizations after the first cycle is closely dependent on the process leading to the remanent magnetization. The rate of recovery is generally large in the case of thermo-remanent magnetization compared with isothermal remanent magnetization. It is also found that in the case of thermo-remanent magnetization the larger the applied magnetic field, the smaller the rate of recovery, while in the case of isothermal remanent magnetization the relation between the rate of recovery and the applied field is reversed. In the very strong field, the rate of recovery in both cases is confused.

Another interesting fact obtained in this work is that the intensity of remanent magnetization changes systematically in response to the number of cycles for the repeated temperature cycling. As seen in Fig. 1, the marked decrease in the first cycle is followed by a 'zig-zag' change for the repeated cycling, in a series of experiments the intensity of remanent magnetization corresponding to any even number of cycles ($2n$th cycle) being generally larger than that corresponding to the consecutive odd number of cycles ($2n \pm 1$ th cycle). This 'zig-zag' phenomenon is also dependent on the process leading to the remanent magnetization. A relative amplitude of the 'zig-zag' to the initial remanent magnetization is largest in the isothermal remanent magnetization produced by a weak magnetic field and smallest in the thermo-remanent magnetization produced by a weak magnetic field. The amplitude amounts to about 15 per cent of the initial remanent magnetization in the former case, and only a few per cent in the latter case. It is also confirmed from a similar experiment shown in Fig. 2 that this 'zig-zag' phenomenon is conserved for a large number of cooling–heating cycles, though the amplitude decreases almost exponentially.

Considering that a similar phenomenon is observed in the hæmatite powder synthesized from iron (Johnson Matthey and Co., Ltd.), which is believed to be pure, this 'zig-zag' phenomenon may not be attribut-

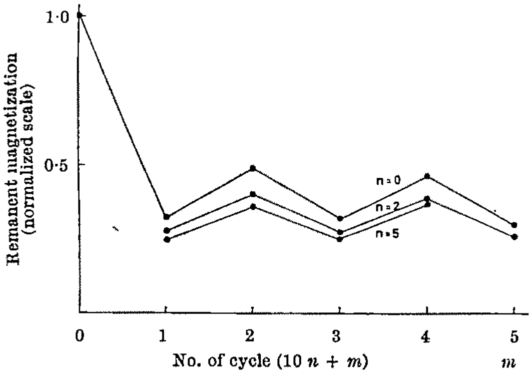

Fig. 2. Systematic change in the isothermal remanent magnetization of synthetic hæmatite powders at room temperature with large numbers of cooling–heating cycles (H = 2,000 oersteds)

able to any special characteristic of the hæmatite specimen. It has been confirmed, too, that the spatial orientation of the hæmatite specimen, relative to the geomagnetic field during the cooling–heating cycles, has no appreciable effect on the 'zig-zag' phenomenon. In view of these results, it may be concluded that this 'zig-zag' phenomenon is essentially related to a parasitic ferromagnetism of hæmatite.

A unified mechanism, through which the combined phenomena, namely, the memory and the 'zig-zag'. of the remanent magnetization of hæmatite at low transition temperature can be interpreted simultaneously, may be required in future work.

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The Use of the Term 'Flux'

Recent suggestions[1] for the adoption of a unit of neutron intensity in reactor physics and technology provide an opportunity to raise the issue of non-ambiguity. In the physical sciences, the term 'flux density' has been used since the earliest days to denote units of the relevant quantity per unit area traversed normally per unit time. The term 'flux', then, describes the product 'flux density × area'. Apparently owing to the isolation of nuclear physics during the period 1939–45, the practice seems to have become established in certain branches of this subject and its associated technologies of using the term 'flux' as a short hand for 'flux density'. This ambiguous use tends to deprive both terms of their original significance, and has led to considerable confusion.

The ambiguity is quite unnecessary and its continuance is surely most undesirable. We should like to propose, therefore, that any unit such as the chad, whatever its magnitude, should be defined as a unit of flux density, its dimensions being $NL^{-2}T^{-1}$.

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CRYSTALLOGRAPHY

Structure of Vapour-deposited Carbon

Although considerable information is available in the published literature on the effect of temperature and pressure of deposition on the microstructure and density of vapour-deposited carbon, only little has been reported on the pyrolytic graphite formed under these conditions from the crystallographic point of view. The purpose of this communication is to present X-ray results obtained in these laboratories from analysis of a series of experimental deposits produced over a range of high temperatures at a pressure of 50 mm. mercury. The perfection of three-dimensional crystallinity exhibited in some specimens produced at moderate deposition temperatures at this pressure is, to our knowledge, a new observation.

Pyrolytic graphite is deposited on a substrate by passing a vapour source of carbonaceous material at reduced pressure and raised temperature over a heated surface. The pyrolytic graphite produced in this way exhibits stacking such that the atomic basal planes are parallel, as in natural graphite, but the atomic layers are randomly oriented to each other. This arrangement destroys the periodic repetition of atom positions in the c axial direction, and produces greatly different electrical and thermal conductivities in the directions normal and parallel to the substrate surface.

X-ray patterns of pyrolytic graphite specimens produced in these laboratories, as well as a commercial graphite and a commercial pyrolytic graphite sample, were obtained with a 114·59-mm. Debye–Scherrer powder camera using copper K_{α} radiation. The details of the X-ray analyses of these samples are shown in Table 1 and Fig. 1. These results indicate that the

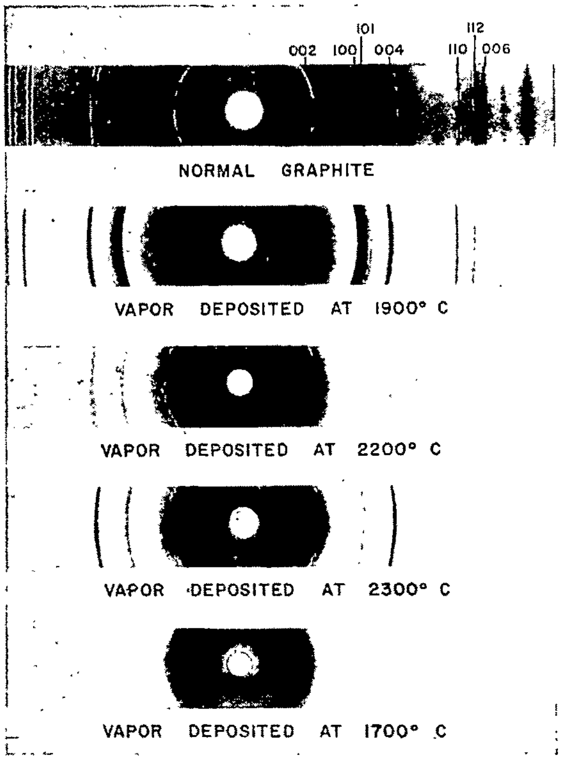
101
112
002
100
004
110
006
NORMAL GRAPHITE
VAPOR DEPOSITED AT 1900° C
VAPOR DEPOSITED AT 2200° C
VAPOR DEPOSITED AT 2300° C
VAPOR DEPOSITED AT 1700° C

Fig. 1. X-ray photographs of graphite

Table 1. PREPARATION DATA AND X-RAY EXAMINATION OF GRAPHITE

Temperature of deposition (deg. C.)	X-ray data	Method of deposition
2,300	Some disorder in the *c* direction	Resistance furnace 50 mm. mercury
2,200	Some disorder in the *c* direction	Resistance furnace pressure unknown commercial source
1,900	All (*hkl*) reflexions present	Induction furnace 50 mm. mercury
1,700	No (*hkl*) reflexions, lack of three dimensional structure	Resistance furnace 50 mm. mercury
—	Well-defined (*hkl*) reflexions	Commercial graphite substrate material

X-ray diffraction powder patterns obtained differ significantly from results of previously reported investigations. The published literature indicates that as the temperature of deposition decreases, there is a loss of the three-dimensional structure as evidenced by the increasing absence of (*hkl*) reflexions. From Fig. 1, it can be seen that at a deposition temperature of 1,700° C. and pressure of 50 mm. mercury, all the (*hkl*) reflexions are absent. At 1,900° C. there seems to be good atomic alignment in the *c* direction. At higher temperatures of 2,200° C. and 2,300° C., the ordering of the structure again becomes less perfect.

These preliminary observations indicate that high temperature *per se* is not the controlling factor for producing an ordered three-dimensional structure of pyrolytic graphite. The variables of pressure, rate of gas-flow, and method of heating the carbon-bearing gas phase, all have a marked influence on the resultant density and structure. Further investigations are now in progress to determine the effect of these variables on the structure and properties of vapour-deposited material.

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CHEMISTRY

Flash Photolysis of Ferrous Sulphate Solutions

KEEN has recently[1] described the kinetics of oxidation of Fe(II) by the transient species produced when a 2-μsec. pulse of 4-MeV. electrons is absorbed by an aqueous solution. We have observed that a similar oxidation occurs in the flash photolysis of ferrous sulphate solutions.

Using a quartz reaction vessel about 1 cm. in diameter and 20 cm. long and a parallel quartz discharge lamp (4 μf., 15 kV.), we find that about 5 μ*M* Fe(III) is produced for each flash irradiation of aerated ferrous sulphate solutions (10^{-4} *M*–5×10^{-3} *M* in 0·01 *N* sulphuric acid) as measured by its absorption at 3130 Å. The effective wave-length in the flash must be less than 2000 Å. since the solutions do not absorb appreciably above this wave-length, and furthermore we find that the oxidation is prevented when an atmosphere of nitrous oxide surrounds the flash tube and reaction vessel.

The oxidation does not result from the direct absorption by Fe(II), since the extent of oxidation is almost independent of concentrations from 10^{-4}–10^{-3} *M*, although it increases at higher, and falls off at lower, concentrations. Moreover, the oxidation occurs in a time-period well outside that of the flash. We have followed the reaction using the usual photomultiplier-oscilloscope technique and find that at low concentrations there are three distinct stages. Fig. 1, using a time-scale of 40 msec. per division, shows a rapid initial absorption followed by a slower one. With a time-scale of 1 msec. per division the rapid rise is resolved into the curve of Fig. 2. The third stage follows these and extends over seconds at 10^{-4} *M* Fe(II).

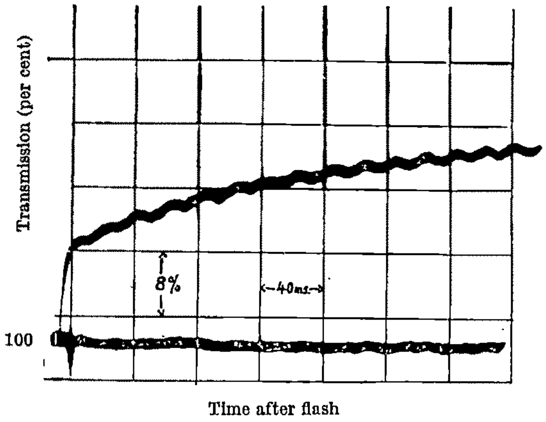

Fig. 1. Optical absorption of 10^{-4} *M* ferrous sulphate in 0·01 *N* sulphuric acid at 313 mμ after illumination with a 40-μsec. flash. Time-scale, 40 msec. per division

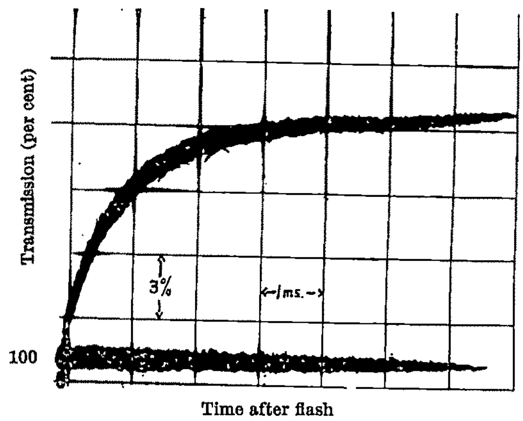

Fig. 2. As Fig. 1. Time-scale, 1 msec. per division

A precise kinetic analysis of each of these processes is difficult since they overlap to some extent, but each appears to be first order in the species which oxidizes Fe(II). The slowest step is also first order in Fe(II) concentration (Table 1) and has a second-order rate constant which is exactly that for the $Fe^{2+} + H_2O_2$ reaction[2] (60 ± 2 m.$^{-1}$ l.sec.$^{-1}$). The second stage is kinetically similar to a second-order constant 6×10^4 m.$^{-1}$ l.sec.$^{-1}$, but the first stage is apparently zero order in Fe(II) concentration (Table 1).

The similarity of rate constants suggests that the species oxidizing Fe(II) in the final step is hydrogen peroxide. The other two are more difficult to identify

Table 1. FIRST-ORDER RATE CONSTANTS FOR THE THREE STAGES OF OXIDATION OF FE(II) IN AERATED 0·01 *N* SULPHURIC ACID

Fe(II) m*M*	k_1 sec.$^{-1}$	k_2 sec.$^{-1}$	k_3 sec.$^{-1}$
0·10	370	6·0	—
0·20	380	12·3	—
2·0	—	—	0·120
5·0	—	—	0·310

but presumably one of them, possibly HO_2, is a precursor to the hydrogen peroxide, for example, by the reaction:

$$Fe^{2+} + HO_2 \rightarrow Fe^{3+} + HO_2^-$$

If, as we suggest, absorption by Fe(II) is not the primary reaction, the species oxidizing Fe(II) must arise from absorption by one or more of water, sulphuric acid and oxygen. The effective wavelength cannot be much below 1850 Å. because of absorption by the quartz, and if we ignore oxygen for the moment, the absorption coefficients for water[3] and ions from sulphuric acid[4] indicate that about 60 per cent of 1850 Å. light will be absorbed by water in 0·01 *N* sulphuric acid solution. Water is known to be photolysed[5]:

$$H_2O \xrightarrow{h\nu} H + OH$$

and the sulphate ion may ultimately give the same products:

$$SO_4^{=} + H_2O \rightarrow SO_4^{-} + H + OH^- \xrightarrow{H_2O} HSO_4^- + OH + H + OH^-$$

The hydrogen atom with oxygen will rapidly give water, so that we might expect HO and HO_2 to be the oxidizing entities.

However, on this basis the extent of the final slow step would be equal to the sum of the two preceding fast ones, whereas we find it is somewhat less than this. This may indicate that other species, possibly resulting from absorption by oxygen, are responsible for at least some of the oxidation. We would also expect both fast steps to be first order in Fe(II) concentration. The zero order found for the first stage could arise from the relatively slow formation[6] of the absorbing species $Fe^{3+}SO_4^{=}$ but we found the presence of 0·01 *N* sodium sulphate to have no effect on this rate.

Subsequent work with perchlorates (to be published, J. H. B.) shows that in the absence of oxygen the final slow step is absent and that the rate constant of the second step is unaffected. This suggests that the second step with a rate constant 6×10^4 m.$^{-1}$ l.sec.$^{-1}$ is:

$$Fe^{2+} + HO \rightarrow Fe^{3+} + OH^-$$

If this is the case it would imply that the reaction:

$$HO + HO \rightarrow H_2O_2$$

must have a rate constant $\ngtr 10^7$ in order for it not to compete with the Fe^{2+} reaction.

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Use of Flash Photolysis to initiate Detonation in Gaseous Mixtures

Thrush[1] has reported on the apparent heterogeneity of initiation of reaction in gaseous mixtures of acetylene and oxygen sensitized by nitrogen dioxide, when flash photolysis was used to produce a region of homogeneous chemical reaction. The techniques of using flash photolysis to initiate explosions of hydrocarbon–oxygen–nitrogen dioxide systems are well known[2,3]. This apparent heterogeneity has been explained by the appearance of detonation waves over very small distances. It occurred to me that flash photolysis might be used to initiate detonation waves of certain geometries in a current detonation and shock-wave shaping programme in this laboratory.

The results of one-dimensional experimentation are reported here.

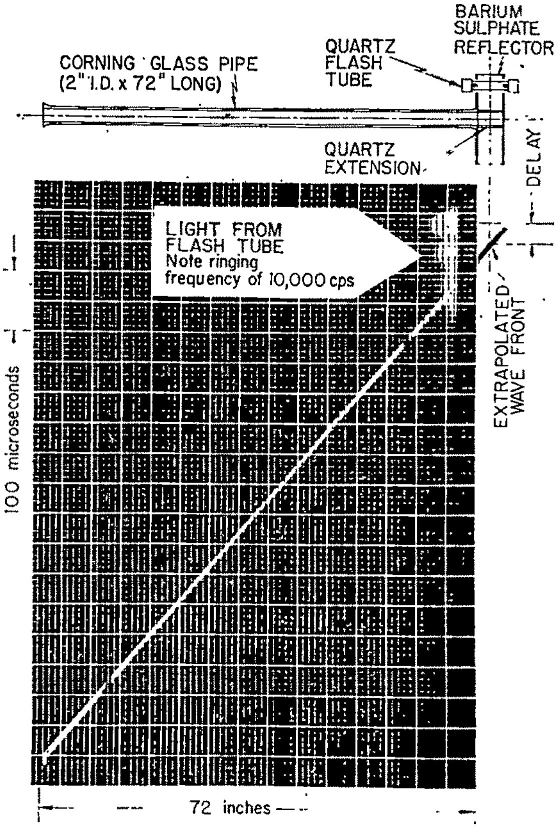

Fig. 1

Fig. 1 gives details of the apparatus employed together with a typical smear photographic record of the detonation wave. A 4-in. length quartz extension was fitted to a 6-ft. length of 2-in. internal diameter Corning glass pipe. The extension was provided with an ultra-violet reflector of barium sulphate and a simple linear quartz discharge tube produced the photolytic flash. The pipe was then oriented with respect to a longitudinal slit so that the detonation wave could be recorded on a simple revolving drum camera. The discharge through the flash lamp in an argon atmosphere was approximately 2,700 joules at 9 kV. The lamp was fired by reducing the pressure of the argon. It will be seen in the smear photograph that the light from the discharge tube was recorded as well as the luminous detonation wave. This gave

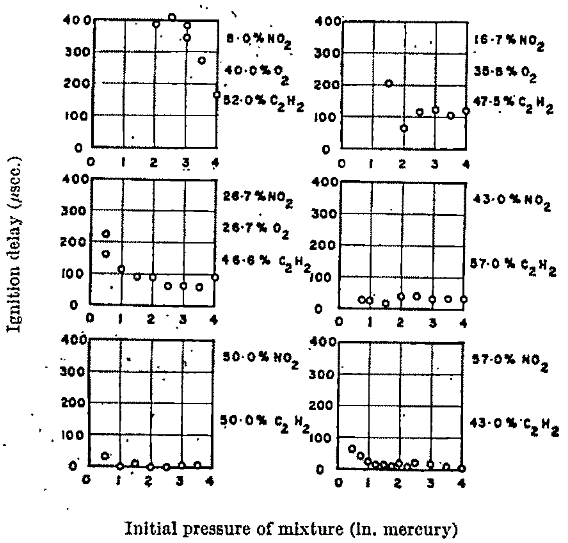

Fig. 2

a measure of the time of the flash with respect to the occurrence of detonation. The time between the first peak in the flash and the occurrence of the extrapolated detonation wave, at the centre of the quartz extension, was arbitrarily taken as the ignition delay.

This ignition delay has been the subject of the early experimentation, and Fig. 2 presents the range of the examination. The variables were the mixture composition and the initial pressure of the mixture. The effect of increasing concentration of nitrogen dioxide was to reduce the ignition delay, while increasing initial pressure had less effect.

In conclusion, stable detonation has been initiated in acetylene, oxygen, nitrogen dioxide mixtures immediately in space, but with a time-delay dependent on mixture composition and initial pressure of the mixture.

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Radiolysis of Carbon Tetrachloride in the Presence of Oxygen

Carbon tetrachloride containing oxygen from the air is very often used as a solvent for the isolation of radioisotopes, as a radiation–polymerization inhibitor, and as a basic material in chemical dosimetry and in sulphur-35 production as well. Its radiolysis is very significant in all these cases.

When carbon tetrachloride in the presence of oxygen is irradiated, free chlorine, phosgene and hexachlorethane are liberated. This system was investigated by Kailan *et al.*[1–3] many years ago, but their results cannot be interpreted quantitatively. Several authors recently investigated this system in the absence of oxygen and the results obtained by them differ greatly from each other[4–8]. It is impossible to deduce from them the mechanism of radiolysis of carbon tetrachloride in the presence of oxygen. Only Durup[9] occupied himself with the influence of oxygen on radiolysis of carbon tetrachloride, but hitherto nobody has measured the production of phosgene.

We have measured the production of chlorine in carbon tetrachloride irradiated in the presence of oxygen with soft X-rays[10]. Now we present further details about the origin of chlorine and phosgene produced by γ-radiation.

Irradiation was carried out at 22° C. using a cobalt-60 source with a dose-rate of $2·5 \times 10^{16}$ eV./min./ml. carbon tetrachloride. Carbon tetrachloride used was a product of Lachema, grade p.a., dry, without further purification. For the measurement of chlorine the spectrophotometer at wave-length 335 mµ was applied. Phosgene was estimated partly by the colorimetric phenylhydrazine modified method, partly by the acidimetric titration. Doses from a cobalt-60 source were measured by the Fricke ferrous sulphate dosimeter ($G_{Fe^{3+}} = 15·6$) and a correction on different electron densities was applied.

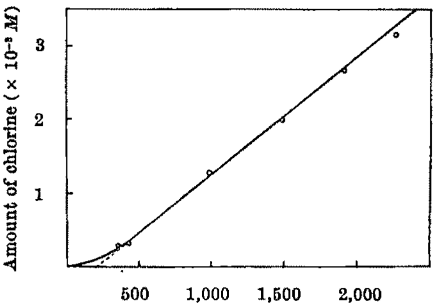

Fig. 1

From Fig. 1 we see that the initial production of chlorine is exponential (induction period up to 140 min. with our conditions) and then linear up to very high doses with $G_{Cl_2} = 3·6 \pm 0·3$. These results can be explained by the following reactions:

$$CCl_4 \xrightarrow{k_1 I\ CCl_4} CCl_3 + Cl$$

$$Cl + CCl_4 \xrightarrow{k_2} Cl_2 + CCl_3$$

since the calculated concentration of chlorine has the same graphical shape as the experimental curve, then:

$$[Cl_2] = k_1 I t - \frac{k_1 I}{k_2\,[CCl_4]} + \frac{k_1 I\, e^{-k_2 [CCl_4] t}}{k_2\,[CCl_4]} \qquad (1)$$

Through the analysis of the experimental curve from Fig. 1 by the help of equation (1) we then obtain the rate-constants $k_1 I$ and k_2:

$$k_1 I = 2·5 \times 10^{-8}\ \text{mole-litre}^{-1}\ \text{sec.}^{-1}$$
$$k_2 = 1·1 \times 10^{-5}\ \text{mole-litre}^{-1}\ \text{sec.}^{-1}$$

The G-value of chlorine is higher than in our earlier work[10], because there we used 30-kV. X-rays and the doses were applied in the induction period range.

In Fig. 2 the amount of phosgene is plotted against the radiation dose. We see that the production of phosgene is linear up to the dose 5×10^{19} eV./ml. with the G-value of phosgene $= 12·1 \pm 1·0$. The situation is rather complicated at high doses and the shape is non-linear. The production of phosgene can be explained by the reactions:

$$CCl_3 + O_2 \xrightarrow{k_3} COCl + Cl_2O$$

$$COCl + CCl_4 \xrightarrow{k_4} COCl_2 + CCl_3$$

$$2\,CCl_3 \xrightarrow{k_5} C_2Cl_6$$

according to which the concentration of phosgene is proportional to the dose:

$$[COCl_2] = \frac{2\,k_1 k_3}{k_5}[O_2]\,I^{1/2}t \qquad (2)$$

We are working now on confirming equation (2).

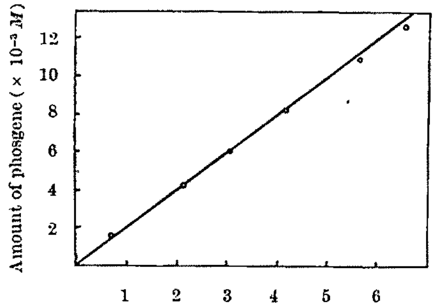

Fig. 2

Both G_{Cl_2} and G_{COCl_2} obtained here are comparable with the results of Durup[9] and other authors[5,7] where $G_{Cl^\cdot} = 3\cdot9$ and $G_{-O_2} = 8\cdot3 \pm 1\cdot0$. According to our reactions the value of $G_{Cl^\cdot}$ must be the same as G_{Cl_2} and approximately $G_{-O_2} \doteq G_{COCl_2}$. For the time being, we have no explanation for the difference existing between G_{Cl_2} in the absence of oxygen[5-8] and in the presence of oxygen.

We are indebted to Prof. Běhounek for his interest and to our co-workers from the Dosimetric Department of the Nuclear Research Institute for their help.

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Liquid Chromatographic Separations using Rubber as the Stationary Phase

In a previous communication[1] it was reported that certain polymers could be separated from mineral oil by a liquid chromatographic method. n-Heptane was used as the mobile phase and small pieces of thin vulcanized natural latex as the stationary phase. It has now been found that compounds of lower molecular weight can also be separated by this method.

The use of rubber for the separation of compounds of low molecular weight was first described by Boldingh[2]; he found it advantageous first to swell the rubber with a different solvent from that used as the mobile phase. The use of a cross-linked dextran ('Sephadex') for separating compounds in aqueous solution has been described by Gelotte[3]. Vaughan[4] has mentioned the possibility of using cross-linked polystyrene beads for this purpose.

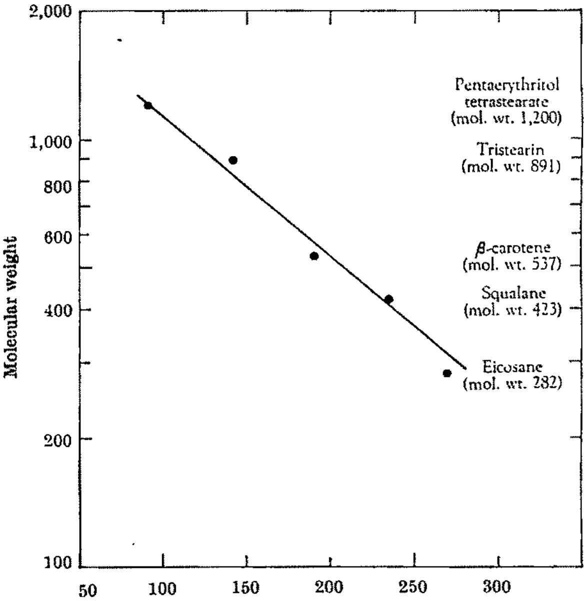

Fig. 1. Effect of molecular weight on corrected retention volume

Boldingh used a fraction of a commercially available rubber powder; but it has been found in these laboratories that columns packed with a similar rubber powder were less efficient than those packed with pieces of surgeons' finger-cots. A column 25 mm.

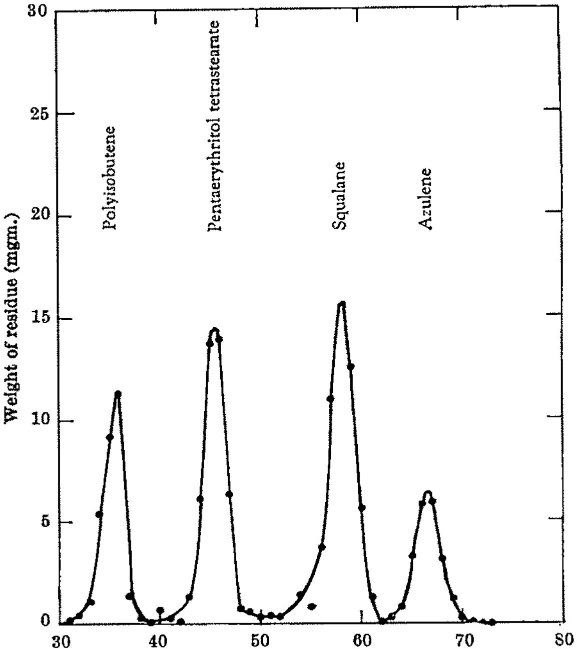

Fig. 2. Separation of a mixture of polyisobutene (mol. wt., 18,000), pentaerythritol tetrastearate (mol. wt., 1,200), squalene (mol. wt., 423) and azulene (mol. wt., 128)

in diameter surrounded by a water jacket at 30° C. was used, packed tightly to a length of 107 cm. with pieces of surgeons' finger-cots passing an 8 mesh B.S. sieve when dry, and retained on a 325 mesh B.S. sieve when swollen with toluene.

The substance (50–100 mgm.) under study was dissolved in 5 ml. of toluene and placed on top of the packing. After the solution had passed into the packing, further toluene was added and the flow-rate adjusted to about 15 ml. an hour. Fractions of 10 ml. were collected in which the solute was determined either by evaporating the toluene and weighing the residue, or colorimetrically in the case of β-carotene and azulene. A graph of corrected retention volume (the retention volume of the elution curve maximum less the void volume of the column) against logarithm molecular weight is shown in Fig. 1. It will be seen that the relationship is approximately linear. An attempt to determine the retention volume of 'Carbowax' polyethylene glycol 1540 was unsuccessful, a very flat elution curve being obtained.

The column used for the above work gave peaks with irregular trailing edges due to the uneven flow of the mobile phase through the column. A second column, 15 mm. in diameter and 207 cm. long, was prepared. Also, the rubber packing was reduced in size so that it passed through a 60 mesh B.S. sieve; and it was packed loosely by allowing it to settle under its own weight. This column gave symmetric elution curves, and the linear relationship between corrected retention volume and logarithm of the molecular weight was again obtained.

The elution curve obtained in the latter column for a mixture of 23·1 mgm. of poly*iso*butene (average molecular weight 18,000), 46·4 mgm. of pentaerythritol tetrastearate, 50·6 mgm. of squalane and 20·8 mgm. of azulene is shown in Fig. 2. Fractions of 5 ml. were collected; recoveries of the compounds ranged from 91 to 103 per cent.

The Directors of Esso Research Ltd. are thanked for permission to publish this communication.

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BIOPHYSICS

Influence of Atmospheric Oxidation on Supercontraction of Wool Fibres reduced with Thioglycollic Acid

The rate of supercontraction of a keratin fibre is progressively enhanced by chemical treatment which results in the breakdown of the disulphide cross-linkages in the fibre. The maximum supercontraction value is, however, not greatly affected by such treatment[1]. Fibres, in which the disulphide cross-linkages have been reduced with thioglycollic acid, contain a high percentage of sulphydryl groups[2]. These sulphydryl groups tend to undergo atmospheric oxidation, which causes the formation of new —S—S— cross-linkages[3], a reaction which can be prevented by blocking the reactive —SH groups. The question arises as to what the effect of such oxidative changes will be on the supercontraction of reduced fibres.

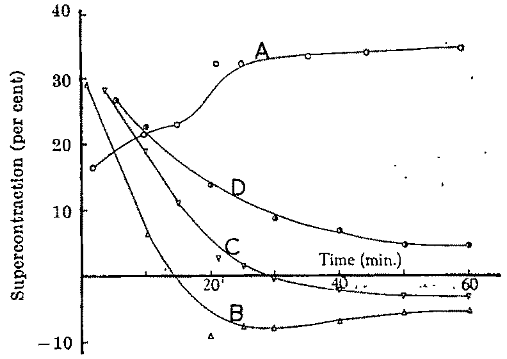

Fig. 1. Fibre reduced for 3 hr. in 1 *M* thioglycollic acid. *A*, normal untreated fibre; *B*, immediately after reduction; *C*, 8 days after reduction; *D*, 20 days after reduction

The microscopic technique, described by Haly and Griffith[4], was employed for obtaining the kinetic supercontraction curves of the fibres.

A sample from a fleece of 64's Merino wool was washed successively in petroleum ether, 0·1 per cent aqueous solution of 'Lissapol *N*' (Imperial Chemical Industries, Ltd.), water, ethanol and finally ether, all at room temperature, and then dried in a stream of cold air.

Sections of about 1·5 cm. were cut from the root end of a fibre. Snippets, 0·5–1 cm. in length, from both ends of this section, were supercontracted in 7·82 *M* lithium bromide solution at 90° C. If both snippets showed the same supercontraction behaviour, it was assumed that the fibre section was homogeneous along its length. The fibre section was then treated for 3 or 7 hr. at 35° C. with a 1 *M* solution of thioglycollic acid in *p*H 5·2 acetate buffer[5]. After the required reaction-time had expired, the fibre was washed well in oxygen-free distilled water on a sintered glass filter. It was then dried rapidly in a stream of air at 40° C. When dry, a snippet was cut off and supercontracted. The rest of the reduced fibre was stored in the absence of direct light. At various

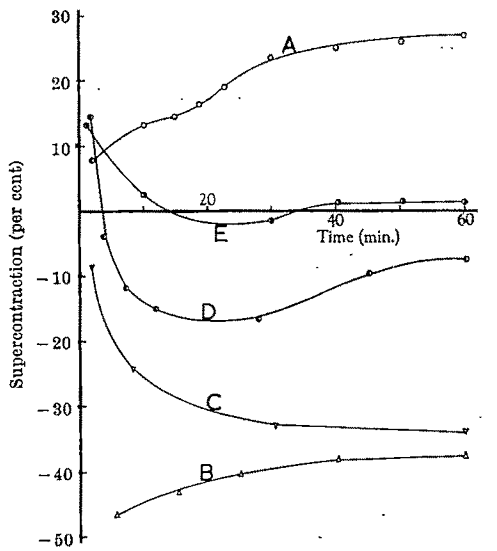

Fig. 2. Fibre reduced for 7 hr. in 1 *M* thioglycollic acid. *A*, normal untreated fibre; *B*, immediately after reduction; *C*, 4 days after reduction; *D*, 10 days after reduction; *E*, 26 days after reduction

time-intervals, snippets were cut off and supercontracted.

The results are shown in Figs. 1 and 2. It is obvious that reduction of 3 hr. is sufficient in the case of Merino wool to break the fibre down to such an extent that partial solution takes place in the contractant. This partial solution could, in fact, be observed under the microscope, particularly at the snippet ends. Due to this partial solution, progressive elongation takes place after the initial rapid contraction. This contraction, which gave values in good agreement with normal equilibrium contraction, was in certain cases so rapid that it could not be photographed effectively at the one-second exposure-time used, and, consequently, no contraction values are shown in the figures. However, as the fibre is progressively exposed to atmospheric oxidation, the extent of partial solution decreases until final contraction values are eventually obtained corresponding to positive contraction. This is probably indicative of the restoration, through atmospheric oxidation, of the disulphide network which is mainly responsible for keratin stability. It would, therefore, be inadvisable to perform supercontraction studies on reduced wool unless precautions are taken to prevent reformation of cross-linkages.

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Ultrasonic Detection of Choledocholithiasis

Present-day methods of detecting gall-stones in the common bile duct at operation are not wholly satisfactory. Stones may be missed in spite of careful palpation, cholangiography and direct exploration of the common bile duct. An attempt has been made to use pulsed ultrasound at the time of operation as an adjunct to their detection.

The apparatus used was a Kelvin and Hughes Mark V *F* flaw detector which is used industrially for the detection of flaws in homogeneous structures such as steel. The detecting probe contains both transmitting and receiving transducers and operates at 2·5 Mc./sec. The transducer emits pulsed ultrasound which is transmitted through a liquid or solid medium with attenuation and is reflected wholly or in part from the surface of any obstruction, the acoustic impedance of which differs sufficiently from that of the medium. The reflexion or echo is picked up by the receiving transducer and the electrical signal generated is amplified and displayed on a cathode ray oscilloscope with a linear time base to give a rough depth calibration (referred to as *A*-scope presentation).

Gall-stones of differing composition (cholesterol, pigment and mixed) and varying in size from 3 to 30 mm. were examined in a tank of saline and definite echoes were obtained from them. They were located by adjusting the position of the transducer head. The optimum range was 3–10 cm. Very slight movement of the transducer produced a marked difference in the size of the echo.

Similar findings were obtained from gall-stones in fresh post-mortem bile ducts and in gall-bladders removed at operation. There were, however, two main differences; the minimum size of gall-stone detectable in bile ducts was 6 mm., and detection proved difficult when the walls of the biliary ducts were grossly thickened by disease. Similar results were obtained when gall-stones in the common bile duct were detected at operation. To provide a liquid medium between the probe and the common bile duct, about 0·5 l. of sterile water was poured into the upper abdomen. The probe was enclosed in a sterile latex sleeve.

Although it is possible with *A*-scope presentation to use ultrasound to detect gall-stones in the common bile duct at operation, the technical difficulties are considerable[1]. It would seem that further research using *B*-scope with plan position indicator[2], which gives a two-dimensional picture, may have something more reliable to offer the surgeon.

We wish to express our gratitude to Prof. W. R. Spurrell, Prof. C. B. Allsopp, Prof. R. Warwick and Prof. G. W. Taylor in whose departments at Guy's and St. Bartholomew's Hospitals the work was carried out. We would also like to acknowledge a grant for this work from the Clinical Research Committee, Guy's Hospital, and to thank Messrs. Kelvin and Hughes for the generous loan of the ultrasonic flaw detector, and the London Rubber Co. for providing the latex sleeves.

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BIOCHEMISTRY

Introduction of the 16α-Hydroxyl Group into Œstrogens by Streptomycetes

Papers concerned with the microbiological transformation of steroids have appeared frequently in the past seven years; however, no examples of the hydroxylation of steroids with a benzenoid *A* ring have been observed. We have been able to introduce a hydroxyl group into the 16α-position of œstrone and œstradiol with two strains of *Streptomyces halstedii* (*ATCC* 13,499 and *NRRL B*-2138) and *Streptomyces mediocidicus ATCC* 13,278.

The cultures were grown for two days in shake flasks at 28° on a medium which contained yeast extract, cerelose, 'NZ amine' type *B* (an enzymatic digest of casein purchased from Sheffield Chemical, Norwich, N.Y.) and calcium carbonate at an initial *p*H of 6·7. This growth in 100 ml. was used to inoculate 2 l. of the same medium contained in a 4 l. fermenter. This mixture was incubated at 28° with stirring at 1,750 r.p.m. and aeration at one volume per volume per minute for 22 hr. After this period, 500 mgm. of œstrone were added in powdered form. The *p*H of the broth was approximately 6·5. The fermentation was continued under the same conditions, for 24 hr. At this time, the whole broth was extracted twice with an equal volume of chloroform. The concentrated extract was qualitatively examined by means of paper chromatography[1] to determine the transformation products. A ferric chloride colour test was used. Two major transformation products plus œstrone were observed. These products were isolated from the extract by silica-gel partition chromatography in which ethanol was the stationary phase and methylene chloride the mobile phase. The effluent fractions which contained the first more polar compound, were combined. Crystals were obtained, m.p. 214–216°, $[\alpha]_D^{24} + 174{\cdot}8$ (methanol) λ(KBr, max.) 2·97μ (OH) 5·80 (17-ketone). The melting point and optical rotation are in good agreement with those published for 16α-hydroxyœstrone[2]. The second more polar compound which migrated on papergrams in the œstriol region was also obtained crystalline, m.p. 281–283°, $[\alpha]_D^{24} + 56$ (dioxane). These properties are the same as those reported for œstriol[3]. The infra-red spectrum is identical with that of authentic œstriol.

Œstradiol was also converted to 16α-hydroxyœstrone and œstriol. These results indicate the presence of both a hydrogenase and dehydrogenase as well as a 16α-hydroxylase in the above-mentioned micro-organisms.

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Effects of *Tris*(*p*-Aminophenyl)-Carbonium Salts and Related Compounds on Experimental Schistosomiasis and Paragonimiasis

IN 1952, we initiated a programme directed toward the synthesis and biological evaluation of a variety of triphenylmethane derivatives as potential schistosomicides in the hope that a useful curative drug could be developed. To date, more than two hundred have been tested in albino mice and several in rhesus monkeys experimentally infected with a Puerto Rican strain of *Schistosoma mansoni*. Several compounds have also been tested against the lung fluke *Paragonimus kellicotti* in dogs. In the summary presented below, all amounts of drug are expressed as carbonium ion.

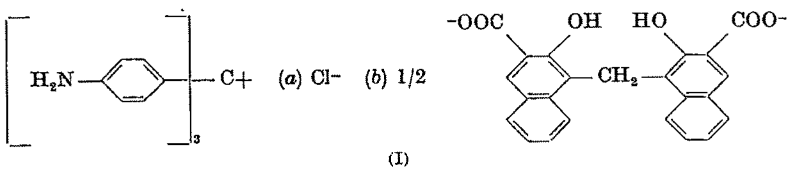
In the early stages, we found that a commercial sample of *para*rosaniline hydrochloride (also known as *para* magenta, basic magenta, magenta, parafuchsin (*e*), *para*-roseine, *CI*-676 (old) and *CI*-42500 (new)) exhibited a lethal effect on both mature and immature worms in mice when fed in the diet and against mature worms in monkeys when administered by gavage. These results demonstrated that a moiety present in the commercial dye might have not only therapeutic but also protective properties.

Chemical and physical analyses on various commercial samples of the dye indicated that *tris*(*p*-aminophenyl)carbonium chloride (TAC chloride) (I*a*) was usually the major component, although varying amounts of metal salts, *bis*(*p*-aminophenyl)-(4-amino-*m*-tolyl) carbonium chloride, 4,4′,4″-methylidynetrianiline, and N-aryl-*tris*(*p*-aminophenyl)carbonium salts were also present. Since the major moiety was the most likely active principle and such complex mixtures are not suitable for systemic use as drugs, we isolated a highly purified form of TAC chloride. The structure of this compound was substantiated by reduction to 4,4′,4″-methylidynetrianiline which was identical with authentic samples of this material prepared by the condensation of *p*-acetamidobenzaldehyde or 4,4′-diaminobenzhydrol with aniline. Subsequent testing of TAC chloride demonstrated that it had the antischistosome activity previously observed with the commercial dye and, in addition, protective activity in both mice and monkeys.

Although TAC chloride and its base [*tris*(*p*-aminophenyl)methanol](TAC hydroxide) were well tolerated in mice and monkeys at levels necessary for antischistosomal activity, such amounts of both compounds caused emesis and gastritis in dogs. We then searched for other TAC salts[1] which might retain good activity but cause less gastrointestinal reaction and this led to *tris*(*p*-aminophenyl)carbonium salt with 1/2 f.wt. 4,4′-methylene*bis*(3-hydroxy-2-naphthoic acid) (TAC pamoate) (I*b*). It was prepared[1] by allowing purified TAC chloride to react with the disodium salt of 4,4′-methylene*bis*(3-hydroxy-2-naphthoic acid) in aqueous methanol and was isolated as a hydrated green amorphous solid with an indefinite melting point, beginning at 220° C. A representative sample containing 2·5 per cent water of hydration has the following absorption spectrum: ($\lambda_{\max.}$ in 50 per cent MeOH–50 per cent *p*H 4·4 phosphate buffer (ε) 237 (133,000), 289 (50,300), 545 (188,000) mμ; $\lambda_{\max.}$ in 50 per cent MeOH–50 per cent 0·1 normal sodium hydroxide (ε) 288 (19,400), 365 (6,560) mμ).

Curative studies in mice established that TAC pamoate was active against mature worms over a wide range of well-tolerated doses (200–1,600 mgm./kgm./day) when given orally either in the food for 2–4 weeks or by gavage for 10 days. The dose-response curve was relatively flat. When administered intraperitoneally, the compound was only feebly active. The acute oral LD_{50} in normal mice is greater than 7 gm./kgm. In the monkey, TAC pamoate was well tolerated and suppressed ova production at gavage doses of 100–400 mgm./kgm./day for 10–20 days. TAC pamoate was much less emetic in dogs than TAC chloride.

Inasmuch as 4,4′,4″-methylidynetrianiline is a likely metabolite of the TAC salts[2], it was of interest to compare the schistosomicidal activity of this compound with TAC chloride *in vitro*. TAC chloride proved to be rapidly schistosomicidal *in vitro* at 50 γ/ml. and slowly lethal at 12·5 γ/ml., while 4,4′,4″-methylidynetrianiline was inactive within 72 hr. at these concentrations. These results suggest that TAC salts are schistosomicidal *per se*, but do not, of course, rule out the possibility that there may be active metabolites. Further investigations revealed that the antischistosomal effects of TAC hy-



(I)

droxide in mice were not reversed by massive doses of *p*-aminobenzoic acid, whereas the antitrypanosomal activity of commercial pararosaniline in mice is antagonized by the acid[3]. Worms in mice treated with TAC pamoate began to shift to the liver after seven days of medication; death of the worms lagged about six days behind the shift to the liver. Live schistosomes from mice treated for nine to eleven days were stained and examined microscopically for evidence of drug-induced damage. The principal changes were a reduction in the amount of pigment in the cæca of the females and atrophy of the vitellaria. In addition, there was a suggestion of atrophy of the oocytes in the posterior end of the ovaries. The general impression gained from these investigations is that TAC pamoate acts slowly by interfering with nutrition but has little obvious specific organ or tissue toxicity for the schistosomes.

When administered orally to dogs infected with *P. kellicotti*, both TAC chloride and TAC pamoate induced a sustained dimunition in egg production and egg abnormalities, which included shape variations, opaqueness, the appearance of a diffuse germinal mass, and indistinct vitelline cells. When normal adult lung flukes from an untreated dog were incubated for 4 days with 12·5 or 50 γ/ml. of TAC chloride in horse serum, the worms survived but produced similar abnormal eggs; the number was proportional to drug concentration. However, when normal eggs were incubated with 125–500 γ/ml. of TAC pamoate *in vitro*, the only change was a slight staining of the outer covering of the egg. Therefore, it seems likely that these TAC salts act on the reproductive system of these worms rather than on the intact eggs after oviposition.

Among the various triphenylmethanes we have synthesized and tested against *S. mansoni* in mice, the structural requirement for schistosomicidal activity is highly specific. Thus, *tris*(*p*-aminophenyl)-carbonium salts, or derivatives that afford such salts in normal use, such as *tris*(*p*-aminophenyl)methanol and 4,4′,4″-(methoxymethylidyne)trianiline, kill a high proportion of *S. mansoni* in mice. However, curative activity is abolished or drastically reduced when these structures are modified by: (1) reduction to the leuco base (4,4′,4″-methylidynetrianiline); (2) removal of one or two amino-groups; (3) alkylation, halogenation or sulphonation of the benzene rings; (4) alkylation of the amino-groups; (5) removal of one *p*-aminophenyl group; (6) replacement of one or more of the amino groups with a hydroxyl group or a hydrogen or chlorine atom; (7) replacement of the central carbon atom with a sulphur, nitrogen or arsenic atom. These results are in agreement with early reports by Wright *et al.*[4] and Schubert[5] that rosaniline, gentian violet and alphazurine lack appreciable action against mature *S. mansoni*.

During the last stages of this work, Yea-lin and Kuang-sheng[6] reported that rosaniline hydrochloride exhibits certain therapeutic effects on *S. japonicum* infections in mice and rabbits. Recently, Yea-lin[7] revised downward the claims of activity of rosaniline hydrochloride and reported *para*rosaniline hydrochloride to be effective against *S. japonicum* in mice. In several respects our results do not appear to parallel those reported by Yea-lin[7] on the activity of fourteen compounds related to rosaniline against *S. japonicum* in mice. However, uncertainty regarding the composition of the compounds he tested and differences in species and test methods employed preclude a satisfactory assessment of these differences.

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Starch-gel Electrophoresis of Anterior Pituitary Hormones

SEPARATION of pituitary hormones by zone electrophoresis on paper has not proved very successful, possibly due to complex formation between proteins[1]. Starch-gel zone electrophoresis introduced by Smithies[2,3] separates proteins not only by their net charge but also by their molecular size. This procedure has superior resolving power to paper or agar-gel zone electrophoresis and has been successfully applied to the analysis of thyrotrophic hormone[4–6]. Starch-gel electrophoretic analyses of various standard preparations of anterior pituitary hormones are presented here together with some evidence of the distribution of hormonal activities.

A modification of the discontinuous buffer system of Poulik[7] was used. This system was found to separate pituitary proteins better than the original method of Smithies. The electrode vessels contained 0·1 *M* lithium hydroxide and 0·38 *M* boric acid. The gel was prepared with partially hydrolysed potato starch (Connaught Medical Research Laboratories, Toronto) and a buffer containing 90 per cent v/v 0·003 *M* citric acid, 0·016 *M* *tris*(hydroxymethyl)-aminomethane and 10 per cent 0·02 *M* lithium hydroxide, 0·076 *M* boric acid. Electrophoresis was carried out for 4–5 hr. at average voltages of 15–20 V. per cm. During this time sheep albumin moved about 16 cm. and the fastest components in sheep pituitary preparations about 30 cm. from the origin.

The gels were fixed and stained overnight in 0·01 per cent nigrosin (Gurr), in methanol/acetic acid/water 50 : 10 : 40 by volume.

Fig. 1 shows the patterns obtained on the bottom surface of the gels after electrophoresis of various anterior pituitary hormone preparations.

Ox growth hormone (*NIH B2*) has two main components (labelled *A* and *B*) migrating towards the cathode in addition to several minor components migrating to the anode. Several other purified ox and sheep growth hormone preparations have all shown components *A* and *B* but variable minor anodal components.

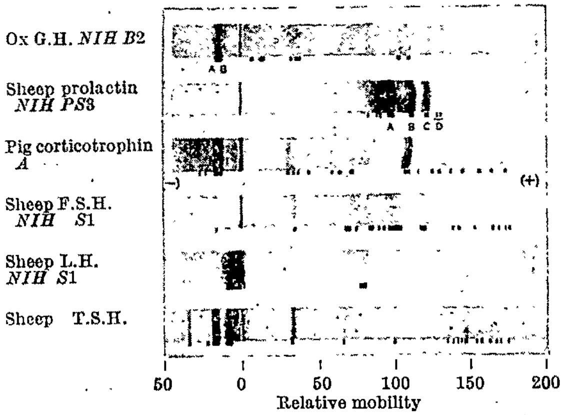

Fig. 1. Photographs of starch gels stained with nigrosin showing the patterns obtained with anterior pituitary hormone preparations. The samples were inserted at the position marked zero on the relative mobility scale and this scale was adjusted so that the prolactin component *A* had a relative mobility of 100

Components *A* and *B* were located in the unstained gel by serrating the edges of the gel with a template as described by Moretti *et al.*[8] and staining a slice from the top of the gel. The pieces of gel containing these components were homogenized with saline and assayed for growth hormone activity in hypophysectomized rats. Both components had approximately equal activity using the width of the tibial epiphysial plate and body-weight as responses. When the components were re-run by inserting the gel sections in a fresh gel they retained their characteristic mobility with no detectable overlapping.

Sheep prolactin (*NIH PS*3), 15 International Units per mgm., shows more than 20 components on the original gel, not all of which can be seen with the amount applied in the experiment illustrated. Purified sheep prolactin preparations from other sources showed similar distribution of the main components. Biological assay in pigeons showed at least 4 regions of the gel, labelled *A–D*, having prolactin activity. The main components in regions *A* and *B* in crude sheep pituitary extract have been separated on diethylaminoethyl-cellulose and have been differentially sedimented in intracellular particle preparations from sheep pituitary homogenate (unpublished experiments). However, separation of the components was less readily achieved when purified prolactin preparations were subject to chromatography on diethylaminoethyl-cellulose under the same conditions used for crude extract. Fractions from adjacent peaks after chromatography showed common starch-gel components. This suggests that purification procedures may have increased the tendency of the components to form complexes. A preparation of ox prolactin showed two active components, electrophoretically different from the sheep preparations.

Pig corticotrophin *A*, 108 International Units per mgm., obtained from a pharmaceutical firm, shows a main cathodal component and at least 21 minor components. The cathodal component is common to two other adrenocorticotrophic hormone preparations examined and has a mobility intermediate between the two growth hormone components.

Sheep follicle stimulating hormone (*NIH S*1) shows several minor components, some poorly resolved, but does not have any strongly staining component, suggesting that most of the material in the preparation does not stain heavily with nigrosin. Sheep luteinizing hormone (*NIH S*1) shows a main cathodal component with very little other material. No biological assay of components in corticotrophin *A*, follicle-stimulating or luteinizing hormone preparations has been carried out.

An impure preparation of sheep thyrotrophic hormone, 1·5 U.S. Pharmacopeia units per mgm., prepared in this laboratory shows at least 15 components. Wynston *et al.*[5] have described four components with thyrotrophic hormone activity in preparations from sheep pituitaries.

The existence of several proteins with one hormonal activity suggests that degradation of the hormone has occurred during fractionation. However, the same components have been found in fresh pituitary extracts subjected to no procedures other than homogenization and centrifugation. Polymorphism does not appear to account for the two active components of growth hormone, since extracts of individual sheep pituitaries all show both components. This hypothesis has not, however, been disproved for prolactin and thyrotrophic hormone. The various preparations examined were obtained from pituitary tissue and it remains to be shown whether multiple forms of anterior pituitary hormones are secreted.

We are grateful to the Endocrinology Study Section, National Institutes of Health, Bethesda, United States, for the supply of growth hormone, prolactin, follicle stimulating hormone and luteinizing hormone. An account of these experiments will be published in more detail elsewhere.

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Polymorphism of α_1-Acid Glycoprotein

α_1-ACID glycoprotein[1,2], a human plasma protein of low molecular weight, has been prepared in a state of extremely high purity as evidenced by electrophoresis and ultracentrifugation over a *p*H range of 1–12 and by terminal amino-acid and immunochemical analyses. Starch-gel electrophoresis of this blood constituent at *p*H 8·5 according to Smithies[3,4] also demonstrated homogeneity. α_1-Acid glycoprotein is further distinguished by the unusually low isoelectric point reported to be at *p*H 2·7 and the high carbohydrate content accounting for 40 per cent and consisting of sialic acid, galactose, mannose, fucose and N-acetyl-glucosamine.

In view of the recently established genetically determined variations of certain plasma proteins[5–8], it appeared of interest to investigate whether or not a comparable polymorphism could be found for α_1-acid glycoprotein. Starch-gel electrophoresis was carried out at the following *p*H values: 7·2, 5·0, 3·8, 2·7 and 2·0. At its isoelectric point this glyco-

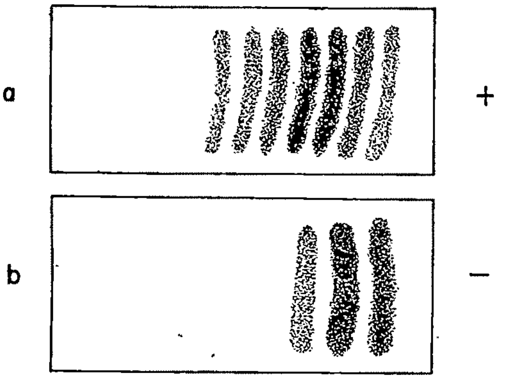

Fig. 1. Starch-gel electrophoresis of α_1-acid glycoprotein at pH 2·7 in $\Gamma/2$ 0·02 phosphate buffer (*a*) and of the sialic acid free form of this protein at pH 5·0 in $\Gamma/2$ 0·02 acetate buffer (*b*). Position of application of the proteins at the right end of the starch gel. Direction of movement from right to left

protein resolved into several bands, whereas on the acid and alkaline side of pH 2·7 homogeneity was observed. A typical preparation[1], derived from 248 litres of pooled outdated normal human plasma (from 1,100 donors of the Commonwealth of Massachusetts), exhibited seven zones (Fig. 1*a*). However, α_1-acid glycoprotein preparations from individual blood specimens separated into different numbers of zones.

To corroborate the validity of these findings, α_1-acid glycoprotein prepared from pooled plasma was treated with neuraminidase, effecting cleavage of essentially the total content of sialic acid, and again subjected to starch-gel electrophoresis. At pH 2·7 the resulting protein revealed a single band, but at the isoelectric point of the enzymatically modified glycoprotein, that is, at pH 5·0, three zones were noted (Fig. 1*b*). At higher pH values apparent homogeneity was restored.

Further work is in progress to determine the genetic implications of these observations.

We are obliged to Dr. J. A. McComb and his associates, Division of Biologic Laboratories, Massachusetts Department of Health, for providing the supernatant solution of Cohn's fraction V derived from pooled normal human plasma, and to Dr. E. H. Eylar, Harvard Medical School, and Dr. E. H. Schultze, Behring-Werke, Marburg-Lahn, Germany, for the gift of neuraminidase.

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Role of Glutathione and of a Self-stabilizing Chain of SH-Enzymes and Substrates in the Metabolic Regulation of Erythrocytes

WE have already described the influence of intracellular glutathione on the activity of inorganic pyrophosphatase and triosephosphatedehydrogenase[1,2].

As shown in column 2 of Table 1, other SH-enzymes such as hexokinase, glutathione reductase, glucose-6-phosphatedehydrogenase are inactivated under conditions of oxidation of glutathione by phenylhydrazine according to Beutler[3]. Glucose affords partial protection as may be seen in column 3. The mechanism of the effect of phenylhydrazine is hæmin-catalysed oxidation of glutathione in the presence of oxygen[4,5]. Glutathionedisulphide, which is formed as an intermediate, causes oxidation, perhaps by formation of mixed disulphides, and inactivation of the essential SH-groups of the enzymes[6]. The oxidized enzymes, if not reduced, undergo within hours irreversible changes[1,6].

Table 1. INFLUENCE OF PHENYLHYDRAZINE ON THE ACTIVITY OF SH-ENZYMES

	Control	1	2	3
Incubation (hr.)	0	12	12	12
Phenylhydrazine (5 mgm./ml. suspension)	—	—	+	+
Glucose (2 × 10^{-2} M)	—	—	—	+
Glutathione reduced (GSH) (mgm./100 ml. cells	73·3	64·8	14·6	31·4
Hexokinase (M × 10^{-3}/l. cells/hr.)	18·1	21·9	2·7	23·4
Glutathione reductase (M × 10^{-2}/l. cells/hr.)	26·3	31·0	10·2	15·8
Glucose-6-phosphate dehydrogenase ΔE × 10^3/0·02 ml. hæmolysate/min.	30·2	24·6	7·0	17·8

The determinations of GSH (ref. 16), hexokinase (ref. 17), glutathione-reductase (ref. 18), glucose-6-phosphate dehydrogenase (ref. 19) were performed according to published methods.

Protection of the SH-enzymes is ensured by the reduced triphosphopyridine-nucleotide-forming reactions of the oxidative breakdown of glucose and the glutathione reductase[7]. The interrelation between the glutathione system (GSH/GSSG) and the SH-enzymes are depicted in Fig. 1.

The enzymes, which are concerned with the reduction of glutathione, carry essential SH-groups[8-10]. They are also inactivated when glutathione is oxidized.

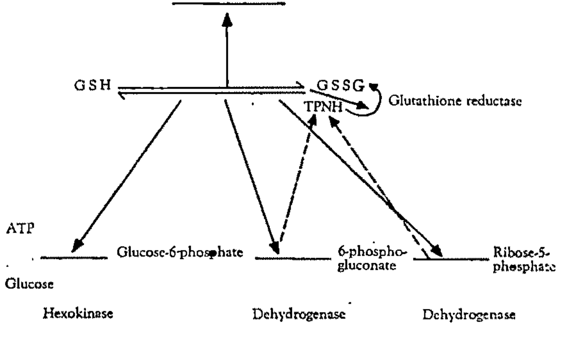

Fig. 1. Scheme of interrelations of enzymes and substrates of SH-metabolism

Thus the extent of enzymatic glutathione reduction is diminished. In case of prevalence of oxidative conditions, more oxidized glutathione would be formed, which in turn would inactivate the SH-enzymes, which participate in the reduction of glutathione. Thus a vicious cycle with destruction of SH-enzymes and glutathione would arise. The same chain of events would occur when one member of the enzymatic self-stabilizing chain of glutathione reduction is missing. Since the chain of SH-enzymes comprises hexokinase, the pacemaker of glycolysis[11], as well as the key enzymes of the oxidative breakdown of the glucose, the oxidation of glutathione might also lead to a decline of the metabolism of glucose and a diminution of formation of adenosine triphosphate with consequent breakdown of cell structure and hæmolysis.

It would appear that the system of SH-enzymes and their substrates plays a significant part in the regulation of metabolism and maintenance of structure of the cell, perhaps particularly for the processes of maturation and senescence[12–15].

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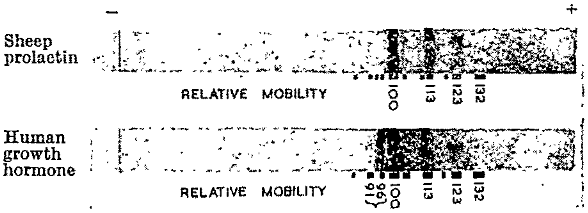
Prolactin Activity of Human Growth Hormone

Zone electrophoresis on starch-gel revealed that purified sheep prolactin contained at least four components with crop-sac stimulating activity in the pigeon[1]. Recently three preparations of purified human growth hormone made by different procedures in different laboratories have been examined by the same method. They all show a striking similarity to sheep prolactin in the mobility and relative amounts of the principal components. This similarity has also been observed by Barrett, Friesen and Astwood[2]. By contrast the patterns are quite unlike the patterns we have observed with ox, sheep, pig and whale growth hormone.



Growth activity percentage recovered 29, 39, 23, 10
Prolactin activity percentage recovered 41, 12, 9, 8

Fig. 1. Starch-gels stained with nigrosin showing patterns obtained with sheep prolactin and human growth hormone

A comparison of one human growth hormone preparation, prepared by Dr. Raben, with the standard sheep prolactin *NIH PS*3 is shown in Fig. 1. Amounts of the two preparations applied to the gel were 2 mgm. for the human growth hormone and 0·5 mgm. for sheep prolactin. Based on staining intensity, these amounts supply approximately the same amount of the principal components in each preparation. Biological assay of the two preparations for crop-sac stimulating activity gave relative potencies of the same order, the human growth hormone being 2·8 International Units per mgm. compared with the prolactin, which is rated at 15 International Units per mgm. The human growth hormone assayed against the ox growth hormone standard *NIH B*2, using the width of the tibial epiphyseal plate in the hypophysectomized rat, was found to be equal to it in potency. If, as may be inferred from the intensity of the components in relation to amount of human growth hormone applied, more purified preparations are possible, then a growth-promoting potency several times that of pure ox or sheep growth hormone may be attainable.

The major components are labelled by their mobility relative to the principal component of sheep prolactin, taken as 100. The amount of prolactin applied to the gel is insufficient to show up the minor components in the photograph. Components 100, 113, 123 and 132 appear in both preparations and correspond to those labelled *A–D* which had prolactin activity in the earlier investigation[1]. Components 123 and 132 in sheep prolactin can each be resolved into two components, but this did not occur in the analysis of the human growth hormone preparation.

In another experiment, components 91 and 96 combined, 100, 113 and 123 of the human growth hormone were cut out of the gel, having been located in the unstained gel as described earlier[1]. The amount of gel with each component was equalized by adding blank gel to the narrower components. The separate fractions were assayed for prolactin and growth hormone activity. For both assays single dose levels and six pigeons or rats per fraction were used. The same proportion of the total amount of each component present in the gel was injected. In the same assays were included two dose-levels of standard prolactin, *NIH PS*3 and standard growth hormone, *NIH B*2, respectively, homogenized with the same amounts of starch gel as the separate components. Also included was a single dose-level of the human growth hormone similarly treated.

All the components of human growth hormone tested were found to have both prolactin and growth hormone activity. The only estimate of the relative amounts of each component at present available is based on staining intensity and may be biased by different affinities of the components for the dye. Accordingly, rather than give doubtful relative potency figures, only the proportion of the activity applied to the gel (corrected for the amount removed in the top section and edges) appearing in each of the components is shown in Fig. 1.

Components 91 and 96 contain a considerable proportion of activity and must have a high potency. The minor components which occur in this region of sheep prolactin have not been tested for prolactin activity. The recovery of activity in the components accounts for somewhat less than the estimated activity applied to the gel for prolactin. However, the discrepancy is within the error of the assay. The ratio of growth hormone to prolactin activity is not constant for the different components, but further bioassays are required to confirm this.

Although the significance of the multiple active components remains to be discovered, it seems likely that the prolactin activity of human growth hormone is not due to contamination, but is a function of the same molecules that possess growth hormone activity.

The similarity of the starch-gel electrophoretic patterns of human growth hormone and sheep prolactin suggests that human growth hormone as now prepared may be chemically and phylogenetically more closely related to the prolactins than to the growth hormones of the ox and sheep, the species most studied in this respect. Further similarity is seen in the chemical and physical properties of the two hormones[3,4]. In molecular weight and amino-acid composition, the growth hormones of different species investigated appear to fall into two groups quite distinct from one another, except that both possess carboxyl and amino-terminal residues of phenylalanine. The group comprising man, monkey and pig is similar to sheep and ox prolactin except in terminal amino-acids, and it is tempting to suggest that monkey and pig growth hormone may also have prolactin activity. However, the starch-gel electrophoretic pattern of pig growth hormone does not resemble that of sheep prolactin.

It is well established that primates do not respond to ox growth hormone[5]. However, it is not certain that a growth hormone related to the hormone of the ox, sheep and whale is absent in primate pituitaries or indeed in the pig. For example, Wilhelmi's method[6,7] for the extraction of ox growth hormone applied to human pituitaries[13] leaves behind much of the growth activity which can then be extracted with solvents which normally extract sheep prolactin. It is possible that some of the growth hormone initially extracted is not identical to that which is brought out by the later extraction.

Sheep prolactin has been shown to have growth hormone-like effects on glucose-tolerance and insulin-sensitivity in dogs[8] and monkeys[9], while stimulation of nitrogen retention in humans[10] and body growth in pigeons[11] and rats[12] has been reported. Such evidence makes it less surprising that human growth hormone preparations should possess both growth hormone and prolactin activity, and suggests that the metabolic effects of prolactin may be a more important part of its function than has been generally recognized.

We are indebted to Dr. M. S. Raben for the gift of human growth hormone and to the Endocrinology Study Section, U.S. National Institutes of Health, for the gift of sheep prolactin.

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Nitrogen and Sulphur at the Active Centre of Carboxypeptidase *A*

A series of metallocarboxypeptidases has been prepared by the substitution of manganese, cobalt, nickel, copper, cadmium, or mercury for zinc, the element present in and essential to the enzymatic activity of native carboxypeptidase *A* [1]. The stability constants, peptidase and esterase activities of these derivatives have been studied and compared[1-3]. Zinc is bound to the single sulphydryl group of the apoenzyme[4-6], as is cobalt as evidenced by its exchange with zinc and by the spectral changes which accompany its binding[2,6]. It has been suggested that a sulphur and a nitrogen atom of carboxypeptidase, together with a metal atom, constitute the active centre of the enzyme, their interaction being required for activity[5].

We now wish to present further evidence in support of the hypothesis that a nitrogenous group is involved in the binding of metals to the active site of carboxypeptidase *A*. The stabilities of the complexes of these metals with a series of nitrogen–sulphur bidentate ligands correlate closely with the affinity of these metals for the binding site on apocarboxypeptidase. The apparent stability constants of the metallocarboxypeptidases in Table 1 (col. 1) have recently

Table 1

Metallocarboxypeptidase	1	2
[(CPD)Me]*	Log stability constant (app.†)	Log stability constant (corr.)‡
[(CPD)Mn]	5·6	5·6
[(CPD)Co]	5·8	7·0
[(CPD)Ni]	5·7	8·2
[(CPD)Cu]	5·1	10·6
[(CPD)Zn]	8·3	10·5
[(CPD)Cd]	7·5	10·8
[(CPD)Hg]	6·7	21·0

* 1 gm. atom of methyl per mole of protein.
† Determined in 1 *M* sodium chloride, 0·05 *M tris*, *p*H 8·0, 4° C.
‡ Corrected for the reduction of (Me_{free}) by the competing *tris* and Cl^- complexes.

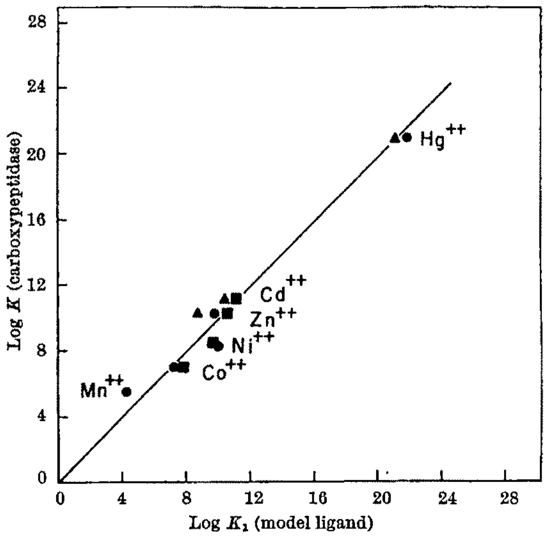

Fig. 1. Correlation of log $K_{corr.}$ for carboxypeptidase with log K_1 for sulphur–nitrogen ligands. ●, cysteine; ■, mercaptoethylamine, corrected for the presence of 0·1 M KCl; ▼, glutathione. All log K_1 values are taken from ref. 7

been measured at pH 8, 4° in 0·05 M *tris*(hydroxymethyl)aminomethane and 1 M sodium chloride, the latter required to dissolve the enzyme, which is otherwise quite insoluble. In these circumstances, zinc is bound more firmly than any of the other metals studied. Allowing for the complexation of the different ions by *tris* and chloride, an appropriate correction must be made for each to ascertain the true stability constants. As is evident from Table 1 (col. 2), this correction is particularly large for mercury. We have compared the stability of these metallocarboxypeptidases with similar data from the literature[7] for cysteine, glutathione, and 2-mercaptoethylamine, which form complex metal ions with nitrogen and sulphur as the donor atoms (Fig. 1).

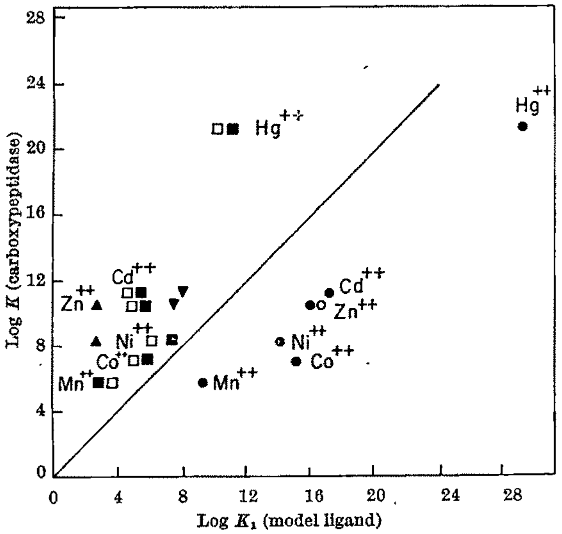

Fig. 2. Lack of correlation of log $K_{corr.}$ for carboxypeptidase with log K_1 for nitrogen–oxygen, nitrogen–nitrogen, sulphur–sulphur, and sulphur–nitrogen–oxygen–oxygen ligands. □, glycine; ■, ethylenediamine; ▲, α-mercaptobutyric acid; ▼, mercaptoacetic acid; ●, 2-mercaptoethyliminodiacetic acid; O, dimercaptosuccinic acid. All symbols refer to the ion appearing on the same horizontal level as the symbol, except for Cd^{++}, which is slightly displaced from the symbols in both instances. All log K_1 values are taken from ref. 7

The high correlation of the stability constants of the metallocarboxypeptidases with those of the complexes of the corresponding metals with these nitrogen–sulphur ligands is striking, and implies a common denominator. We believe that these results strongly support the hypothesis that, together with the single —SH group of the enzyme, a nitrogen atom is involved in binding zinc and other metals to the apocarboxypeptidase.

In Fig. 2 these enzyme stability constants are plotted against those of nitrogen–oxygen, nitrogen–nitrogen, and sulphur-oxygen bidentate ligands as well as a sulphur–nitrogen–oxygen tetradentate ligand. In contrast with Fig. 1 there is marked scatter of the data ruling out any of the modes of binding shown by these ligands as being consistent with that of carboxypeptidase A. The constants for disulphide ligands are orders of magnitude higher than those of carboxypeptidase, as exemplified by the chelate of zinc with dimercaptosuccinic acid, also shown in Fig. 2.

It is therefore suggested that the active centre of native carboxypeptidase A includes one sulphur, one nitrogen, and one zinc atom. Present evidence indicates that the nitrogen atom is either part of an α-amino or, perhaps, an imidazole group[3]. Studies to differentiate between these are in progress.

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Similarity of Peptides in Two Antigen-combining Fragments released from a Rabbit Antibody by Papain

DURING an examination of the enzymatic hydrolysis of rabbit γ-globulin, it was noted that only one-half to two-thirds as many peptides could be differentiated in the hydrolysates as was expected from the amount of base needed to maintain pH during hydrolysis: approximately 50 peptides were distinguished following hydrolysis of rabbit antibodies with trypsin, 70 following chymotrypsin and 100 following subtilisin, whereas approximately 100, 150 and 210 μmoles of sodium hydroxide per μmole of antibody respectively were required to maintain pH at 8·0. The results suggested the possibility that similar or identical peptides were released from different parts of the same molecule resulting in a smaller number of

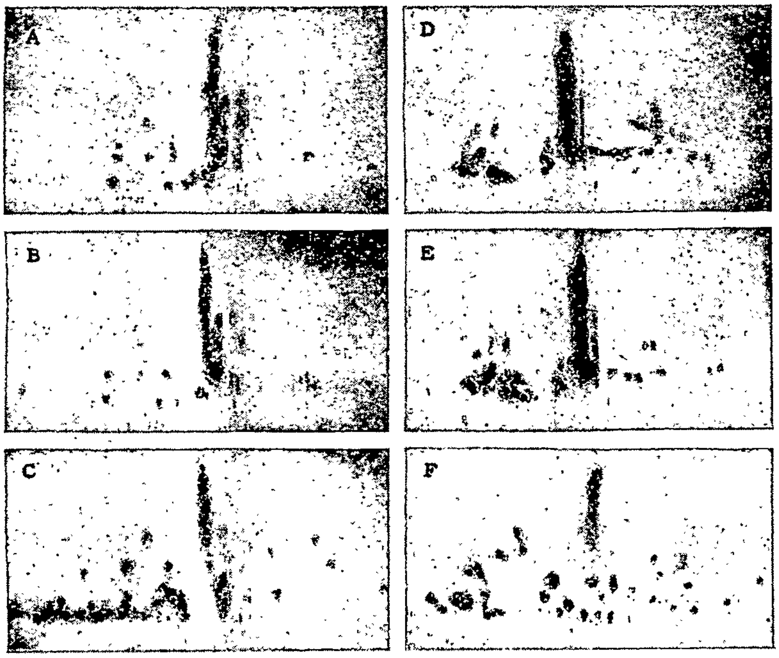

Fig. 1. Peptide patterns of chymotrypsin hydrolysates of I (*A*), II (*B*) and III (*C*) and of the subtilisin hydrolysates of I (*D*), II (*E*) and III (*F*). In each figure, the length of the vertical stroke demarcating the origin at the bottom centre was 7 mm., the anode was to the right and solvent chromatography was in vertical direction

detectably different peptides than the number of peptide bonds broken. An opportunity to test this hypothesis was afforded by Porter's isolation of three large peptide fragments from papain hydrolysates of rabbit γ-globulin[1]. Two of these, I and II, had antibody characteristics and together comprised 50–60 per cent of the antibody molecule; the third fraction, III, was easily crystallized but had no antibody activity. In the work reported here, the peptides of these three fragments were compared.

Rabbit antibodies against type VII *Pneumococcus* polysaccharide were isolated from pooled type-specific rabbit γ-globulin by precipitation with homologous antigen. The specific precipitate was washed four times with 0·15 *M* sodium chloride, suspended in 0·1 *M* potassium chloride containing 0·01 *M* cysteine and 0·002 *M* sodium ethylenediaminetetraacetate and hydrolysed in the *p*H-stat[2] at *p*H 7·0 [1] with twice-crystallized papain in an enzyme to antibody ratio of 1 : 1,000 by weight. The hydrolysate was kept at 2° C. for 2 days and the crystals which formed, fraction III, were collected and recrystallized twice. The remainder of the hydrolysate was dialysed against distilled water and fractionated on a carboxymethyl cellulose column using a *p*H 5·5 acetate gradient, 0·005–0·9 *M*[1]; all column fractions other than I and II were discarded. Type VII polysaccharide was readily detected immunochemically in I and II but not in III. In accord with this, S_{w20} for III in the ultracentrifuge was 3·5 while I and II sedimented much more rapidly, the sedimentation constant depending on the ratio of antibody to antigen in the specific precipitate; the rate of sedimentation of I and II could be decreased by the addition of type VII polysaccharide, in keeping with a decrease in the size of an antigen-antibody complex.

The three fractions were oxidized with performic acid[3] and hydrolysed in the *p*H-stat at *p*H 8·0 with chymotrypsin or subtilisin. 0·1–0·4 mgm. of peptides were applied to a 23 × 57 cm. sheet of Whatman No. 1 filter paper and electrophoresis performed at 52 V./cm. for 45–50 min. at 21–28° C. under 'Varsol' (Esso Standard Oil Co.) at *p*H 4·2 (0·5 per cent pyridine in 0·2 *M* acetic acid). The papers were dried and subjected to ascending chromatography with 2,4,6-collidine, 2,6-lutidine and water in a volume ratio of 1 : 1 : 1. The peptide patterns were developed with ninhydrin.

Approximately 35 peptides could be differentiated in the chymotrypsin hydrolysates of I and II (Fig. 1, *A* and *B*) and about 50–55 in those of III (Fig. 1 *C*). The peptide patterns obtained for the subtilisin hydrolysates of I and II revealed 55 to 60 peptides (Fig. 1, *D* and *E*) and 70 to 75 peptides in the subtilisin hydrolysates of III (Fig. 1 *F*). Per μmole of I, II and III[4], 35, 35 and 60 μmoles of sodium hydroxide were used respectively during chymotrypsin hydrolysis and 60, 60 and 90 μmoles during subtilisin hydrolysis.

As can be seen from Fig. 1, the peptide patterns for I and II hydrolysed under the same conditions were almost identical; a few reproducible differences were apparent. The patterns for III differed quite markedly from those of I and II, but overlapping of some peptide positions in hydrolysates of III with those of I and II was noted.

It would appear, therefore, that approximately half the spots in the patterns obtained for total antibody hydrolysed with chymotrypsin or subtilisin contained peptides derived from at least two different parts of the same molecule. Since rabbit γ-globulin appears to have a single peptide chain[5], it is interesting that two large portions, each equivalent to almost one-third of the chain and each containing an antigen-combining site of the antibody, should have very similar peptide structures.

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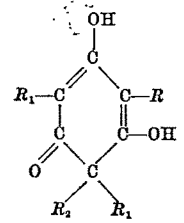
'Sunlight' Flavour of Beer

The fact that an unpleasant flavour occurs on exposing beer to sunlight was first reported by Lintner in 1875 [1]. Since that time much work has been carried out on this phenomenon. No explanation, however, has yet been given.

We have observed by quantitative determination that volatile mercaptan was formed in beer exposed to sunlight[2]. It has been found that humulones (*a*, *b*, *c*) and lupulones (*d*, *e*, *f*) present in hops are related to the formation of 'sunlight' flavour[3]. Furthermore, it has been observed that 'sunlight' flavour was formed even when humulone or lupulone was added, without being heated, to a fermented solution of sucrose or Japanese *sake*[4]. Among the synthetic lupulone analogues (*g*, *h*, *i*, *j*) only acetolupuphenone (*g*) caused sunlight flavour as does humulone and lupulone (ref. 4 and unpublished results).



a, *d*, $R=(CH_3)_2\cdot CH\cdot CH_2CO—$
b, *e*, $R=(CH_3)_2\cdot CH\cdot CO—$
c, *f*, $R=CH_3\cdot CH_2\cdot (CH_3)\cdot CH\cdot CO—$
a, *b*, *c*, $R_1=(CH_3)_2\cdot C=CH\cdot CH_2—$ $R_2=OH$
d, *e*, *f*, $R_1=R_2=(CH_3)_2\cdot C=OH\cdot CH_2—$
g, *h*, *i*, *j*, $R=CH_3CO—$
g, $R_1=R_2=(CH_3)_2\cdot C=CH\cdot CH_2—$
h, $R_1=R_2=CH_3\cdot CH_2\cdot CH_2\cdot CH_2—$
i, $R_1=R_2=CH_3CH_2CH_2—$
j, $R_1=R_2=CH_2=CH\cdot CH_2—$

We have now shown that humulone and acetolupuphenone are degraded by photochemical action and that the 3-methyl-2-butenyl group may be split off. 'Sunlight' flavour is recognized when a mixture of humulone or acetolupuphenone and cysteine is exposed to sunlight under conditions in which oxygen in the solution is replaced by carbon dioxide.

3-Methyl-2-butenyl mercaptan (b.p. 125–127° C.) was synthesized by the method of Braun and Plate[5]. This mercaptan produced the typical sunlight flavour of beer when it was added in trace amounts to a fermented solution of sucrose.

On the basis of this experimental evidence, it may be concluded that sunlight flavour of beer is 3-methyl-2-butenyl mercaptan and that it is formed by a photochemical reaction between the 3-methyl-2-butenyl group in the molecules of humulones or lupulones and certain sulphur-containing compounds present in beer.

Further details will be published in *Agricultural and Biological Chemistry, Japan.*

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PHYSIOLOGY

Metabolic Origin of Urinary Methylamine in the Rat

It has been shown[1] that certain monoamine oxidase inhibitors containing the hydrazine moiety (pheniprazine and iproniazid) besides increasing the level of urinary tryptamine[2] and β-phenylethylamine[3] produce a rise of as much as 300 per cent in the amount of urinary methylamine excreted by rats. To demonstrate the origin of this additional methylamine, rats were injected with compounds which, on the basis of their structure, appeared to be possible precursors of this substance. The compounds studied were glycine, sarcosine, dimethylglycine, betaine, creatine and DL-methionine. 100 mgm. of the compound to be tested was made up in isotonic solution to neutral *p*H and injected into the rat intraperitoneally.

To determine the effect of a monoamine oxidase inhibitor on the metabolism of these substances 1 mgm. of pheniprazine was injected intraperitoneally into each of three rats three hours before they received the possible methylamine precursors ; a second group injected with saline functioned as controls. The rats (Wistar, male, 250 gm.) were placed after injection in metabolism cages in groups of three, fed on Wayne Lab. 'Blox' and water *ad libitum.* Urine was collected for 24 hr. and the level of both methyl- and dimethylamine in the sample determined as previously described[1]. The levels of methylamine, expressed as percentages of the controls, are shown in Table 1. Levels of dimethylamine are not shown because they were not significantly altered by any of the treatments.

Table 1. LEVELS OF METHYLAMINE IN URINE FROM RATS INJECTED WITH POSSIBLE PRECURSORS OF METHYLAMINE IN THE PRESENCE AND ABSENCE OF PHENIPRAZINE
(Expressed as per cent of controls (upper and lower values in parentheses))

Treatment	Untreated: methylamine-level	No. of exp.	Pheniprazine treated: methylamine-level	No. of exp.
Saline	100	–	305 (257–375)	4
Glycine	176 (130–250)	6	468 (360–555)	3
Sarcosine	230 (178–280)	4	612 (540–690)	3
Dimethylglycine	108 (100–120)	4	300 (260–356)	4
Betaine	102 (88–124)	3	460 (405–515)	2
Creatine	159 (146–173)	2	1,240 (1,160–1,320)	2
DL-Methionine	101 (88–114)	4	337 (255–405)	5

In the absence of pheniprazine, three compounds, sarcosine, glycine and creatine, produced significant increases in levels of methylamine, sarcosine being considerably more active in this respect than the other two. Betaine, dimethylglycine and methionine had no effect. Injection of pheniprazine raised the level of methylamine about 300 per cent as was shown previously. This increase was not significantly affected by subsequent injections of either dimethylglycine or methionine. It was raised to more than 400 per cent by glycine and betaine, to 612 per cent by sarcosine and to 1,240 per cent by creatine. Urine collected on the second day showed even greater increases with sarcosine (1,800 per cent) and betaine (730 per cent).

According to the present results, sarcosine and creatine (of the compounds studied), appear to be the most likely precursors of methylamine. Sarcosine may give rise to methylamine through the enzyme glycine oxidase first reported by Ratner *et al.*[4]. Deamidination of creatine would produce sarcosine, which may in turn be demethaminated to yield methylamine and glyoxylate. Should an alternative route of metabolism of sarcosine (namely, sarcosine oxidase) be blocked by pheniprazine the level of sarcosine available for metabolism by way of glycine oxidase would be increased.

Horner and MacKenzie reported that both methionine and betaine may serve as methyl donors for the methylation of glycine to sarcosine[5]. However, in the present work only betaine facilitates production of methylamine in the presence of pheniprazine. This may well indicate that betaine or its biologically active derivative is the major methylating agent for glycine.

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Ubiquinone and Tocopherol in Birds

UNLIKE the rat and the rabbit, it seems from nutritional studies made by several groups of workers that the chicken needs little vitamin E, providing its diet contains an adequate supply of selenium and only limited amounts of unsaturated fat[1-3]. Since tissue-levels of ubiquinone in the rat[4] and rabbit[5] are high compared with those given for some other animal species[6] and since these levels have been found to be dependent on vitamin E [4,5], it was of some interest to investigate tocopherol- and ubiquinone-levels in the chicken and some other avian species, with particular reference to those which show considerable differences in their basal metabolic rates. Chickens were reared to 7 weeks on an ordinary commercial ration, and a group of 4 pigeons and 12 canaries were purchased as adult birds from a commercial supplier. Selected tissues from each species were examined for ubiquinone, ubichromenol and α-tocopherol by analytical methods described previously[7].

As shown in Table 1, extremely low levels of tocopherol were confirmed in the young chicken, and the ubiquinone-levels in this species were among the lowest yet observed by us in animal tissues. The tissues of the pigeon and canary contained considerably higher concentrations of both ubiquinone and tocopherol. The level of ubiquinone in the powerful pectoral muscle of the pigeon is particularly interesting in view of the very high metabolic activity of this tissue (which is known to contain high levels of other enzymes and co-factors concerned with oxidation): it is quite an exceptional concentration compared with those found in muscles of other species we have examined. The uniformly high levels of ubiquinone in all the tissues of the canary can also be related to the high basal metabolism in this species[8]. On chromatographic examination, all the tissues of these birds, which were fed ordinary mixed diets, were found to contain only ubiquinone-50. However, other ubiquinones could easily be introduced into the tissues of the birds by feeding them diets containing yeasts, which contain lower isoprenologues of ubiquinone-50: in the latter case there was a marked difference in the isoprenologue pattern of the various avian tissues.

Table 1. UBIQUINONE (UQ), UBICHROMENOL (UC) AND TOCOPHEROL (T) IN THE TISSUES OF SOME BIRDS

Species	Tissue	UQ (μgm./gm.)	UC (μgm./gm.)	T (μgm./gm.)
Fowl	Heart	18	3	1·4
	Liver	13	1	0·9
	Breast muscle	1·4	0·2	1·5
Pigeon	Heart	192	12	6·8
	Liver	47	not detected	15·4
	Breast muscle	136	8	0·6
	Gizzard	3	0·2	1·4
Canary	Heart	242	17	17·9
	Liver	52	3	8·0
	Breast muscle	38	3	5·0
	Gizzard	43	4	3·9

Although these results are preliminary in some respects, they are not without significance, and tend to support the concept that there is a relationship between tocopherol, ubiquinone and oxidation processes in animals. Although the three species examined were not on identical diets and the latter may have differed somewhat in vitamin E content, this is a factor that, as Edwin *et al.*[4] have shown, has only a minor bearing on the concentration of tocopherol accumulated by the tissues. The differences described in Table 1 undoubtedly reflect real species variations. The role of vitamin E and selenium in the control of ubiquinone in avian species will be discussed in a more detailed report elsewhere.

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Metabolism of *Gerbillus pyramidum*

THE genus *Gerbillus* comprises about thirty species which are distributed throughout Africa. *G. pyramidum* or the greater Egyptian gerbil, as it is also called, is found in Arabia, across North Africa to Morocco on the west and down eastern Africa through the Anglo-Egyptian Sudan to Kenya and Tanganyika[1].

The species discussed in this communication, *G. pyramidum*, differs somewhat from the Mongolian gerbil, *Meriones unguiculatus*, in that it is a larger and heavier animal with a less-docile temperament.

Because there are geographical and climatological differences in the habitats of the two species, inherent differences in their energy metabolism and body temperature regulation might also occur.

Oxygen consumption was measured by means of a closed circuit apparatus which incorporated a commercial recording servo-spirometer (Mouse Spirometer, model 160, Custom Engineering and Development Co., St. Louis, Missouri).

Twenty-one animals were used in the metabolic phase of this investigation, ten males and eleven females, ranging in weight from 72 to 145 gm. The conditions under which the oxygen consumption was measured are those described in a previous paper dealing with the Mongolian gerbil, *Meriones unguiculatus*[2]. The animal was housed, during the test period, in a small glass chamber, which contained soda lime for absorption of carbon dioxide. The chamber was immersed in a thermostatically controlled water-bath which could be set at any desired ambient temperature. After the animal was put in the chamber, an equilibration period of at least 1 hr. was allowed to elapse before the experimental period was begun. The experimental period lasted about 50 min. At least two consecutive runs had to agree closely before the resultant value was accepted. Metabolic measurements were made at ambient temperatures of 15–40° C. and expressed as oxygen consumption in ml./gm. body-weight/hr. This measurement should be regarded as being that of the resting metabolism, since the animals were not in a fasting state.

Least squares fitting of curves to the data, for temperatures below 30° C., yielded the equation $X = 3{\cdot}88 - 0{\cdot}102\ Y$, where Y represents ambient temperature, and X oxygen consumption in ml./gm. body-weight/hr. The correlation coefficient of this curve is 0·904. The critical temperature for *G. pyramidum* seems to be 30° C. Below this temperature, metabolism increases in a linear fashion, with decreasing ambient temperature, at the rate of 0·102 ml. oxygen/gm./hr./deg. C.

Oxygen consumption rises sharply at temperatures above 30° C. The regression line between 30 and 40° C. is $X = -3{\cdot}92 + 0{\cdot}146\ Y$. Between 30 and 40° C. there is a somewhat greater scatter of the results for each of the temperatures at which metabolic measurements were made. As a result the correlation coefficient for the regression line is 0·611.

It was not feasible to measure simultaneously the body temperature and oxygen consumption. Therefore, in order to ascertain thermo-regulatory capacity, it was necessary to expose another group of animals to ambient temperatures similar to those encountered in the metabolic chamber. Five animals were used in this phase of the investigation.

The gerbils were individually housed in large open-top plastic boxes in the hot room. Water was provided, although very little, if any, was consumed. Rectal temperatures were taken with a small thermocouple just before entering the room, and thereafter at hourly intervals. The probe was inserted to a point approximately level with the lower lobe of the liver. This point in our experience approximates the true reading of core temperature. The ambient temperatures chosen were 35 and 40° C. Table 1 summarizes the results of these exposures. The 2-hr. value is included both to enable comparison with the other species, *M. unguiculatus*, and to indicate the thermal state of the animal at the end of a metabolic measurement which usually took 2 hr.

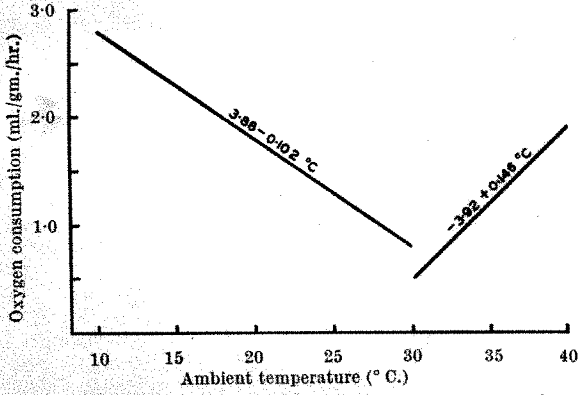

Fig. 1. Effect of ambient temperature on oxygen consumption in *Gerbillus pyramidum*

Table 1. Summary of the Results of Exposure of Gerbils to Heat

Ambient temperature (° C.)	Body temperature (° C.)						
	Mean control at 23° C.	After exposure to heat			Change		
		2 hr.	4 hr.	6 hr.	2 hr.	4 hr.	6 hr.
35	38·7	39·4	38·7	39·1	0·7	0·0	0·4
40	38·1	40·9	42·6	—	2·8	4·5	—

It is clear that while the African gerbil is not greatly inconvenienced at 35° C., even after 6 hr. exposure, it is incapacitated at 40° C. within a very short time. Visual observation alone confirms this. The animals salivate and wet their chests and lie down in a sprawled position, frequently shifting their position as if to seek a cooler location. This behaviour is much like that described by Schmidt-Nielsen[3], Herrington[4] and others[5,6] for the common laboratory animals. It is in sharp contrast to the behaviour of the Mongolian gerbils, which at a similar temperature did not appear to be uncomfortable even though they were exposed for a longer period. Two of the African gerbils exposed to 40° C. died in less than 3½ hr.; two others which seemed severely affected were removed to a cool environment at 4 hr., but died later. Their body temperature at the time they were removed had reached 43·5°.

It is evident from the foregoing results that the metabolic pattern of the African gerbil, *G. pyramidum*, is similar to that of the albino rat. In both animals the resting metabolism is increased between two- and three-fold as the temperature is decreased from 25–10° C. The critical temperature for both species is about 30° C. with a very narrow zone of thermoneutrality. This result is in accord with the pattern for tropical mammals as discussed and illustrated by Scholander[7]. The present results are unlike the metabolic pattern displayed by the Mongolian gerbil, *M. unguiculatus*. The Mongolian species shows an increase in metabolism between 25 and 10° C. (similar to that of the African species) but quite unlike the African species *M. unguiculatus* has a zone of thermal neutrality extending from 30–40° C. This difference between the two species may be due, in great part, to the superior capacity of *M. unguiculatus* to regulate its body temperature in a hot environment. It is likely that *G. pyramidum* spends the daylight hours in burrows where the conditions of temperature and humidity do not tax the animal's heat-regulatory mechanism.

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Spectral Sensitivity of Single Visual Cells

SINCE the work of Young and Helmholtz, the highly developed colour vision in man and many animals has been thought to depend on several, usually three, retinal receptors with different spectral sensitivity[1,2]. Such a theory is best suited for the explanation of human sensations, of animal reactions, and of electrical responses of the whole eye. It is, however, very difficult to determine the spectral sensitivity of the postulated single receptors using these methods. The existence of different photosensitive pigments has been demonstrated by chemical methods in vertebrates[3], and *in vivo* photometric measurements have shown them for the mammalian retina[4]. But this again does not give us an entirely satisfactory explanation of the principal unit-level functions, and the very successful chemical analyses of the visual pigments do not help us understand colour vision[5].

Hitherto, electrical recordings from single elements in the vertebrate retina have been carried out only on the level of ganglion cells and not on the level of single receptors[5,6]. It is clear from these experiments that in the layer of bipolar cells and in the inner plexiform layer we are dealing with the integrated activity and not with the primary reactions of visual cells. Electrical recording from single visual cells of the blowfly (*Calliphora erythrocephala*) was made successfully, using microelectrodes with a tip diameter of approximately 0·1μ (ref. 7).

On the basis of results of electrophysiological and behavioural experiments, colour vision appears to exist in *Calliphora*, and the spectral sensitivity of the whole eye is well known[8]. The study of the spectral sensitivity of single visual cells in this animal may provide information necessary for the understanding of colour vision in arthropods and might probably provide a model for colour vision in general. Basic properties of the receptor potentials, the mechanism of analysing the plane of polarized light in the ommatidia, and details of the methods are described elsewhere[7,9].

In the present experiments, stimulating lights were monochromatic flashes of equal quanta and of 0·1 or 0·02 sec. duration with an interval of 10 sec. The spectral range 315–681 mμ was covered by a series of narrow-band interference filters with a mean wave-length interval of 19 mμ between filter peaks.

The spectral sensitivity of 39 single visual cells was determined for the wild form of *Calliphora*; additional experiments were performed with the white-apricot mutant, in which the red screening pigment is absent.

All cells in the dorsal part of the eye have a uniform sensitivity curve with one maximum at 489 mμ, $\sigma = \pm 7$ mμ, and a second maximum in the ultra-violet at 345 mμ, $\sigma = \pm 3$ mμ. Three different receptor types were found in the ventral part of the eye: (1) a green receptor similar to that in the dorsal part with one maximum at 491 mμ, $\sigma = \pm 6$ mμ, and a second maximum in the ultra-violet, again at 345 mμ, $\sigma = \pm 1$ mμ; (2) a blue receptor with one maximum in the visible part of the spectrum at 468 mμ, $\sigma = \pm 6$ mμ, and a second maximum again at 345 mμ, $\sigma = \pm 2$ mμ in the ultra-violet; (3) a yellow-green receptor with one maximum at 524 mμ, $\sigma = \pm 7$ mμ, and another maximum also at 345 mμ.

Statistical treatment for the 491-mμ and 468-mμ peaks gave $P_{20} < 0\cdot0002$; for the 491-mμ and for the 524-mμ peaks, $P_{19} < 0\cdot0002$; for the 468-mμ and 524-mμ peaks, $P_{5} = 0\cdot0002$. Comparison of the maximum for the dorsal part at 489 mμ with the maximum for the ventral part at 491 mμ gives $P = 0\cdot8$. All maxima in the ultra-violet are very close to each other, with P between 0·48 and 0·66.

If one compares sensitivity curves of receptors belonging to the most common type with the 491-mμ maximum by setting the maximum at 491 mμ as 100 per cent amplitude, the curves follow one another with one exception; some of them have a hump or even a third peak at 616 mμ. If the elements are compared according to the absolute value of potential amplitudes, the following rule becomes clear: the lower the response of an element, the more significant becomes the hump at 616 mμ, and only elements with very feeble action potentials show a peak at 616 mμ. This observation shows that the red screening pigment does not absorb light of this wave-length, and in weakly illuminated elements the portion of scattered red light becomes much greater than the portion of light coming through the dioptric apparatus of the ommatidium. On the other hand, except in the case of weakly illuminated elements and in the far red, the screening pigments are not relevant to the shape of the sensitivity curves. It appears from these experiments that *Calliphora erythrocephala* has at least three visual receptors with different spectral sensitivities. All sensitivity curves have a uniform peak at 345 mμ. This corresponds very well to the rather stable position of the β-absorption in all derivatives of vertebrate visual pigments. The correct value of this wave-length may be the same in *Calliphora* and in vertebrates, because our measurements may have been affected by the quality of filters available. It may be assumed that the visual pigment of the most common type of visual cell in *Calliphora* is a retinene with the maximum absorption near 490 mμ. This pigment is already known to occur in insects[10].

All the cells in the upper part of the eye yielded only one uniform curve. This situation is similar to that in other insects, which have been found to have one part of the eye sensitive to colours and another part colour-blind; for example, *Periplaneta*[11], *Notonecta*[12], and *Libellula*[13]. The most common receptor in the ventral part functions like the dorsal one. The numerical relationship among green, blue and yellow-green receptors is 18 : 4 : 3. If the assumption is made that five of the seven visual cells within each ommatidium are green receptors, and, of the remaining two, one is a blue and the other a yellow-green receptor, the numerical relationship would come very close to that actually found.

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A Potential Source of Error in the Hexobarbitone Sleeping-Time Test in Mice

RECENTLY, Bhide[1] directed attention to the effect of hydration on the hexobarbitone sleeping time of mice. We have been able to confirm this work and that of earlier workers with respect to the effects of hydration in mice[2], but it is not generally appreciated that dehydration, whether achieved by means of diuretic agents or deprivation of water for relatively short periods, has a significant effect on hexobarbitone sleeping time; these effects may lead to error in the screening of potential central nervous system depressants where these agents also have some diuretic or antidiuretic activity and where water supplies are accidentally removed from the animals for short periods.

We have found that a series of diuretic substances, mainly unrelated either chemically or in their mode of action, reduced hexobarbitone sleeping time in mice as did deprivation of water for relatively short periods.

Unfasted female albino mice weighing 17–20 gm. received hexobarbitone sodium 100 mgm./kgm. at different intervals after receiving a dose of diuretic. The interval at which maximum effect was obtained is the only one reported. Sleeping time was taken as the interval between the loss and the regaining of the righting reflex. All drugs were given by intraperitoneal route in a volume of 0·2 ml. distilled water per 20 gm. body-weight. The diuretics examined were acetazolamide ('Diamox sodium' 25 mgm./kgm. and 500 mgm./kgm.), sodium sulphate (100 mgm./kgm.), sodium chloride (200 mgm./kgm.), sodium bicarbonate (200 mgm./kgm.), ammonium chloride (10 mgm./kgm.) and injection of 'Mersalyl' B.P. (0·1 ml./kgm.). Control groups received 0·2 ml. of distilled water at an appropriate time-interval before the anæsthetic. Ten mice per group were used throughout.

The results in Table 1 show that all the diuretics used in these experiments significantly reduced the hexobarbitone sleeping time.

Before the administration of hexobarbitone in the water-deprivation experiments, the animals were deprived of water for varying intervals of time but they were not deprived of food. Table 2 shows the results of these experiments. Deprivation of water for 2½ hr. before the administration of the hexobarbitone has a highly significant effect on the sleeping time. Mice have a high turn-over rate of water, about five times that of man[3], and it is reasonable to conclude that they are very susceptible to dehydration whether occasioned by withdrawal of water supplies or by diuretics. A water load of 1·5 ml./kgm., when given intraperitoneally ½ hr. before the anæsthetic, significantly increased the sleeping time.

Table 1

Diuretic	Hours between administration of diuretic and hexobarbitone	Control sleeping time	Sleeping time after diuretic ± *S.E.*	Values of *P* (*t* test)
Acetazolamide, 25 mgm./kgm.	2·0	31·2 ± 3·2	13·4 ± 2·4	0·001
Acetazolamide, 500 mgm./kgm.	2·0	31·2 ± 3·2	10·3 ± 1·7	0·001
Ammonium chloride	3·0	30·1 ± 3·1	20·2 ± 1·8	0·05
Sodium chloride	2·0	26·7 ± 1·6	20·1 ± 1·1	0·01
Injection of 'Mersalyl'	5·0	26·7 ± 1·6	13·5 ± 1·1	0·001
Sodium bicarbonate	0·5	26·7 ± 1·6	18·3 ± 1·2	0·001
Sodium sulphate	2·0	26·7 ± 1·6	15·1 ± 1·7	0·001

Table 2

Hours between deprivation of water and administration of hexobarbitone	Control sleeping time	Sleeping time after water deprivation ± *S.E.*	Value of *P* (*t* test)
0·5	31·5 ± 2·1	27·9 ± 1·2	Not significant
2·5	31·5 ± 2·1	16·9 ± 1·8	0·001

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HÆMATOLOGY

Stathmokinetic (Mitosis-inhibiting) Method for estimating Plasma Erythropoietic Hormone

ASSAY methods for estimating the erythropoietic activity of plasma samples usually involve the evaluation of altered hæmatological values in test animals, or the change in incorporation of radioiron into the red cells of test animals—usually rats with polycythæmia induced by transfusion. *In vitro* bone marrow techniques do not seem to be widely used.

This communication describes a method for assaying plasma extracts, the stimulating properties of which appear to be confined to erythroid cellular division as observed in the bone marrow of the intact rat.

Activated plasma was obtained by bleeding rabbits (2·5–3·5 kgm.), 10 ml./kgm. body-weight, for 3 consecutive days, to a hæmoglobin-level between 5 and 6 gm./100 ml. On the fourth day the animals were exsanguinated under nembutal anæsthesia and blood taken by aortic catheter was received into bottles containing heparin as anticoagulant. The cells were then centrifuged off at −3° C., and the plasma stored at 3° C. Samples were pooled and then extracted, using Lowy and Keighly's modification[1] of the method described by Borsook[2].

Adult Wistar rats, weighing 150–180 gm., from a closed colony were the recipient animals. No animal was used unless it had been in the laboratory for at least 4 days and more usually the period was more than a week.

Injections of plasma extract were made subcutaneously for 3 consecutive days. At 10 a.m. on the fourth day, 18 hr. after the last injection, a subcutaneous injection of colchicine, 0·05 mgm./100 gm. weight, was given. 5 hr. later the animals were killed, a femur removed, and marrow smears prepared. Duplicate smears prepared from each animal were air-dried, fixed in methyl alcohol and stained with May-Grunwald Giemsa stain.

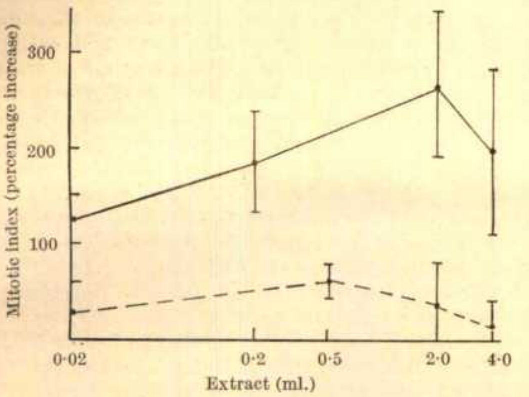

Fig. 1. Response of five groups of rats (4 or 5 rats in each group) injected with extract prepared from the plasma taken from bled rabbits. The interrupted line shows the response to extract from normal rabbit plasma

The films were examined under a high-power lens using oil immersion. Five hundred nucleated red cell precursors were counted, the number of mitotic divisions noted and the result expressed as a percentage (mitotic index). Red-cell precursors at all stages of development were counted, no attempt being made to differentiate between various stages of maturation. Cell differentiation was improved by the florid metaphase patterns resulting from colchicine blocking. A number of cells defied identification. The mitotic index of normal unstimulated animals receiving colchicine was estimated in 8 rats and the mean found to be $3 \cdot 8 \pm 1 \cdot 4$ *S.E.* All results were expressed as a percentage increase above this figure.

Fig. 1 shows a dose response curve using the method in groups of 4 or 5 rats. The interrupted line shows the response with extract prepared from plasma obtained from unbled rabbits. It is at once apparent that a very considerable increase in the mitotic index occurs with very small doses of the active extract. The fall in the response to the 4-ml. (active) dose is what one would expect in such a biological system. The rather sharp fall at 2- and 4-ml. doses for the extract from unbled animals suggests a tachyphylaxis which might result from antibody formation due to injection of relatively large quantities of protein-containing extract of low potency.

Grant[3] used colchicine to demonstrate its mitotic-inhibiting properties in bled rats, and in rats subjected to an atmosphere with low oxygen tension; my results are in accord with his findings.

The main advantage of the method appears to be its sensitivity in the lower dose-range—an advantage in striking contrast to other assay procedures. A major disadvantage is its lack of objectivity—a disadvantage partially removed by re-labelling the specimens by an independent observer, who disclosed the key only when the results were known.

Using this method, I have shown (unpublished work) that although mitotic erythroid hyperactivity is induced, the reticulocyte counts are not altered to a significant degree. Injections continued for 10 days did not produce changes in the hæmoglobin-level; this may mean that the factor necessary to cause this change was lacking, or alternatively that the potency of the material was low and that larger doses than those used (0·75 ml./100 gm.) might be effective. So far as these experiments have proceeded, the only effect has been on the rate of mitosis in the erythroid marrow cells.

It seems probable that this factor responsible for cellular division is identical with that demonstrated by others, for example, the ether-soluble fraction described by Linman, Bethell and their co-workers[4]. Further work is proceeding to characterize this factor.

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Paracrystalline Hæmoglobin as a Species Difference between *Rattus norvegicus* and *Rattus rattus*

The common laboratory white rat is almost always the albino variety of the Norway rat (brown rat, *Rattus norvegicus*)[1,2] and only exceptionally the albino variety of the black rat (*Rattus rattus*)[3]. The two species are morphologically similar, which makes it rather difficult to distinguish them. Nevertheless, there are qualitative characters which make distinction sure.

On suspending the erythrocytes of albino rat in isotonic sodium citrate in a refrigerator their hæmoglobin attains a certain paracrystalline state characterized by general birefringence of these red cells in polarized light (Fig. 1) combined with enormously increased osmotic resistance[4].

Comparative studies showed that the red cells of the brown rat (*Rattus norvegicus*) only, be it the albino or the wild variety, sustain this change. It could not be observed, even after prolonged suspension, in the red cells of either the nearest zoological relative—the wild black rat (*Rattus rattus*)—or other animal species—white mouse, mouse (*Apodemus agrarius*), rabbit, hamster (*Cricetus cricetus, Mesocricetus auratus*), guinea pig, cat, dog, horse, pig, horned cattle, sheep and man.

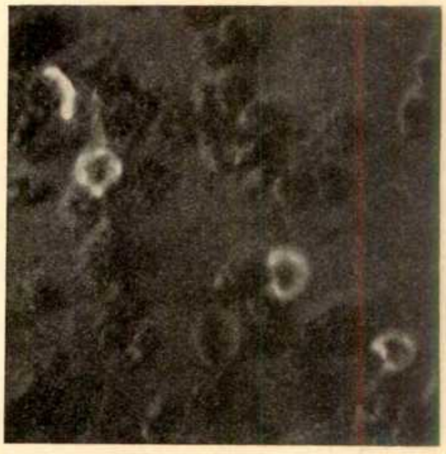

Fig. 1. Diffuse birefringence of paracrystalline red cells in the albino rat (*Rattus norvegicus*) in polarized light after 28 hr. storage in cold isotonic sodium citrate

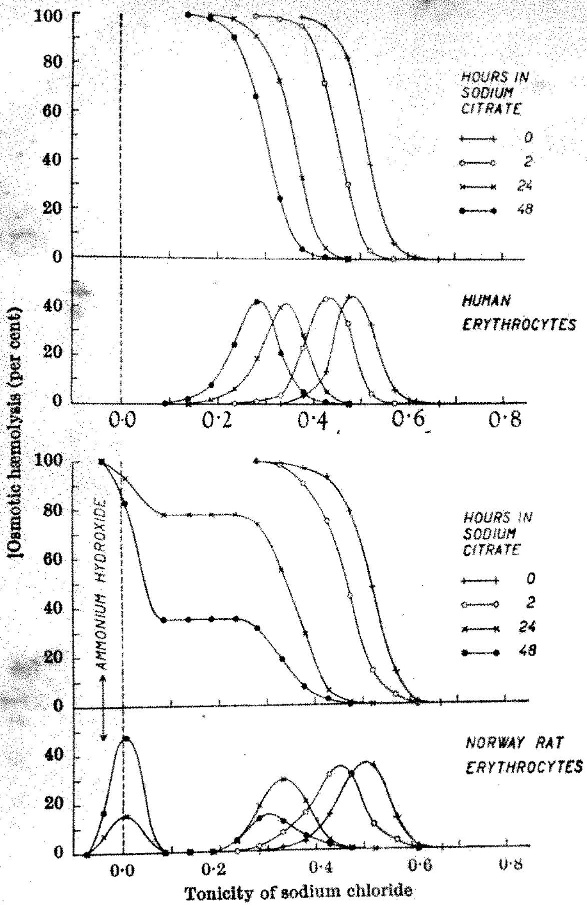

Fig. 2. Integral and distribution curves of osmotic resistance of human and albino rat (*Rattus norvegicus*) red cells during storage in cold isotonic sodium citrate

Similar differences between the erythrocytes of the Norway rat and other animal species were found in changes in osmotic resistance. The storage of erythrocytes in cold citrate is accompanied with gradual and marked increase of their osmotic resistance, especially in the first hours of suspension, and the maximum is attained within 60–72 hr. The increase is even in all red cells as evident from the Gauss-like type of the distribution curves[5]. All but the red cells of the Norway rat react thus, and even extremely resistant cells hæmolyse in distilled water (Fig. 2, upper part).

In contrast to that, the osmotic resistance of the red cells in the Norway rat changes in a different way, the cells being unevenly affected by cold citrate (Fig. 2, lower part): one part increases in resistance in roughly the same way as in other species, but the other attains resistance of such a degree that it withstands hæmolysis even in distilled water. The respective distribution curves are bimodal, reflecting a change of the originally homogeneous suspension into one with bimodal variability[6].

Two qualitatively different types of red cells thus exist side by side in the citrate suspension of brown rat red cells, the fraction of the paracrystalline birefringent and highly resistant erythrocytes being proportional to the duration of storage in the cold citrate.

The increase in resistance is due to change of colloid chemical properties of hæmoglobin proteins resulting from the escape of intracellular colloid water. The relation between mean corpuscular volume of the suspended red cells and the amount of their intracellular potassium and changes in concentration is an indirect proof of this.

The mean red cell volume decreases abruptly during the first 2 hr. of suspension in citrate through the escape of water while the absolute potassium quantity (μμgm. in one red cell) falls much slower. As a result, its osmotically effective concentration (expressed in m.equiv./l. of red cells) is higher than before storage. Red cells become more hypertonic than their environment and the initial decrease of their volume cannot be ascribed to loss of free water which only solves crystalloids but rather to water bound on colloids of the protein red cell core. Through this dehydration of intracellular proteins the red cells become more rigid and gel-like, only swell with difficulty and therefore show increased osmotic resistance.

Red cells with sodium for their main cation (sheep, cattle, cat)[7] and hence having still less possibility of decreasing their osmotically effective intracellular concentration in an environment rich in sodium react in the same way.

Dehydration of intracellular protein is so far advanced in some red cells of the Norway rat that the hæmoglobin molecules are forced, owing to lack of room, to arrange themselves into a paracrystalline birefringent space system. The structural orderliness and rigidity of these cells are so great that the cells withstand hæmolysis even in distilled water, being unable to swell sufficiently.

The birefringence described and variability of bimodal osmotic resistance in the Norway rat were found in both common laboratory white rat strains and inbred Wistar, *LE*, *PVG* and *WAG* strains. This supports the suggestion that all commonly kept laboratory rats belong to the Norway rat species, not to the black rat (*Rattus rattus*) species.

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PATHOLOGY

Zymograms in Human Pathology

ENZYME histochemistry is sometimes a valuable aid in differentiating tumours in human pathology[1] and in studying several other pathological processes[2].

The methods used in histochemistry are based on the localization of enzyme activity. The action of the enzyme is demonstrated, not the enzyme as such. Moreover, in principle, it is possible to demonstrate any enzyme as a 'substance'. The method of Hunter and Market[3,4], in which tissue homogenates are

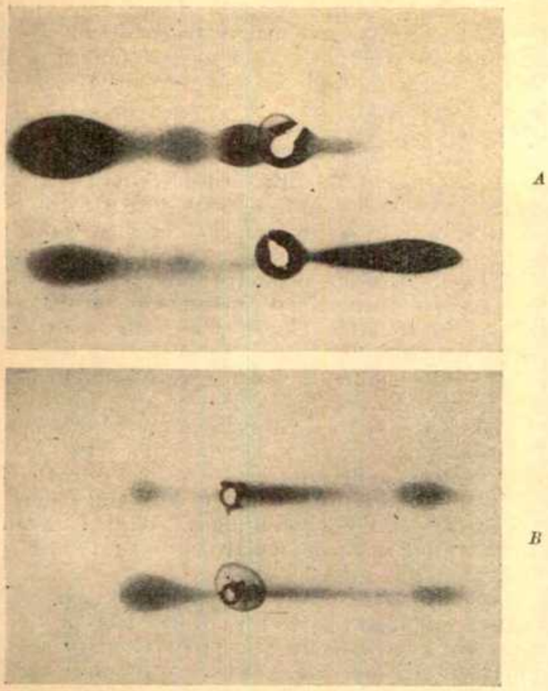

Fig. 1. *A*, Zymogram of homogenate of nodular goitre (upper) and of an adenocarcinoma of the large intestine (lower); *B*, amidoschwarz stained electrophoretic diagrams of the same homogenates ('albumin' moving to the right)

differentiated by means of starch-gel electrophoresis after which the enzymes are stained in the electrophoresis diagram according to a technique derived from histochemistry, offers a possibility of combination of characterizations. The histochemical technique employed locates the action of the enzyme; the position on the electrophoresis diagram more or less characterizes the enzyme as a protein. With the aid of this technique, Market and Hunter[4] succeeded in demonstrating various esterases in homogenates of organs of the mouse. The pictures obtained, indicating the localization of enzymes in the electrophoresis diagram, are called zymograms.

We used a somewhat modified and rather simpler technique, namely, agar electrophoresis[5], followed by histochemical location of the enzyme esterase in various anomalies in human pathology.

Histochemical slides (2·5 × 7·5 cm.) are immersed in 1 per cent agar solution and dried. Next 3 ml. of 1·5 per cent agar solution in veronal buffer pH 8·6 is poured on the slides. Beforehand, strips of electrophoresis paper are attached to the ends of the slides by means of buffer. These are hung in the electrode trays. With an injection needle, straight holes are punched in the agar which are filled with homogenate. This is prepared by homogenizing tissue in a Bühler apparatus with equal volume of distilled water. After electrophoresis (4 hr., 5 V./cm.) the electrophoresis slides are stained for 0·5 hr. by Gomori's esterase method[6]. The substrate is prepared by adding 1 ml. of 1 per cent α-naphthol acetate in 50 per cent acetone and 5 ml. of 0·2 M disodium hydrogen phosphate to 50 ml. of distilled water. In it some fast blue salt *RR* or *B* is dissolved. After incubation it is washed in distilled water, followed by overnight fixation in ethanol 50 per cent. After drying at 37° C. (glasses covered with filter paper) it is covered with cover-glass and glycerin gelatin. Other glasses are stained for protein after electrophoresis by means of amidoschwarz.

Fresh human material, mostly obtained by operation, was prepared in this manner. Several anomalies in various organs could be studied. Also in human material the zymograms thus obtained show distinctly different esterases. Fig. 1 gives an illustration. In Fig. 2 a few zymograms of anomalies in the digestive tract, thyroid gland and pulmonary tumours are grouped schematically. These illustrations are representative of what was observed in larger material. With our technique, in the main, 6 esterases are encountered in human material (I, II, III, IV, V, VI). Three of these (I, II, III) move in the direction of the albumin from the blood, the other three in the direction of the globulins.

Some general conclusions may already be drawn from our observations. It appears that different organs do not show the same zymogram. There are qualitative differences, for example, in the homogenates of the thyroid gland, the I and II esterases are lacking, and quantitative differences, for example, the predominance of I esterase in the digestive tract. Histochemically the highest esterase activity in thyroid gland, digestive tract and lung carcinoma was localized in the epithelium. The zymograms show highest activity of VI, I and II esterase respectively. The question arises whether electrophoretically different esterases show characteristic sites of localization. Further, it is noteworthy how the tumours of a single organ (lung carcinoma) show a fairly identical pattern. An adenocarcinoma of the appendix is clearly different from it. This displays a pattern similar to some non-tumour-like anomalies of the digestive tract.

Protein staining of the electrophoresis slides naturally shows a pattern quite different from the zymogram. It is noteworthy that protein is often scarcely demonstrable at the site of highest enzymatic activity (Fig. 1). This opens up possibilities for isolation.

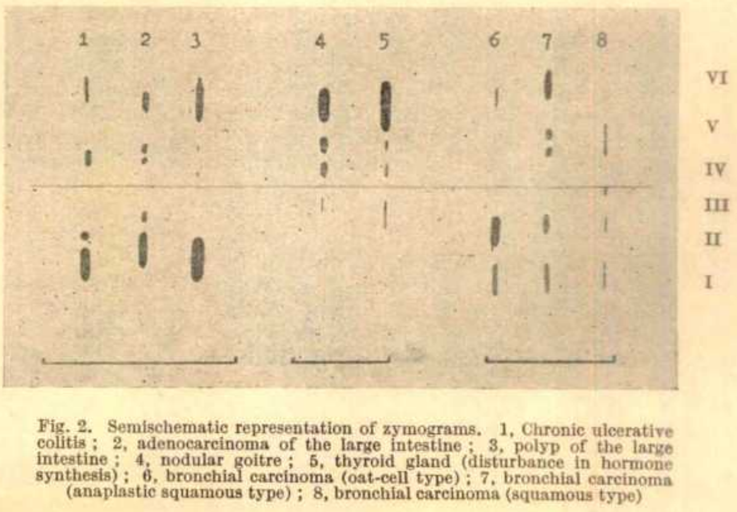

Fig. 2. Semischematic representation of zymograms. 1, Chronic ulcerative colitis; 2, adenocarcinoma of the large intestine; 3, polyp of the large intestine; 4, nodular goitre; 5, thyroid gland (disturbance in hormone synthesis); 6, bronchial carcinoma (oat-cell type); 7, bronchial carcinoma (anaplastic squamous type); 8, bronchial carcinoma (squamous type)

In principle the method employed is also suitable for other enzymes. It has already been applied by us in the case of acid phosphatase, for example.

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A Heteroglycan from *Toxoplasma gondii*

LITTLE is known about the biochemical determinants of virulence and antigenicity in Protozoa. So far as we are aware, no immunochemical studies on *Toxoplasma gondii* have yet been reported, except the work of Hook and Faber[1]. They showed that cytoplasm-modifying and complement-fixing antibodies were related to the protein, but not carbohydrate fractions, obtained from sonically fragmented cells. Lunde and Jacobs[2] concluded that the *Toxoplasma* hæmagglutination test antigen was a protein contained in the crude lysate of washed parasites; but no attempts were made to isolate and identify the soluble antigens in a relatively pure state.

During the course of our comprehensive investigations on the immunochemistry of this protozoan species, we happened to isolate and study a polysaccharide fraction with special reference to its immunobiological properties, which are described in the present communication. The *RH* strain of *T. gondii* used was obtained from Dr. Leon Jacobs, National Institutes of Health, Bethesda, Maryland, and has been maintained by serial passage in mice, using peritoneal exudate. Pure suspensions were prepared from artificially infected laboratory-bred, serologically negative Swiss albino mice, using the method of Fulton and Spooner[3], and, on an average, each yielded 2×10^7 toxoplasms. Suspensions containing 20 mgm./ml. dry weight toxoplasmata were lysed by the addition of 10 times their weight of cold (4° C.) distilled water. After centrifugation and restoration of isotonicity by addition of an equal volume of 1·7 per cent sodium chloride solution, the carbohydrate fraction was separated by fractional precipitation with cold *iso*propanol, and deproteinization was carried out with chloroform and *n*-butanol. The actual yield of polysaccharide ranged from 2·8 to 4·2 per cent, with a mean value of 3·6.

Chemical analysis showed some unusual features, the nitrogen content varying from 0·8 to 1·2 per cent, and phosphorus from 0·2 to 0·4 per cent. No lipid could be detected on degradation with dilute acetic acid. The carbohydrate fraction was readily soluble and physical homogeneity was demonstrated by electrophoretic and ultracentrifugation techniques, and the absence of protein was confirmed by low nitrogen content, lack of ultra-violet absorption in the region of 280–290 mμ and failure of products to give the usual protein reactions. No nucleic acid could be detected by examination of their ultraviolet absorption spectra.

Agar-diffusion[4] experiments demonstrated immunological homogeneity when pure polysaccharide antisera reacted with homologous fractions and proliferative cell suspensions to give only one line of precipitation. When similar fractions obtained from ten other strains of *T. gondii* were examined in the gel-diffusion test, the lines that developed indicated identical specificity since they were, in every case, contiguous when reacting from adjacent reservoirs. No reaction was evident when antiserum against *Besnoitia jellisoni* (kindly furnished by Dr. J. K. Frenkel, University of Kansas Medical Centre) was tested against these preparations, or vice versa.

Chromatographic analysis revealed the presence of glucose, glucosamine and a rare pentose which could not be identified with reagents at our disposal. The presence of these sugars was confirmed by the corresponding precipitation inhibition tests on Ouchterlony double-diffusion plates. No aldoheptoses were found, and there is suggestion that the diffuse streaks on the stained chromatograms may refer to some of the newly discovered dideoxy sugars; this point is under examination in Prof. Dr. O. Westphal's laboratory at the Dr. A. Wander Forschungsinstitut, Freiburg–Zahringen, West Germany. Tests for uronic acid and reactive acetyl gave negative results.

The polysaccharide was a poor antigen when isolated, but high-titred antisera could be produced in rabbits by combining the preparations with the lipoprotein of *Shigella dysenteriae*, or by injecting the polysaccharide after coating on erythrocytes or on killed group *A* hæmolytic streptococci.

The polysaccharide sensitized sheep erythrocytes to agglutination, and lysis (120 μgm./ml.), and the specificity of the reaction was demonstrated in the corresponding inhibition tests. The fraction possessed no pyrogenic, skin-sensitizing or toxic activity, and after treatment with heat or alkali no alteration in these properties was detected. All the preparations fixed complement, did not interfere with the cytoplasm-modifying reaction, became coated on collodion particles (rendering them specifically agglutinable), inactivated the properdin system *in vitro*, had antiphagocytic and virulence-enhancing activity and acted both as a preparing and shocking reagent in the elicitation of generalized Schwartzmann reaction in rabbits.

Studies on its topographic distribution on intact cells of *T. gondii* indicated that it is at least a partial surface antigen. Resting cell-suspensions of *T. gondii* failed to oxidize the polysaccharide, which could not deplete the dye-test or complement-fixing activity of whole-cell antisera. In all cases, the polysaccharides, derived from as many as eleven strains that were examined so far, stimulated non-specific immunity to an extent approaching that induced by *Escherichia coli*, 2380, when tested by the method of Rowley[5].

The polysaccharide specimens could neither actively immunize mice to artificial infection with *T. gondii* nor did their corresponding antisera possess any passive mouse-protective power. Besides, all the preparations were found to lack (1) Forssman heterophile reactivity; (2) direct hæmagglutinating capacity; (3) capacity to inhibit lysozyme *in vitro*; and (4) ability to interfere with hæmagglutination of fowl erythrocytes by Newcastle disease virus. No heterogenetic relationship could be demonstrated between these soluble antigens and many species of bacteria that were included in this work.

No serological cross-reactivity was found between polysaccharide antisera and carbohydrates of known monosaccharide composition and linkage, such as synthetic polyglucoses with 1–4, 1–6 and 1–4–6 linkages, glycogens with 1–4–6 branching points, gum arabic and rhamnose-containing polysaccharides such as Karaya gum.

A comparative investigation is in progress to delineate the role of this antigenic fraction in infection and immunity. A detailed account of these results will be published elsewhere.

We are indebted to Dr. T. Ramakrishnan, Indian Institute of Science, Bangalore, who carried out the physical examination of the polysaccharide fraction.

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BIOLOGY

Electron Microscopy of Mossy Fibre Endings in Ammon's Horn

The endings of the axons of the granule cells of the dentate fascia in relation to Lorente de Nó's areas *CA*3 and *CA*4[1] of Ammon's horn have certain special morphological characteristics as seen in Golgi preparations. These have been described by Cajal[2], who termed them mossy fibres, analogous to those of the cerebellum. He found that these axons have a tortuous course and that they show characteristic varicosities consisting of triangular or star-shaped masses of protoplasm, from the angles of which both short and long processes arise. The main course of this dentate-ammonic tract runs across the bases of the apical dendrites of *CA*4 and *CA*3 neurons at right angles to them. The protoplasmic masses come into intimate contact with the apical dendrite or occasionally with the neuronal perikaryon, and were considered by Cajal to be sites of synaptic contact. Lorente de Nó[1] was also of this opinion, but could not decide whether the structures were part of the pyramidal neuron or of the axon terminating on it. According to Cajal[2] these 'mossy endings' could not be stained by reduced silver techniques.

Electron microscopy shows that these structures are large presynaptic bags into which dendritic spines, sometimes branched, are invaginated.

Appropriate regions of the hippocampus of the rabbit were fixed with buffered osmium tetroxide, stained with alcoholic phosphotungstic acid and embedded in araldite for sectioning[3].

Sections through the apical dendrites near their bases show the presence of structures having the characteristics of presynaptic processes, but which have diameters of 3–4μ (Fig. 1, *pre*). These large bags are full of synaptic vesicles, and the enclosing membrane may be seen to be in close contact with the apical dendritic membrane for distances of up to 4μ. The opposed membranes are thickened at intervals (*b*), such thickenings having the appearance of synaptic contacts as described by previous authors[3].

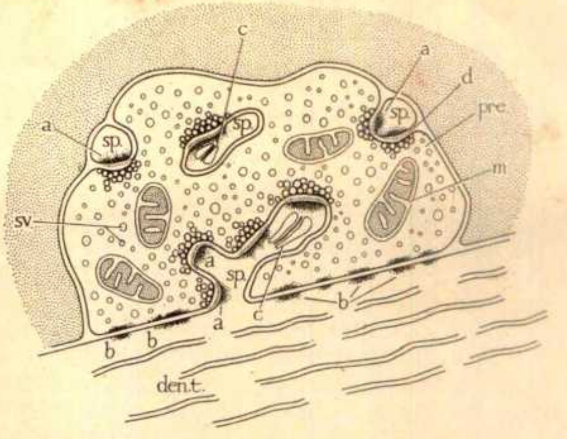

Fig. 1. Diagram of a presynaptic bag showing areas of synaptic contact with dendritic spines and with the main dendrite. The stippled regions represent neuronal and glial processes of the neuropil

In addition, the profiles of several dendritic spines (*sp*) may be seen within the bag, and in favourable planes of section continuity of these with the main dendrite can be observed. Such dendritic spines may be branched. The tubules of the main dendrite (*den. t.*) do not appear to enter these spines. Identification of these small dendritic profiles (diameter is usually 0·5–1μ) depends on the presence in many cases of a spine apparatus (*c*) similar to that which has been described in neurons of the visual cortex of the rat[3]. Also, the membranes of the spine show numerous synaptic contacts (*a*), in which the thickening of the post-synaptic membrane is wider and denser than that of the presynaptic membrane, with, in addition, widening of the intermembranous space (~300 Å.) in the region of the synaptic area. These appearances are in contrast to those of the synaptic contacts between the presynaptic bag and the main dendrite (*b*), where the thickenings of the two membranes are equal and there is no increase in width of the space (~200 Å.) between the pre- and post-synaptic membranes. These two varieties of synaptic contact thus correspond to the types 1 and 2 described by Gray[3] in the visual cortex of the rat.

It is of interest that in this situation a single presynaptic bag may be observed to form two types of synaptic contact, suggesting that the differences may be due to variations in the properties of the post-synaptic processes rather than in the nature of the axonal ending. Observations of Golgi preparations[2] with the light microscope show that these synaptic structures are in many cases not terminal, but of the 'bouton de passage' type. It has not been possible so far to verify this point in electron-micrographs, owing to the difficulty of obtaining a suitable plane of section. As well as the synaptic vesicles, the presynaptic bags may be seen to contain from three to six mitochondria, but in no case have neurofilaments been observed. The absence of neurofilaments may explain the fact that these endings do not stain with reduced silver techniques, as the argentophilia which is found in the axonal terminals of certain regions appears to depend on neuro-filaments being present[4].

A full report of this work will be published at a later date.

I am indebted to Dr. E. G. Gray and Prof. J. Z. Young for their interest and advice.

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Juvenile Stages of *Fasciola hepatica* in the Liver of the Mouse

MOST biologists agree that in the later stages of its life-cycle *Fasciola hepatica* wanders through the liver towards the bile ducts, where maturity is attained. When cysts containing metacercariæ are ingested along with herbage to which they are attached, the larvæ are activated in the intestines of the host and are then able to emerge from the cysts, each of which develops a minute perforation. The young flukes then penetrate the wall of the intestines, creep about over the abdominal viscera and eventually enter the liver. Much research and some controversy were necessary before this migration became established beyond any doubt. Schumacher[1] has reviewed the literature bearing on this problem, and himself carried out experiments of a classical nature, which produced much valuable information concerning the time relations of the various stages of the migration in guinea pigs. He also gave an account[2] of the histological appearance of young flukes in the liver of the host 64 hr. after cysts were fed, but this is a very incomplete study and practically no other information of the same kind is available in the literature of fascioliasis. I have studied the early stages of migration through the liver of the definitive host, the mouse having been chosen to minimize the rather laborious task of serializing sections of infected liver.

The young flukes assemble in the abdominal cavity of the mouse about 24 hr. after cysts are administered *per os*, and during the next 2 days some of them enter the liver. At this stage, they are about 0·3 mm. long, but growth is so rapid that the length is about 0·5 mm. in 6 days, 0·6 mm. in 7 days, 0·8 mm. in 8 days and 1·2–1·6 mm. in 11 days. During little more than 1 week spent in the liver, therefore, the young fluke quadruples its length, at the expense of hepatic tissue. The burrow of the entering fluke is only a little broader than its body, but it is equal to about six hepatic cells in breadth, so that by the time the body of the fluke is just accommodated several hundred hepatic cells have been damaged and excavated. As the fluke grows, the burrow becomes progressively larger, and as the liver may be riddled with burrows, in even moderate infections, the toll of hepatic tissue must become truly enormous. Two photomicrographs of this damage and also the mode of progression of the young flukes are reproduced here (Fig. 1). Four days after the feeding of cysts, the young fluke may be seen advancing into a mass of hepatic cells (Fig. 1,*A*). Pyknotic nuclei of hepatic and reticulo-endothelial cells, and some cytoplasm, form a wedge-shaped mass which is entering the oral sucker. Damaged hepatic tissue is seen in the wall of the burrow and a mass of debris, largely nuclear but also containing leucocytes and erythrocytes, occurs in the wake of the fluke. This appearance is characteristic. The fluke advances into normal tissue, effectively devouring it, and broken tissue which is not ingested forms a concentrate, largely of nuclear origin, which is smoothed down by the movements of the fluke and eventually left behind in the most recently vacated part of the burrow. The burrowing fluke moves along a slightly zig-zag path, the direction of advance changing slightly from time to time, and the older burrow thus assumes the appearance of a number of ovoid chambers with narrower connexions. Change of direction is indicated by slight compression of the hepatic tissue on the right of the fluke seen in sagittal section in Fig. 1, *B* ; this fluke, which is at the 8-day stage, illustrates the way in which the rim of the oral sucker, beak-like in section, bites into masses of hepatic cells, and the anterior end of the fluke is flexed ventrally in a characteristic feeding posture.

The importance of studying the feeding activities of such young flukes will be obvious to helminthologists, who are well aware that such flukes are able to resist the action of any drugs which have so far proved successful for the destruction of adult flukes residing in the bile ducts. A fuller account of the early stages of migration through the liver is now published[3], and in this an attempt has been made to demonstrate the preference which young flukes seem to have for a

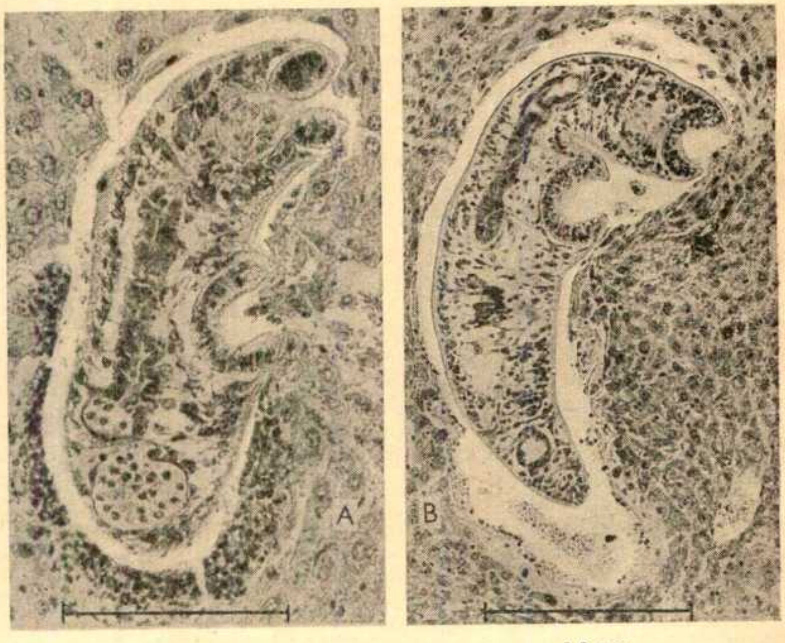

Fig. 1. Sagittal sections passing through two young flukes in the liver. In *A*, 4-day stage, note damaged tissue in the wall of the burrow, hepatic cells with pyknotic nuclei about to be ingested, and debris accumulating in the rear of the fluke. In *B*, 8-day stage, note a characteristic feeding posture, oral sucker approaching hepatic cells, and change of direction of burrowing indicated by compression of hepatic tissue

diet of hepatic cells. It is inevitable that damage to a vascular organ such as the liver should produce hæmorrhages, but in these early stages it is seen that much of the blood which seeps into the burrows lodges in the regions vacated by the flukes, which ever seem to seek fresh hepatic cells. It is claimed that the young fluke is never a blood-feeder in the accepted sense of the term, showing a distinct preference for the hepatic cells, which must form a succulent diet.

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Biochemical Indications as a means of distinguishing between Iron and Manganese Deficiency Symptoms in Citrus Plants

IRON and manganese deficiencies are widespread in many citrus-growing areas throughout the world, including Israel. Similarity of the visual symptoms of these deficiencies, in some stages (Fig. 1), makes differentiation difficult[1].

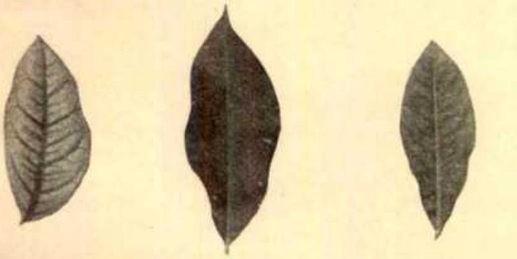

Fig. 1. Control (centre) and manganese- (left) and iron- (right) deficient lemon leaves

Moreover, due to the rather wide range of leaf patterns associated with manganese insufficiency, there are contradictory descriptions of these symptoms in the literature[1-3]. Since it is assumed that the visible symptoms are, in some way, an expression of the disturbed metabolic processes in plant cells, it was decided to investigate these metabolic pathways in order to throw some light on the reasons for this visual similarity. Different metabolic patterns, however, may be used as reliable indicators in differentiating between iron and manganese deficiency symptoms.

This communication deals with only a part of the different metabolic patterns that were observed.

For the purpose of the experiment, several varieties of 3-month-old citrus seedlings—rough lemon, sweet lime and sour orange (generally used as root-stocks) and Shamouti orange, Duncan grape fruit, and Eureka lemon (used as scions)—were transferred to aerated water culture and were grown in a green-house. Purification and composition of the nutrients were according to Hewitt[4].

The solutions contained gradually decreasing amounts of iron and manganese and were changed every fortnight. Characteristic deficiency symptoms developed after 3–6 months. Leaf samples were taken from the plants exhibiting different stages of the developing visual symptoms, especially when iron and manganese deficiencies could be confused.

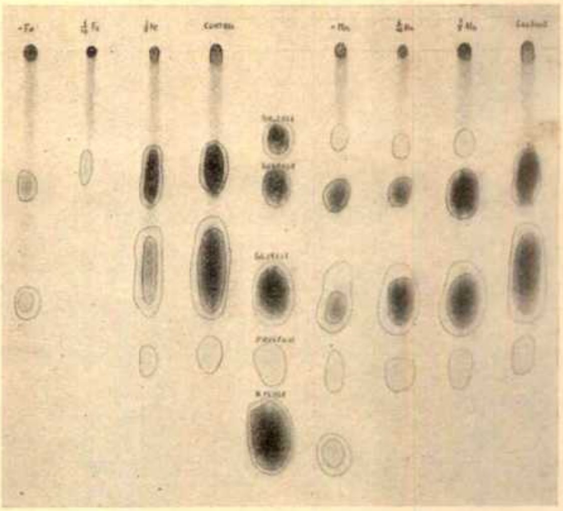

Fig. 2. Ascending paper chromatograph (butanol/acetic acid/water, 9 : 1 : 3) of lemon leaves extract from plants supplied with decreasing amounts of iron and manganese, compared with the markers (in the centre). Spots developed by anilin/butanol/phosphoric acid/water (1 : 5 : 1·2 : 10) spray

Five gm. of fresh leaf material were macerated in 80 per cent ethanol, the ethanol was evaporated *in vacuo*, and the solution was passed through 'Zeokarb 225' and 'Deacidite *G*' resin columns which retained amino-acids and organic acids respectively. The solution, after passage, contained sugars which were detected by means of paper chromatography[5].

Fig. 2 clearly shows the degradation of sugars, due to decreasing amounts of iron in the nutrient solution, and the appearance of some free pentose in the case of manganese-deficient lemon plants.

Since pentoses can be more rapidly detected by methods other than chromatography, it was decided to do so by means of an orcinol test, which can be used as a simple colorimetric determination[5].

For this purpose 0·5 gm. of fresh leaf material was macerated in water and acetone, and besides the pentoses, the peroxidase activity and the chlorophyll content of the sample were estimated[6]. Chlorophyll serves as a means of indicating degree of chlorosis, and peroxidase activity has been found to vary, according to different micronutrient deficiencies, in tomato plants[7].

After centrifugation the supernatant was purified by active carbon and 'Zeokarb 225', and the colourless plant sap was used for the orcinol reaction[5].

Table 1 shows that, in addition to free pentose accumulation, which is enhanced by manganese deficiency, peroxidase activity and chlorophyll $a : b$ ratio may give some substantial contribution to the problem of rapid and correct evaluation of this type of deficiency symptom. This 'biochemical approach' should be appraised in the light of other diagnostic methods, namely, visual symptoms, mineral analysis of the leaves and different methods of diagnostic supply of minerals to deficient leaves.

Chemical analyses of leaves for iron and manganese are not only troublesome in comparison with these tests, but also do not always give a conclusive answer; for example, it has been found[2] that when manganese is deficient, in many cases, less iron also appears to accumulate in the leaves. Diagnostic sprays consume considerable time and, sometimes due to impurities

Table 1. EFFECT OF MANGANESE AND IRON DEFICIENCIES ON CHLOROPHYLL, PENTOSE CONTENT AND PEROXIDASE ACTIVITY OF CITRUS LEAVES*

Citrus variety	Treatment	Chlorophyll (percentage of fresh material) Total	*a*	*b*	*a* : *b*	Peroxidase activity†	Free pentose content‡
Rough lemon	Control	0·1040	0·0770	0·0270	2·84	33	100
	–Mn	0·0507	0·0332	0·0175	1·89	12	145
	–Fe	0·0501	0·0364	0·0137	2·64	180	85
Sweet lime	Control	0·120	0·0843	0·0356	2·40	27	100
	–Mn	0·0709	0·0459	0·0250	1·83	13	133
	–Fe	0·0411	0·0288	0·0123	2·34	61	68
Sour orange	Control	0·0954	0·0680	0·0274	2·48	47	100
	–Mn	0·0852	0·0540	0·0312	1·70	14	147
	–Fe	0·0477	0·0326	0·0151	2·16	73	92
Eureka lemon	Control	0·0822	0·0596	0·0226	2·63	32	100
	–Mn	0·0339	0·0223	0·0116	1·92	15	222
	–Fe	0·0362	0·0268	0·0094	2·85	59	109
Duncan grapefruit	Control	0·0849	0·0615	0·0234	2·62	32	100
	–Mn	0·0687	0·0452	0·0235	1·92	16	194
	–Fe	0·0231	0·0154	0·0077	2·13	125	88
Shamouti orange	Control	0·0951	0·0668	0·0283	2·36	48	100
	–Mn	0·0645	0·0423	0·0222	1·93	21	180
	–Fe	0·0212	0·0152	0·0060	2·51	154	114

* Each value is an average of three or four leaf samples of different ages.

† Peroxidase activity is expressed as number of seconds which were required to complete oxidation of 75 μgm. pyrogallol at *p*H by 5 mgm. fresh leaf material.

‡ Each leaf sample, even the control, developed some colour with the orcinol reagent. The value of the control has been taken arbitrarily as 100.

in the chemical compounds used in these sprays and injections, the results are open to doubt. This 'biochemical approach', therefore, may serve as an additional means of diagnosis in cases of uncertainty.

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Oxidation of Reduced Pyridine Nucleotides and of Sulphite by Pea Root Mitochondria

IT has been shown[1] that freshly isolated pea root mitochondria oxidize α-oxoglutarate poorly or not at all if the oxygen uptake is measured by a polarographic method (in which the mitochondria are not shaken), and the oxygen uptake is not stimulated by diphosphopyridine nucleotide (oxidized form, DPN). However, if the mitochondria are first shaken for 30 min. in a water bath at 25°, they do oxidize α-oxoglutarate and DPN increases the rate of oxygen uptake (Table 1).

The ability of these mitochondrial preparations to oxidize the reduced pyridine nucleotides has been examined by the same procedures. At a low concentration (0·1 m*M*) reduced diphosphopyridine nucleotide (DPNH) was not oxidized by unshaken, but was by shaken, mitochondria (Table 2, exp. 1). This suggests that shaking makes the mitochondrial membrane more permeable to DPNH. With reduced triphosphopyridine nucleotide (TPNH), however, the oxygen uptakes were about the same for both unshaken and shaken mitochondria (Table 2, exp. 2), thus indicating, perhaps, that the membrane of the unshaken mitochondrion is already permeable to TPNH (but not to DPNH). In this respect pea root mitochondria are similar to rat liver mitochondria[2].

Table 1. OXIDATION OF α-OXOGLUTARATE

Additions	q_{O_2} Unshaken mitochondria	Shaken mitochondria
None	450	350
DPN (0·1 m*M*)	460	350
α-Oxoglutarate (10 m*M*)	440	660
DPN (0·1 m*M*) + α-oxoglutarate (10 m*M*)	440	790

The incubation medium contained 0·05 *M* phosphate buffer, *p*H 6·8, 10 μ*M* cytochrome *c*, 100 μl. mitochondrial suspension and additions as given in the Tables, in a total volume of 0·5 ml. Experiments were conducted at room temperature (22–23° C.). Oxygen uptake (measured polarographically) is expressed as q_{O_2} (μl. O_2/mgm. trichloroacetic acid—insoluble N/hr.).

Table 2. OXIDATION OF REDUCED PYRIDINE NUCLEOTIDES

Exp.	Additions	q_{O_2} Unshaken mitochondria	Shaken mitochondria
1	None	500	500
	DPNH (0·1 m*M*)	510	740
2	None	530	560
	TPNH (0·1 m*M*)	630	680
	TPNH (0·7 m*M*)	740	780
3	None	520	490
	DPNH (5 m*M*)	2,110	1,610
	TPNH (5 m*M*)	1,430	890

At much higher concentrations (5 m*M*), both DPNH and TPNH were oxidized by unshaken mitochondria (Table 2, exp. 3). They were also oxidized by shaken mitochondria, but at a much slower rate. At a concentration of 1·0 m*M*, DPN enhanced the low oxidation of α-oxoglutarate by unshaken mitochondria[1] whereas there was no enhancement at a concentration of 0·1 m*M* (Table 1). It thus seems that at external concentrations of 1·0 m*M* and more, the nucleotides can enter the mitochondria.

Table 3. OXIDATION OF SULPHITE

Exp.	Additions	q_{O_2} Unshaken mitochondria	Shaken mitochondria
1	None	500	430
	ADP (m*M*)	510	440
	Na_2SO_3 (0·3 m*M*)	500	580
	ADP (m*M*) + Na_2SO_3 (0·3 m*M*)	500	660
2	None	400	410
	DPN (0·1 m*M*)	400	400
	Na_2SO_3 (0·3 m*M*)	410	560
	DPN (0·1 m*M*) + Na_2SO_3 (0·3 m*M*)	420	560

Sulphite is oxidized by mitochondrial preparations from pea internodes[3] and oat seedlings[4]. It is also oxidized by pea root mitochondria, but only if these were first shaken (Table 3). Adenosine diphosphate (ADP) increased the uptake of oxygen in the presence of sulphite only with shaken mitochondria and DPN had no effect on this uptake. This supports the finding[3,4] that ADP, but not DPN, is required by the sulphite-oxidizing system.

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Growth of *Fomes annosus* under Reduced Oxygen Pressure and the Effect of Carbon Dioxide

Although most fungi are regarded as strictly aerobic organisms, some species—under natural conditions—are known to colonize in localities which *a priori* must be expected to offer only poor supplies of free oxygen. This is the case with fungi attacking the interior of roots and stems of living trees. Owing to respiration in the living tissues of these organs, free oxygen dissolved in the water of vessels in sapwood and heartwood or present as free gas in intercellular spaces and non-functioning vessels is soon consumed. Concomitantly carbon dioxide is liberated. Diffusion of gases within a tree trunk probably occurs by the shortest distance, namely, by horizontal diffusion from the outside through the cortex into the interior of the stem and vice versa. However, in warm seasons when respiration-rate is high the very active cambium enclosing the central wood of a stem offers a barrier to the diffusion of gases in either direction[1]. Consequently as oxygen is consumed by living cells (medullary ray cells, parenchyma cells) in the sapwood the oxygen pressure will drop rapidly in the interior and be kept at a low level until diffusion is again made possible by a decrease in temperature.

Seasonal variation in oxygen and carbon dioxide pressures in stems of several hardwood and softwood trees has been investigated by Chase[2], who found that in summer the oxygen content in the wood of *Pinus strobus* would drop to approximately 1 per cent, corresponding to a pressure of 8 mm. mercury. At the same time the carbon dioxide content increased to approximately 10 per cent.

The parasitic basidiomycete *Fomes annosus* (Fr.) Cke. is known to attack roots and heartwood of many conifers and even deciduous trees, thus being exposed periodically to conditions of low oxygen pressure and high carbon dioxide pressure. It was, therefore, of interest to investigate the growth response of this fungus to such conditions.

Round 6-mm. pieces from the periphery of a young colony of *F. annosus* on malt agar were inoculated on to the surface of freshly boiled and poured malt agar in Petri dishes and incubated at room temperature in Brewer jars containing air, hydrogen, nitrogen and/or carbon dioxide. In the hydrogen jars all free oxygen and oxygen dissolved in the agar was removed by catalytic reaction with the hydrogen, the jars thus representing a strict anaerobic environment. No growth occurred in these jars (Table 1). In the nitrogen jars the oxygen was removed by three subsequent evacuations to 40 mm. mercury and slow refillings with nitrogen which had passed through a wash bottle containing alcalic pyrogallol. Theoretically the nitrogen jars contained only 0·02 mm. oxygen although the actual content might have been somewhat higher because of sluggishness in oxygen diffusion from the agar. Nevertheless, these bottles represented a micro-aerophilic environment in which *F. annosus* proved to grow unaffected.

Table 1. Growth of *Fomes annosus* on Malt Agar in Air, Nitrogen, Hydrogen and Carbon Dioxide. Total Pressure 760 mm. Mercury. Incubation 6 days at Room Temperature

Gas pressure (mm. mercury) Q_2	N_2	H_2	CO_2	Linear growth (mm.)	
160	600	0	0·228	30	Atmospheric air
155	582	0	23	44	Carbon dioxide added to air
0	trace	760	(trace)	0	
0	trace	737	23	0	
trace	760	0	(trace)	30	
trace	737	0	23	45	

For growth in pure carbon dioxide the jars were filled in the same way as with nitrogen except that the washing in pyrogallol was omitted. These jars also represented a micro-aerophilic milieu. With 760 mm. carbon dioxide no growth occurred whereas 23 mm. of the gas in combination with air or nitrogen accelerated growth about 50 per cent. As could be expected, no effect was obtained under strictly anaerobic conditions in hydrogen.

That neither hydrogen nor carbon dioxide in high concentration *per se* was toxic to *F. annosus* was shown by subsequent aerobic incubation at which normal growth was resumed.

Nine different strains of *F. annosus* (isolated from fruiting bodies on infected *Pinus sylvestris* and *Picea abies*) have responded qualitatively equal to those conditions. It could be concluded that *F. annosus* is a fungus able to grow equally well under aerobic as under microaerophilic conditions, but not anaerobically, and that a slight elevation of the carbon dioxide pressure has a stimulatory effect, but a high concentration of this gas an inhibitory effect on mycelial growth. These observations seem to agree well with what was to be expected from a fungus parasitizing the interior of roots and trunks.

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Filtration of Phytoplankton by the Octocoral *Alcyonium digitatum* L.

Most of the work carried out on the food and feeding of cœlenterates deals with the true reef-building corals, Actiniae and Scyphomedusae, which harbour zooxanthellae in their tissues. The group Alcyonaria, however, has received less attention. There seems to be considerable controversy as concerns the significance of these symbiotic algae for their host-cœlenterates. Some authors report that the cœlenterates derive no benefit from their zooxanthellae[1,2], while others believe that zooxanthellae form a considerable part of their food, the other part being of animal origin[3–5]. Still others maintain that some cœlenterates, namely the xeniid octocorals, may in some way or other rely on their algae as their only source of food[6,7].

Little work has so far been done to study the food and feeding of alcyonarians which do not harbour zooxanthellae in their tissues, for example, *Alcyonium digitatum* L.[3,8], which has been described as carnivorous. Its food is reported to consist solely of other small animals, mainly zooplankton organisms, which it paralyses by the action of its nematocysts.

In order to investigate whether *A. digitatum* is fully dependent on animal food or may also feed on phytoplankton organisms, fresh colonies were placed in the dark in jars containing membrane-filtered sea water, to which strains of *Sceletonema costatum* labelled with carbon-14 were added. Two different concentrations of algae were used (20 : 1). The rate of removal of algae by the octocoral was estimated by measuring the radioactivity in sub-samples of 10 ml. water at time-intervals starting with half an hour. As a control, the drop in the radioactivity of the algae was measured in the same way in identical concentrations of *Sceletonema*. The considerable decrease (27 per cent) of activity in the control with the higher concentration of algae is probably due to the dense concentration of algae as well as to the bacterial activity (the *Sceletonema* strains used were not bacteria-free) (see Table 1).

Table 1. RADIOACTIVITY (C./MIN.) IN SUB-SAMPLES OF 10 ML. SEA-WATER

Lapse of time (hr. and min.)	Experimental (*A. digitatum*)		Control	
	Concentration of algae (20)	(1)	Concentration of algae (20)	(1)
0	687	45	666	34
0·30	—	34	—	—
1	674	—	—	—
2	—	17	—	—
3	533	25	—	29
4	—	65	—	—
5	492	—	592	32
6·30	496	23	—	—
23	250	—	486	36

In the higher concentration of algae, one colony of *A. digitatum* filtered about 50 per cent of the *Sceletonema* present in 23 hr. while in the lower concentration, another filtered up to 93 per cent of the algae in only 6½ hr. After the experiments, thoroughly washed pieces of the *Alcyonium* colonies were homogenized and the activities of the different parts measured (c./min.) were as follows: ectodermal covering, 4·7; mesoglœal and endodermal tissue, 22·7; and gastric juice, 46·5. In some other experiments still higher activities have been measured in the tissue-homogenates of *A. digitatum*[9]. In all the feeding experiments we have carried out using strains of *Sceletonema costatum*, *Ditylum* sp. or natural phytoplankton populations labelled with either carbon-14 or phosphorus-32, *A. digitatum* has proved to be able to filter such algal organisms.

The ability of *A. digitatum* to filter and obviously digest phytoplankton algae is evident from the above data, demonstrating the transportation of the labelled nutriment to different tissues of the colonial animal. This phenomenon may help to explain why this species can survive for relatively long periods of time in waters deficient in zooplankton, and moreover supports the idea that at least some cœlenterates may also feed on plant material.

The ciliary movements in *Alcyonaria* seem well suited to filter phytoplankton algae out of the surrounding water. In *A. digitatum* the ciliary current maintained in and out of the polyps seems not only to convey oxygen, as already stated[10], but will also constantly bring a new potential source of plant food within their reach. It might, of course, be expected that such a current would play no part in providing *A. digitatum* with its animal-food, since the latter mainly consists of small zooplankton crustaceans which move too fast to be caught by ciliary currents. In the active stage, however, *A. digitatum* expands greatly with a resulting enormous increase in the surface area[11] and this brings the colony in contact with a still larger volume of water masses. Moreover, as may also be expected, expansion and extension of the polyps activate the ciliary currents in their cœlentera. It is not believed, however, that expansion of the *Alcyonium* colony to its normal activity size is induced by any need of oxygen, provided that this gas is not deficient in the surrounding water[11]. It is almost certain that the expansion of the colony in Nature is related mainly to feeding requirements.

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VIROLOGY

Inhibition by Cortisone of the Synthesis and Action of Interferon

THE administration of cortisone and certain other C-21 and 19-nor-steroids increases final yields of influenza virus in chick embryos by inhibition of viral interference[1]. Paradoxically, the rate of viral synthesis is reduced[2] at the same time. Interferon, a substance produced by cells inoculated with interfering virus, has been proposed as the mediator of interference[3]. Therefore, it was important to determine whether cortisone and other steroids capable of inhibiting the induction of interference might influence either the production of interferon or its effect.

We find that interferon is produced most easily by a method suggested by Wagner[4]; this involves the intra-allantoic inoculation of chick embryos with small amounts of infective virus and prolonged (65–72 hr.) incubation before harvesting allantoic fluid. After dialysis of allantoic fluid at pH 2·0, which destroys the virus but leaves interfering (interferon) activity, this activity is measured as the reduction in viral synthesis *in vitro* in chorioallantoic membrane cultures. It has been found that membranes of older (12–13 day) chick embryos are more susceptible to the interferon effect[5], and these have been used in the present experiments.

In Table 1 we present the summary of an experiment in which cortisone (diethylaminoacetate hydrochloride of hydrocortisone) in two dosages clearly prevented the *in ovo* synthesis of interferon. In this

Table 1. INHIBITION OF INTERFERON FORMATION BY CORTISONE

(*A*) Production of interferon (*in ovo*)

Group	−2 hr.	0 hr.	+70 hr.
1	Water	virus*	harvest and dialyse at pH 2·0†
2	*SC*-5914 (1·0 mgm.)	virus	
3	Cortisone (0·1 mgm.)	virus	
4	Cortisone (1·0 mgm.)	virus	
5	Water	saline	
6	—		

(*B*) Test for interferon (*in vitro*)

Group	−20 hr.	0 hr.	Hæmagglutinin yield at 42 hr.
1	Incubate 1 : 2 dilution in medium with *CAM*+	add challenge virus‡	1·2§
2			2·3
3			4·7
4			5·2
5			4·7
6	(Control)		5·5

* Influenza *A* (*PR*8) 10^2 EID_{50}.

† Then readjusted to pH 7·0 ; note that dialysis removes steroids from fluids.

‡ Influenza *B* (Lee) 10^6 EID_{50}.

§ Log_2 viral hæmagglutinin.

+ Chorioallantoic membrane tissue culture.

experiment 11-day-old chick embryos (4/group) were inoculated with $10^{2\cdot0}$ EID_{50} of the *PR*8 strain of influenza *A* virus, or a saline control 2 hr. after the injection of water (control), cortisone, or *SC*-5914, 'Searle' (17-ethyl-19-nortestosterone), a steroid with 1/1,000 the activity (by weight) of cortisone in negating interference with inactivated virus (Kilbourne, E. D., unpublished results). After 70 hr. of incubation, allantoic fluid pools were collected from each group, then dialysed against 0·1 *M* sodium chloride glycine buffer at pH 2·0 for 24 hr. at 4° C. These 'interferon' preparations were then restored to pH 7·0 by dialysis against 0·01 *M* phosphate buffered saline for 24 hr. (two changes) (Table 1, part *A*).

As shown in part *B* of Table 1, the interferon activity of 1 : 2 dilutions of the pools thus treated was determined *in vitro* by inoculation of 12-day-old chorioallantoic membrane cultures with 10^6 EID_{50} of influenza *B* virus (Lee) 20 hr. after their incubation with 'interferon' pools or medium alone (group 6). Measurement of yields of hæmagglutinin 42 hr. later demonstrated reduced titres (evidence of interferon action) only in groups 1 and 2, which had not received cortisone originally. It has been established since then that cortisone does not merely prevent the release of interferon from the chorioallantoic membrane into the allantoic fluid, but also inhibits its formation in the membrane. The design of the foregoing experiment precludes the possibility that cortisone acted *in vitro* after interferon was synthesized, because dialysis separates effectively the (dialysable) steroid remaining in the allantoic fluid from the non-dialysable interferon.

However, it has been shown in other experiments that cortisone and other steroids administered *in vitro* will prevent the *effect* of pre-formed interferon if they are given 2 hr. before the inoculation of interferon, then removed by washing before the incubation of membranes with interferon and the subsequent inoculation of challenge virus. It is of interest that suppression of interferon action is the only biological action manifestly common to the disparate steroids capable of inhibiting interference.

Previous papers from this laboratory have suggested that inhibition of both viral synthesis and interference by cortisone might have a single biochemical basis[1], and that the basis was most probably related to the inhibiting effect of cortisone on protein synthesis[2]. It is, therefore, of great interest to find that cortisone inhibits the synthesis of a protein (interferon) which can interfere with virus replication. If interferon is an abortive product of viral multiplication[3], then further evidence is provided that the effects of cortisone on viral multiplication are on protein rather than nucleic acid synthesis.

It seems possible that endogenous adrenal corticoids may influence the dynamics and outcome of infection *in vivo*, depending on the balance of their effects on viral synthesis and on the synthesis and action of interferon.

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Apparent Release of Tobacco Mosaic Virus in Living Infected Cells

USING a Zeiss *GFL*65 phase microscope with 'Neofluar' objectives we are attempting to record the sequence of changes produced by tobacco mosaic in living cells of Turkish tobacco. Leaves at intervals after inoculation are prepared by exhausting them of air under water with a high-vacuum pump. Hand sections are cut in a pan of water under a binocular microscope with small pieces of razor blade mounted in a handle. Most sections are paradermal, along or beside veins on the underside of leaves inoculated with tobacco mosaic virus on the upper surface, or on systemically infected leaves. One cell thickness, sometimes obtainable, is ideal ; 2 cells may allow images that can be photographed, and occasional observations are possible on pieces 3 cells thick.

A number of tissue pieces have provided images, apparently of the primary emission of virus in recently infected cells. Strains *U*1 and *U*5 [1,2], representing 2 forms of the virus[3], initiated similar series of events, differing in several details. Appreciation of these events, when they were first seen to occur, was aided by previous studies on the state of cells after infection was apparent.

Tobacco mosaic virus aggregates in recently infected cells often appear as translucent monolayers in the parietal cytoplasm. Single or double layers are termed grey plates[4]. The long axes of the virus particles seem to be vertical to the cell wall, and aggregation is lateral. The grey plates vary in size from 1 to 5μ or more in diameter. If the virus is of the *U*1 form, several monolayers may appear over each other, remain attached, and become by accretion the familiar hexagonal crystals or thick virus plates. Virus of the *U*2–*U*5 form fails to aggregate into crystals, apparently because the end-to-end bonding of the particles is too weak to hold a number of monolayers together. Later, the virus particles of *U*2–*U*5 combine in crude acicular aggregates. A second difference between forms is the presence of

x-bodies in $U1$-infected cells, but not in $U2$–$U5$-infected cells.

A condition characteristic of most active plant cells, but particularly noticeable in cells in which the virus is about to appear, is the non-random distribution of organelles in the cytoplasm. The most obvious example is an apparently functional association between nucleus and plastids, in which specialized structures are involved. Chloroplasts become tightly adherent to the nucleus and remain so in spite of rapid streaming of cytoplasm around them. Approaching the nucleus before adhering to it, a chloroplast may put out a fine protrusion that attains a length of 5–30μ. The protrusion touches the nucleus and shortens as the plastid comes closer. A second protrusion from the opposite side of the same chloroplast occasionally appears, and becomes surrounded by spherosomes, mitochondria, etc. When the plastid itself becomes adherent to the nucleus the first protrusion may be still visible, extending over the surface of the nucleus; the second may have retracted. More than 30 chloroplasts have been seen adherent to a large nucleus.

The first sequence of virus emission seen and photographed was in an epidermal cell from the underside of a leaf inoculated 3 days earlier with $U5$. The microscope was focused on the nucleus of a cell, free from any indication of virus infection, between two obviously infected cells. A number of plastids adhered to the nucleus, and plastid projections were visible on its surface. The cytoplasm flowed actively over the nucleus. After half an hour, bubbles were seen around the nucleus, and bubbling continued actively for 5–10 min. Similar bubbling has been induced experimentally by physico-chemical agents (unpublished work) and seems to indicate a radical change in permeability of the nuclear membrane and nearby endoplasmic reticulum. Mitochondria and spherosomes were present and circulated in the adjacent cytoplasm. After the bubbling, plastids were no longer closely adherent to the nucleus, and gradually they became free to drift away in cytoplasmic streams. Cytoplasmic flow from the nucleus became concentrated mainly in one direction. The outflowing cytoplasm was denser than normal, and monolayer grey plates with a dark 2- or 3-layer spot in the centre began to appear (Fig. 1). A slower rate of flow from the other side of the nucleus allowed condensation of a few grey plates on the nuclear surface. The source of virus material seemed to be inside the nucleus, possibly around the nucleolus. In several cells when increase of visible virus ceased, the nucleolus was noticed to have lost its optical density or seemed smaller than it was before virus began to appear. Active flow of virus material from the nucleus continued for 10–15 min. (longer in other examples), and the grey plates circulated through the cell, giving it the typical appearance of a recent infection.

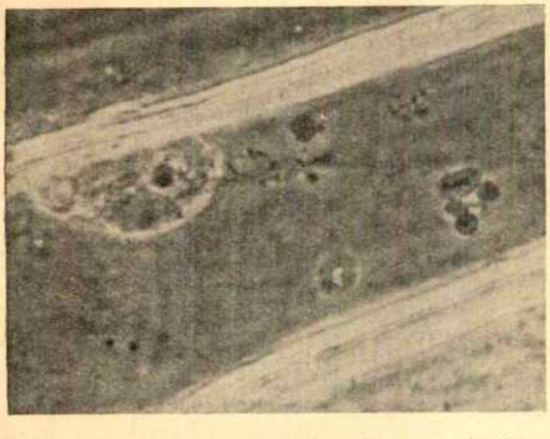

Fig. 1. Late stage in virus emission, virus aggregates (grey plates) formed and at a distance from the nucleus. Flow from the nucleus still apparent

The second full sequence seen and photographed was with strain $U1$ in a sub-epidermal cell from a young systemically infected leaf 4 days after inoculation. The preliminary bubbling around the nucleus was less active than in the $U5$ series, but in this and other examples of $U1$ infection there seemed to be a second site of activity in the cell. From around the nucleus with its adherent plastids, rapid and copious streaming occurred towards a vortex in the cytoplasm, some distance away. The basis of the vortex was a complex of cytoplasmic vesicles. Such vesicles have been interpreted as inclusions of vacuolar material within the cytoplasm[4]. In the vortex, spherosomes and mitochondria were in violent motion. Virus appeared as grey plates or as less-formal aggregates, mainly in the sluggish return flow from the vortex to the nucleus. The equivalent of a cytoplasmic vortex in the $U5$ infections seemed to be near or around the nucleus itself, rather than at a distance, as in $U1$.

In several $U1$-infected cells, seen after emission, amorphous material was present against the nucleus among the plastids. It drifted away with some of the plastids and circulated, apparently as the beginning of an x-body. It should be possible from further observation and biochemical tests to determine whether this material contains any virus protein or nucleic acid, or is debris from cell structures such as plastids.

The whole appearance of these events suggests a burst of virus emission quickly accomplished, rather than a mere assembly of virus on or at various distances from the nucleus. If the events have not been wrongly interpreted, they indicate that the final stages of tobacco mosaic virus multiplication begin within the nucleus. This agrees with Zech's findings[5]. The nucleolus seems to lose material during virus emission, and may be the source of virus nucleic acid. Possibly plastids are concerned with the synthesis of virus protein[6], and in the cytoplasmic vortex most of the nucleic acid and protein may be joined together. Finally, aggregation into grey plates, or other forms, often seems associated with vesicles or vacuolar membranes. Although such suggestions about the steps in virus formation are almost entirely speculative, it can be claimed with reason that whole virus is formed very soon after the burst of activity initiated in and around the nucleus of recently infected cells.

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GENETICS

Frequencies of Hairy Pinnæ among Indian and Sinhalese Peoples

SINCE Tommasi[1,2] published a small pedigree, one of the phenotypes suspected to be holandrically inherited in human beings is that called hypertrichosis of the ears but more accurately hypertrichosis of the pinnæ of the ears. I have shown this to be completely *Y*-linked in my family, publishing a pedigree containing 181 people of 7 generations[3]. The age of onset is usually in late puberty, though in a few cases the hairs are not conspicuous until the men are about 25.

I have examined 495 men in India, and 414 men in Ceylon to count the frequency of this characteristic. These figures are given in Table 1. Andhra Pradesh is a south-eastern State of India with a population of about 33,000,000. In this State I counted the frequency of this characteristic among my co-travellers in buses and railway trains during 11 journeys travelling a distance of about 800 miles[3]. In the different parts of Ceylon where the counts were made many subjects stated that their ancestors had been local people for a long time.

Table 1

Place	Subjects	Total No.	No. with hairy pinnæ	Percentage with hairy pinnæ
India				
Andhra Pradesh	Co-travellers	345	21	6·1
West Bengal	Workers of Indian Statistical Institute	150	24	16·0
Ceylon				
General Hospital, Colombo	Outpatients	124	44	35·5
Coconut Research Institute, Lunuwila	Staff and labourers	109	35	32·1
Coconut Seed Multiplication Farm, Ratmalagara	Staff and labourers	60	26	43·3
Department of Agriculture, Peradeniya	Workshop men	58	23	39·7
Royal Botanic Gardens, Peradeniya	Garden staff	63	25	39·7

Statistical analysis shows that: (*a*) there is no significant heterogeneity between frequencies in different parts of Ceylon; (*b*) the frequencies of samples in Ceylon, Andhra Pradesh, and West Bengal differ significantly. Among the Sinhalese 295 were Buddhists most of whose ancestors had probably lived in Ceylon for 2,000 years; 97 were Christians, mostly Roman Catholics; 12 Muslims and 10 Hindus. Three of the Muslims and 3 of the Hindus had hairy pinnæ. There is no significant difference between the frequencies among Buddhists (37·8 per cent) and Christians (39·2 per cent).

I have also examined many women in the various localities but found none having hairy pinnæ, as is expected on the hypothesis of *Y*-linked inheritance. The fact that the frequency of hypertrichosis is higher in Bengal and Ceylon than in an intermediate region is compatible with the statement in the Mahawamsa, an ancient Sinhalese chronicle, that Ceylon was colonized from Bengal about 2,500 years ago.

The results presented here clearly show significant differences in the frequency of the hairy pinnæ between different populations. It would seem worth while making counts of the frequencies in various parts of the world. This requires very little effort and may prove to be as useful as the survey of blood groups and other biochemical characteristics in obtaining confirmatory evidence about the affinities and differences between different human populations.

I am grateful to all the subjects for their co-operation, and to the following people in Ceylon for helping in the survey: Drs. W. Pallie, V. Basnayake, M. L. M. Salgado, I. Samaraweera, J. Wanigasooriya and Mr. W. A. K. Fonseka.

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Karyological Demonstration of Hybridization of Mammalian Cells *in vitro*

IN a recent publication, Barski, Sorieul and Cornefert[1] described the formation of cells with a hybrid karyotype in mixed cultures of two mouse cell strains *in vitro*. The experiments to be described show that this phenomenon is easily reproducible and that it is not limited to a pair of strains of strictly defined karyotypes.

The two cell lines used in our experiments are strains *NCTC* 2555 and *NCTC* 2472 of Sanford, Likely and Earle[2], ultimately derived from a single cell of mouse subcutaneous adipose tissue, as received from Dr. G. Barski by kind permission of Drs. Wilton R. Earle and Katherine K. Sanford. Both strains are populations composed predominantly of hypotetraploid cells but containing varying proportions of cells of higher ploidy[3]. The karyotypes of the hypotetraploid cells of the two strains, as established in the course of our experiments, are given in Table 1 and illustrated by Figs. 1,*A* and *C*.

Table 1

	NCTC 2555	*NCTC* 2472
Total number of chromosomes, modal	57	55
Total number of chromosomes, variation extremes	51–64	51–59
Number of biarmed chromosomes, modal	15	1
Number of biarmed chromosomes, variation extremes	11–16	1–3
Number of extra long telocentrics, modal	0	1
Number of extra long telocentrics, variation extremes	0	1–3
Minutes	?	0

It can be seen that the two cell types can be unmistakably identified by the presence of (*a*) a high number of bi-armed chromosomes, some of which represent good 'markers', in the cells of line *NCTC* 2555; (*b*) at least one extra long chromosome and a somewhat shorter one (not shown in Table 1, but visible in Fig. 1,*C*) with a sub-median secondary construction in the cells of line *NCTC* 2472.

Mixed cultures were initiated on January 3, 1961, by inoculating equal numbers of cells of the two lines into two media (a modified 199 (ref. 4) and *NCTC* 109 (ref. 5)) containing 10 per cent of heat-inactivated horse serum. Dispersion of cells for transfer was accomplished either mechanically or by trypsinization. The cultures were incubated at 37° C. in an atmosphere of 5 per cent carbon dioxide in air. Control cultures of the two strains separately were treated

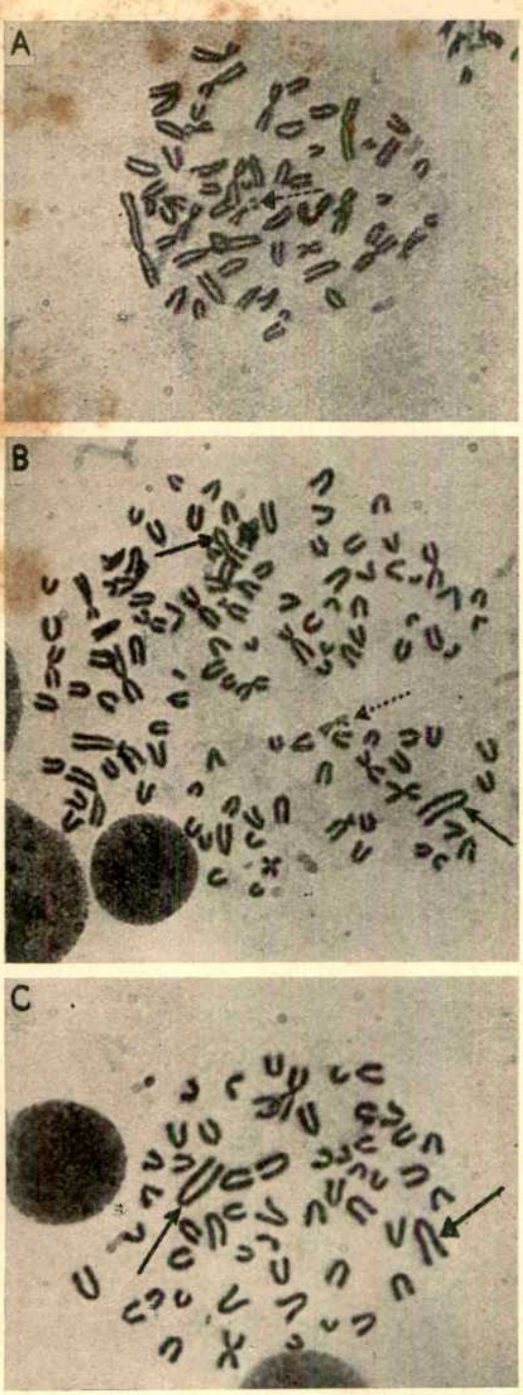

Fig. 1. *A*. Metaphase with 58 chromosomes (of which 15 are bi-armed) in a cell of strain *NCTC* 2555. Note characteristic metacentric (arrow) present in almost all cells of the strain. *B*. Representative example of hybrid cell with 112 or 113 chromosomes, of which 16 or 17 are bi-armed. Note the presence of the three 'markers' (arrows) shown in *A* and *B* *C*. Metaphase with 54 chromosomes (2 bi-armed) in a cell of strain *NCTC* 2472. Note the two 'marker' telocentrics: the extra long one and the somewhat shorter, submedially constricted one (arrows)

similarly. Karyological analyses of the mixed populations and of cells of each line grown separately were carried out at intervals on aceto-orcein squashes of colchicine-treated cultures.

At the time of writing (March 28), the occurrence of hybrid cells was observed in all four of the karyologically analysed mixed populations (two in medium 109 and two in medium 199). The hybrid cells are recognized by the total number of chromosomes and the number of bi-armed chromosomes expected from the fusion of hypotetraploid cells of the two strains, as well as by the presence of 'marker' chromosomes (Fig. 1,*B*). The frequencies of hybrid cells in the four mixed populations are at present approximately 1, 1, 5 and 10 per cent, the highest value having been observed in medium 109.

These results confirm the observations of Barski *et al.*, and furthermore show that the formation of hybrid cells is easily reproducible and is not restricted to two rigorously defined karyotypes: the latter is clear from the fact that strain *NCTC* 2472 used by us and strain *N* 1 *T* (derived from *NCTC* 2472) used by Barski *et al.* have somewhat different karyotypes. Thus the long constricted chromosome of line *NCTC* 2472 used by us has never been observed in Barski's line *N* 1 *T*; the cells of the latter strain very seldom contain bi-armed chromosomes, whereas those of line *NCTC* 2472 used by us all carry at least one such chromosome.

It may be pointed out also that, of the two strains involved in the mixed cultures of Barski *et al.*, at least one was re-isolated from a tumour induced by the inoculation of the original *in vitro* line. On the other hand, the cells used in our experiments never underwent animal passage since their explantation by Sanford *et al.* It is clear, therefore, that the tendency to cell fusion is not a result of animal passage.

These observations suggest that hybridization *in vitro* of other types of somatic cells should be equally possible. If this hope is justified, hybridization may become a useful tool for the investigation of a number of problems of somatic cell genetics, of oncology and of virology[6]. Examples of problems which are common to these fields and which hybridization *in vitro* could help resolving are: the genetic mechanism of variations in resistance to drugs and other chemotherapeutic agents observed frequently in tumour cells[7]; and the possible occurrence of zygotic induction[8] in virogenic cell lines.

The practicability of the method will depend to a large extent on the establishment of selective techniques for the detection of hybrid cells. Its applicability to the study of the mechanisms of cellular differentiation will require further the establishment of techniques for overcoming barriers of tissue specificity.

The technical assistance of Mlle. M. T. Thomas is gratefully acknowledged. This work was supported by a grant from the Rockefeller Foundation.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, May 15

University of London (at the Wright-Fleming Institute of Microbiology, St. Mary's Hospital, Paddington, London, W.2), at 5 p.m.—Dr. P. A. Gorer: "Genetics and the Immunology of Cancer".*

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. E. W. Bastin: "Self-organizing Mechanisms as Models for Scientific Theories".

Institution of Electrical Engineers, Electronics and Communication Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Scientific Papers.

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. M. S. Bartlett: "Probability, Statistics and Time" (Inaugural Lecture).*

University of London (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. A. Engström (Karolinska Institute, Stockholm): "Micro-X-ray Analysis Applied to Biology and Medicine".* (First of three special university lectures in Biophysics. Further lectures on May 16 and 17.)

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Lionel Salter: "The Presentation of Music". (Last of three Cantor Lectures on "The Presentation of Science and the Arts on Television".)

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W.C.2), at 6.30 p.m.—Annual General Meeting, followed by Dr. P. J. Bowen: "The Experimental Investigation of Space".

Tuesday, May 16

University of London (at Birkbeck College, Malet Street, London, W.C.1), at 5.30 p.m.—Prof. G. Martin (University of Bonn): "The Metaphysical Problem of Plato's Theory of Ideas".* (Further lecture on May 18.)

Librarianship Students Association, North-Western Polytechnic (at the Library Association, Chaucer House, Malet Place, London, W.C.1), at 6.30 p.m.—Prof. Hyman Levy: "The Popularization of Science—an Analysis".

Tuesday, May 16—Thursday, May 18

Institute of Physics and the Physical Society, Colour Group, and the Inter-Society Color Council of the U.S.A. (at the Royal Institution, Albemarle Street, London, W.1, and Imperial College, London, S.W.7)—Maxwell Colour Centenary.

Wednesday, May 17

Institute of Physics and the Physical Society, Low Temperature Group (at 47 Belgrave Square, London, S.W.1), at 4 p.m.—Mr. M. Zvegintzov: "Very Low Temperature Engineering".

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 4.45 p.m.—Dr. P. V. Sukhatme: "The World's Hunger and Future Needs in Food Supplies".

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. P. G. Cooray: "The Mode of Occurrence and Geological Significance of the Charnockites of Ceylon". (To be read by Prof. J. Sutton.)

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Scientific Papers.

Royal Society of Medicine, Comparative Medicine Section (at 1 Wimpole Street, London, W.1), at 5 p.m.—Dr. J. L. Stafford: "Iron Metabolism in Man and Animals"; Dr. D. J. Perkins: "Transferrins and Iron Transport".

University of London (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. A. Engström: "Dose–Damage Relationship for Internal Contamination with Radioisotopes".*

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Television Wire Distribution Systems".

Thursday, May 18

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. B. Katz, F.R.S.: "The Transmission of Impulses from Nerve to Muscle, and the Subcellular Unit of Synaptic Action" (The Croonian Lecture).

University of London (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. D. Mazia (University of California): "The Analysis of Cell Reproduction".*

Royal Aeronautical Society, Astronautics and Guided Flight Section (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. R. Smelt: "The Agena Satellite and the Discoverer Programme".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Annual General Meeting. 6.30 p.m.—Mr. J. A. Ratcliffe, F.R.S.: "Experimental Investigation of Space".

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. W. E. Ormerod: "The Study of Volutin Granules in Trypanosomes".

Friday, May 19

University of London (in the Physiology Theatre, Guy's Hospital Medical School, St. Thomas's Street, London, S.E.1), at 5 p.m.—Prof. W. M. Smart: "Some Aspects of Cosmology" (Fison Memorial Lecture).

Institution of Electronics (in the Large Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 7 p.m.—Mr. C. F. Whitbread: "Aerial Techniques".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Graduate Research Assistants (2) (with an honours degree in botany, biochemistry or chemistry) in the Department of Botany, to participate in research on the mechanism and biochemistry of photosynthesis—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (May 20).

Lecturer (with qualifications in biochemistry, and research and teaching experience) in the Department of Chemistry—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (May 22).

Lecturer in Pure Mathematics—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (May 24).

Staff Fellow in the Department of Physics, to contribute substantially to the research programme in low temperature physics being initiated in the Department, or to one of the existing programmes in nuclear physics and the physics of solids—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (May 24).

Lecturer or Assistant Lecturer in Bacteriology—The Registrar, The University, Leeds 2 (May 27).

Lecturer (preferably with a special interest in geomorphology) in Geography—The Registrar (Room 22, O.R.B.), The University, Reading (May 27).

Lecturer (with a good honours degree in metallurgy, and preferably some experience in research and industry) in Metallurgy—The Registrar, The University, Nottingham (May 27).

Research Assistant (graduate or prospective graduate, with a special interest in the field of growth hormones) in the Department of Botany, to undertake work on the physiology of ripening and senescence in annual plants—The Registrar, University College of Wales, Aberystwyth (May 27).

Assistant Lecturer in Botany—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (May 29).

Lecturer in Physics—The Registrar, The University, Hull (May 31).

Official Tutorial Fellow in Engineering—The Secretary, St. Catherine's College, 42 Banbury Road, Oxford (May 31).

Assistant Lecturer in Chemistry tenable in the Department of Physiology—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (June 3).

Analyst (Dairy Chemist) (with a degree in science or agricultural science, or equivalent qualifications with emphasis on chemistry) in the Department of Agriculture, Hawkesbury Agricultural College, Richmond, New South Wales, Australia, for investigational work associated with dairy factory manufacturing problems—New South Wales Agent-General's Office, 56 Strand, London, W.C.2 (June 5).

Lecturer or Assistant Lecturer in Applied Mathematics; and a Lecturer or Assistant Lecturer in Pure or Applied Mathematics—The Registrar, The University, Hull (June 5).

Lecturers or Assistant Lecturers in Chemistry and Mathematics at the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 10).

Senior Lecturer or Lecturer (preferably with a knowledge of tropical soils) in Soil Science in the Department of Agriculture, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 10).

Lecturer in Psychology at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, June 15).

Lecturer or Assistant Lecturer (preferably with special qualifications and interests in protozoology, or in the ecology of terrestrial animals, or with special qualifications and interests in cytology and genetics) in Zoology at the University College of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 15).

Lecturer in Veterinary Physiology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 16).

Lecturer in Philosophy in the School of General Studies, Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 17).

Senior Lecturer in Agricultural Engineering at the University College of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 20).

Chair of Physics (Electronic Computing) in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

Senior Lecturer in Physics at the Royal Australian Air Force Academy (University of Melbourne)—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

ASSISTANT LECTURER (Grade B) IN MATHEMATICS—The Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

CHEMICAL RESEARCH TECHNICIAN/ASSISTANT, to work on steroids with a team investigating endocrinology in relation to human biology and genetics—Dr. J. M. Tanner, Institute of Child Health (University of London), The Hospital for Sick Children, London, W.C.1.

ENTOMOLOGIST (with a university degree in science or agricultural science with specialization in entomology, some qualifications in plant pathology, and preferably experience in either agriculture or pest control) in the British Solomon Islands Protectorate, for entomological work in crop protection with special reference to control of anblypelta and brontispa causing damage to coconut palms; work will also cover insect pests of rice, stored copra, village food crops and cocoa, a new crop in the Protectorate—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/45/04.

RESEARCH ASSISTANT to help with current research on an immunological project concerned with rheumatoid arthritis—The Pathologist, Stoke Mandeville Hospital, Aylesbury, Bucks.

RESEARCH ASSISTANTS (2) (with a first- or second-class honours degree in geology) IN THE DEPARTMENT OF GEOLOGY—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

SENIOR TECHNICIAN (with experience in general laboratory work, photomicrography and histological techniques) IN THE DEPARTMENT OF ZOOLOGY—The Bursar, The University, Sheffield.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

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TAXATION OF SCIENTIFIC BODIES

IN the debate in the House of Lords on November 19, 1960, which arose out of the last report from the Advisory Council on Scientific Policy, Lord Adrian directed attention to the position of scientific societies as affected by the Rating and Valuation Bill now being considered by Parliament. The independent scientific societies in Britain, he pointed out, are a very important part of the organization for fostering progress in both pure and applied science. Their contribution to the encouragement of particular branches of science, as by the work of the Chemical Society or the Geological Society, has been immense, while others, like the Royal Society and the British Association, encourage science in general. They are a main forum for the exchange of ideas, and in retreating during the past decade from the idea of a Science Centre, so stoutly championed by Sir Robert Robinson, the Government has thrown on them a large share of the responsibility for providing scientific and technical libraries service for the country. Even to-day, Government action has scarcely begun to bridge the gap.

Nor is this all. Publication of periodicals as vehicles for original knowledge has become increasingly onerous, partly through the expanding scale of scientific work, and partly through mounting costs of printing, paper and postage. The Nuffield Foundation has already made some tentative inquiries into the possibility of alternative methods of publication and distribution; but, in the meantime, many of the scientific and other learned societies are strained to the utmost in carrying on the task of publication. Nor, as Lord Adrian also noted, should it be forgotten that such societies have also become the representative bodies that can be called on by the State for advice, and membership of which gives status to the individual. In this connexion alone, their value in bringing scientists together is important, and they have even greater direct value in providing incentives and guidance to young men.

Prof. C. F. A. Pantin has directed attention in a letter in *The Times* to another aspect. He emphasizes the extent to which the Government draws on the services of scientific men and women on advisory boards and committees of various kinds in guiding the scientific effort of the nation. For this great service they rarely receive reward of any kind, and their ability to serve efficiently depends directly on the existence of the learned societies which they maintain by their voluntary subscriptions. Their efficiency depends not merely on the maintenance of publication but even more on that personal discourse by which we all keep in touch with rapid advance of knowledge, and which Lord Adrian himself emphasized.

Lord Adrian pointed out that for more than a century the scientific societies of Britain have been assisted by the State in the provision of buildings for some of them, by exemption from rates, and recently by grants for publications. It was a thoroughly British arrangement. The societies have kept their freedom and independence, and the help they receive from the State enables them to shoulder the much greater responsibility that both the rapid advance of science and the national interest demand. There has been no serious complaint about the way the arrangement has been working recently, or about the goodwill of the Government towards scientific societies. It is the danger which threatens their finances through the Rating and Valuation Bill with which Lord Adrian was concerned.

The effect of the proposals of the Bill, in accordance with a recommendation of the Pritchard Committee, would be that societies which are now exempt would in future be required to pay half the full rateable value of their premises. To shoulder these new burdens without any increased help, the scientific societies will be obliged to raise their subscriptions and cut down their expenses; for example, by reducing the size of their periodicals and curtailing the number of meetings they arrange. There may well be room for more careful scrutiny and some reduction in the number of papers published; but it is one matter for that to take place on the initiative of a scientific society itself, and quite another when it is forced on the society by financial stringency alone. No doubt the major national societies will survive the crisis, even if some of them are maimed; but, as Lord Adrian observed, some of the smaller bodies dealing with highly specialized branches of science may be faced with extinction if the recommendations of the Pritchard Report are adopted and there is no countervailing assistance from the State.

When Lord Adrian made this powerful plea for due consideration before action is taken to reduce so seriously the great scientific asset Great Britain has in the independent scientific societies, Lord Hailsham, the Minister for Science, responded by asking if Lord Adrian could induce the societies to inform either him or the Chancellor of the Exchequer on the exact position, which is already under consideration by the Government. Lord Hailsham agreed as to the force of Lord Adrian's remarks, but the Minister of Housing and Local Government, Mr. H. Brooke, made no specific reference to this aspect when moving the second reading of the Bill in the House of Commons on November 30. He merely explained that the Government had decided to follow the Pritchard Committee's recommendation and repeal the total exemption from rates given by the Scientific Societies Act of 1843 to scientific and other societies, the definition having proved too difficult to apply in practice. Instead, under the Bill, practically every organization now obtaining benefit under the 1843 Act would in future qualify automatically for 50 per cent de-rating, and the local authority would have discretion to give up to complete exemption if it thought fit.

Although it was not mentioned in the debate, a small deputation from the Parliamentary and Scientific Committee had already discussed the matter with the Minister on July 27. Then, after the presentation of the Bill to Parliament, at a meeting of the Rating Sub-Committee, it was agreed to take steps to secure certain amendments during the passage of the Bill through Parliament. First, it was proposed to make it clear that the hereditaments to be given mandatory 50 per cent rating relief under clause 8 (1) will include all hereditaments properly established for scientific purposes as well as charitable purposes. Secondly, it is proposed that the power given to rating authorities in subsection (4) of this clause 8 to reduce or remit the payment of rates chargeable in respect of hereditaments, and including hereditaments not established for profit and concerned with science, should be transferred to the Minister of Housing and Local Government, so that there should be central decision.

On the first amendment, it might be observed that scientists may well feel that there is a certain lack of dignity if relief in this matter is made to depend on charity and not on the nature of the scientific society itself. There may be difficulties, as Lord Hailsham suggested; but the need is a national one and deserves a certain dignity of treatment whether from the point of view of the scientist or of the nation. On the second, because the matter is a national one, it is neither just nor appropriate to place on local authorities the financial obligation to meet charges which properly belong to the whole nation, quite apart from the matter of uniformity in policy which central decision should secure.

When this clause was considered in Standing Committee in the House of Commons on February 14*, the position of the scientific and learned societies was pressed by Mr. D. Wade and Mr. J. T. Price; but the Minister maintained that national importance by itself ought not to be taken as entitling a body to relief from rates. This, of course, specifically rejects the argument advanced by Lord Adrian as well as by Prof. Pantin in spite of the fact that Mr. Brooke himself agreed that it would be a grave loss to the nation if some of the societies in question ceased to exist. There was in fact some general support for the view that since the societies in question are mostly bodies of national character, there is no cogent reason why any special financial assistance they received should come from the local rate-payers. Mr. Mitchison had urged on February 9 that the Government should accept the full re-rating of these societies, and that the consequent burden should be met in full by the Government as a Government concerned with science and the high reputation of the country in these matters.

Because of the form and content of the Bill and the ordinary rules of procedure, amendments requiring the Government to give full rating relief could only be proposed by the Government itself. This undoubtedly appears to be the most dignified procedure; but, while declining to give a pledge that whatever body had received exemption from rates hitherto should now be entitled to turn to the Exchequer for re-imbursement of any rates it might have to pay, Mr. Brooke nevertheless stated that the Treasury would be ready and willing to look at the publication grants which it makes, in particular to the Royal Society, and to consider the position which has arisen. The problem, he said, cannot be solved by legislation; he had been in touch with both the Minister for Science and with the Chancellor of the Exchequer and had their assurance that at the time when the effects on the learned societies could be assessed in the light of the new revaluation they would be fully prepared to meet representatives of the societies for a full examination of those effects on the finances of the societies.

Mr. Brooke maintained that the question is what their financial position will be when the Bill becomes an Act and when the new valuations have been made, and that there will be ample time for these conversations. The societies would not begin to pay rates until April 1964, and then on 10 per cent of their rateable value; but we should know some time in 1962 what the actual liabilities for rates of the societies will be. For this reason, he recommended the Committee not to accept the amendments, but that the Government should be held to the assurance he had given.

During the third reading of the Bill on May 10, Mr. Brooke stated that a deputation from the Royal Society and the British Academy, acting on behalf of the national learned societies, had met the Minister for Science and the Financial Secretary of the Treasury, and that it was generally considered that the needs of such societies should be dealt with outside the framework of the Bill. A joint review of the needs of learned societies of national importance is to be undertaken after the Bill becomes law.

There are thus hopes of a satisfactory settlement which meets the needs of the societies and of the nation on terms conducive to mutual dignity and respect. None the less, there is no justification for any relaxation by scientists over the next few years; they must continue to emphasize the place and function of the scientific society, and its value not simply to the advancement of science but also in the service of the Government and nation.

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* Parliamentary Debates: House of Commons—Official Report. Standing Committee D. Rating and Valuation Bill: Ninth Sitting, Tuesday, 14th February, 1961. Columns 409–456. (London: H.M. Stationery Office, 1961.) 9*d*. net.

A SURVEY OF ORIENTAL GRASSES

The Grasses of Burma, Ceylon, India and Pakistan (excluding Bambuseae)

By Dr. N. L. Bor. (International Series of Monographs on Pure and Applied Biology. Division: Botany, Vol. 1.) Pp. xviii+767. (London and New York: Pergamon Press, 1960.) 160s. net.

NOTHING gives greater pleasure than to welcome a comprehensive work based on dedicated study by a recognized authority. The author of this book began his work on the Gramineae more than thirty years ago, and it deals with the species, both wild and introduced, of 243 genera of this important family which occur in the sub-continent of India. Presented mainly as a practical handbook, it surpasses the author's modest claims.

The general part of the text gives an excellent account of the morphology of the grass plant (pp. 1–22), deals with dispersal methods, uses of grasses and enumerates certain obnoxious ones. Unfortunately, not all these unusual observations are indexed. The systematic part of the text (pp. 61–681) presents the genera and species grouped in three tribes of Panicoideae and thirty-six of Pooideae. Critics of the alphabetical sequence should read carefully the reasons given on p. 77, and bear in mind the problems implied, as well as exemplified, in the concise, but cogent, section on taxonomy and phylogeny (pp. 61–76). Taxonomists will wish to know the precise date of publication; this was December 14, 1960. The eighty line-blocks, though uneven in style and quality, are of particular interest in showing a comparison of the work of the ten artists. (Not mentioned in the book, they are: Mr. G. Atkinson, Dr. Bor (Figs. 3, 4 and 70), Miss L. Ripley, Miss S. Ross-Craig, Mr. J. Shaw, Miss M. Smith (Figs. 31 and 77), Miss E. M. Stones, Mrs. O. Tate, Mr. P. Taylor and Mr. W. E. Trevithick). The bibliography is good, including almost 600 items; the 89 works by Dr. Bor testify to his preparatory industry. The index lists about 4,800 entities, but omits all varieties. The author acknowledges his indebtedness to J. D. Snowden in the genus *Sorghum*, and Dr. A. Melderis has given assistance in the *Triticeae*.

The magnitude of Dr. Bor's achievement can be gauged from the number of taxonomic entities involved: the 243 genera include 1,250 (± 5) species, but the index shows that he has also placed some 3,500 specific synonyms. Ninety-seven of the genera are monotypic in the area; 60 per cent have less than three species (high proportions, even for Gramineae, perhaps because of introductions); thirty-two have more than ten. The author gives full synonymy for the genera, he has checked all references, examined almost all the type specimens (but does not indicate which) and gives caryological data. The taxonomy is commendably explicit, with full notes covering difficult cases; but one regrets the author's caution, as so few will reach a better position than he to decide some of the issues he leaves "to later research". Need he have deliberately placed the same nine species in both *Erianthus* and *Saccharum*? As to the nomenclature, the author's deep concern with the plants themselves seems to have made him impatient of some of the niceties of the International Code. The inclusion of the same new variety in both *Andropogon polyptychus* and *Dichanthium polyptychus* makes it invalid (*vide* Article 33), like the new combination similarly duplicated on pp. 151 and 212. The text is unmarred by typographical errors, but users may add *Ischnochloa*, *Sorghastrum* and *Themeda* to the list of Andropogoneae on p. 80.

Dr. Bor has completed a study of noble proportions which will become relied on for Asiatic grasses as is Hitchcock's invaluable manual for the North American; the keys make it more useful, the lack of descriptions being less of a handicap for grasses where keys so often use technical characters. Significant scientifically and superlatively well written, the book shows a beautiful balance of original and derived information; at every opening one observes the author's practical knowledge of the grasses of India and his familiarity with the academic sources. This fitting addition to the literature is especially welcome at the present stage in the development of agriculture in Asia. What a pity the high price will tend to restrict ownership of the book to institutions.

JOHN LEWIS

VIRUSES IN DISEASES OF CHILDREN

Viral Infections of Infancy and Childhood

A Symposium of the Section on Microbiology, The New York Academy of Medicine. Edited by Prof. Harry M. Rose. Pp. xi+244. (New York: Hoeber-Harper, 1960.) 8 dollars.

THIS beautifully produced symposium provides a concise account of recently acquired knowledge and current concepts in its chosen field. The approach is epidemiological, with the accent on laboratory aspects.

The first two chapters summarize recent work on the infectivity of ribonucleic acids extracted from poliovirus (H. E. Alexander) and from virus-infected cells (J. S. Colter)—excellent accounts, though of questionable relevance to many readers of this book. About half the 232 pages of text are devoted to enteroviruses, opening with chapters on coxsackie infections (H. L. Hodes) and virus myocarditis (S. van Creveld). The latter chapter gives a particularly useful review and an account of the outbreak of Coxsackie myocarditis of the newborn in Amsterdam in 1955. This dangerous disease is a threat to infants in the nurseries of maternity units, and van Creveld suggests the possibility of active immunization of pregnant women or gamma-globulin prophylaxis for infants born during epidemics of 'summer grippe'. The general properties of *ECHO* viruses are described by J. L. Melnick, and A. B. Sabin presents a particularly well-arranged review of the role of *ECHO* viruses in human disease. Fever, meningitis, and sometimes skin rash have been prominent features of various outbreaks, but associations with such other symptoms as diarrhœa and respiratory illness also appear to be significant. D. A. J. Tyrrell and his colleagues describe their investigations on the outbreak of meningitis and skin rash in England in 1956, caused by type 9 *ECHO* virus, which resembles a Coxsackie group *A* virus in its virulence for suckling mice. Long-term observations of enterovirus infections among children in a Washington orphanage are summarized by L. Rosen and associates. Dalldorf discusses the increasingly recognized role of enteroviruses other than poliovirus in paralytic disease.

Respiratory infections are introduced with a challenging and controversial exposition of the principles of influenza vaccination by F. M. Davenport and A. V. Hennessy, a subject discussed further by E. C. Curnen. There follows an account by A. Isaacs of interferon, which offers an alternative method of approach to control of virus infections. R. M. Chanock and his associates give a useful review of newly discovered respiratory agents, sounding a proper note of caution in relation to interpretation of associations of these viruses with disease. Adenoviruses are surveyed briefly by W. P. Rowe, who also gives an excellent account of salivary gland virus and related infections of animals. Recent advances in varicella and measles are reviewed by S. L. Katz and his colleagues.

This book will be of particular interest to medical virologists, clinical pathologists and clinicians who are concerned with infectious diseases and children.

N. R. GRIST

FACTS ABOUT INDUSTRIAL GROWTH

Patterns of Industrial Growth, 1938–58

(Statistical Office of the United Nations, Department of Economics and Social Affairs.) Pp. viii+472. (New York: United Nations; London: H.M. Stationery Office, 1960.) 8.50 dollars; 60*s.* 6*d.*

THE statistical staff of the United Nations has made a heroic attempt to present a picture of industrial growth during twenty years in the 'free' world. In many of the tables the countries are grouped by continent or sub-continent; still more valuable is a four-fold classification by degree of industrialization. Data are given for 1938, 1948, 1953 and 1958 or for the periods lying between each. There are also separate data presented for each country.

The volume should be of the utmost use for students of economic growth, but it should also be serviceable to those of more general interests, whether political or technological. Many a generalization can now be checked by reference to the facts; it is likely that many misconceptions will be removed.

Some of the most prominent tables show growth expressed as average annual percentage rates of increase in each of the three periods. This gives the right orientation, since the most interesting problems of modern economics are concerned with the inter-relations between growth-rates. The most central figure in the volume is that giving the average world figure for the annual rate of growth in mining and manufacturing together, namely 4·4 per cent per annum. The increase in output for each person employed in them was 2·0 per cent per annum or 2·7 per cent per annum—depending on the method of measurement.

The lowest class of countries, by degree of industrialization, showed an increase in production of 4·6 per cent per annum, and, it is satisfactory to note, an increase in output per person of 1·7 or 2·1 per cent. But the growth was from a very low level. The value added per person employed in manufacturing in the lowest class group in 1953 was less than a quarter of the value added for each person so employed in Europe, and little more than a twelfth of that per person employed in the United States and Canada (together).

There is a striking table (p. 17) showing a close correlation between the rate of increase of production and the rate of increase of production for each person engaged. No support at all (pp. 26–9) can be found for the view that strong growth tends *per se* to be inflationary; there is no correlation between the rate of industrial growth and the degree of inflation as between countries.

Much selection was needed even for this massive volume. It could be argued that too much space is given to tables of percentage distribution between groups. The explanatory text is workmanlike and raises many interesting points, but in regard to causes we can still only be at the beginning of judgment.

ROY HARROD

PLANT PATHOLOGY

Plant Disease Handbook

By Dr. Cynthia Westcott. Second Edition. Pp. xii+825. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1960.) 101*s.* 6*d.*

THIS is an enlarged edition of the book by the same author written some ten years ago, and except for slight alterations the layout remains the same. This edition, however, deals with a much wider range of host plants, and there is a noticeable expansion in most sections as a result of discoveries in research during the past decade. The book in fact lists more than 2,000 diseases occurring on more than 1,000 host plants.

It is a large book of five chapters preceded by an author's note on "How to use this Book". The first three chapters are very short—Chapter 1 is the usual introduction to the subject describing the nature of plant diseases and the principles underlying their prevention or control. Chapter 2 gives an up-to-date account of the chemicals used in control practices, and Chapter 3 is a list of the various plant pathogens, with special attention paid to the fungi, in the form of a short classification.

The next two chapters occupy nearly 700 pages between them and provide what we can call the substance of the book. Chapter 4 includes no less than 40 different types of disease placed in sections which have been created in somewhat arbitrary fashion according to symptoms shown by the hosts (for example, cankers, galls, leaf spots, mildews, rusts, wilts and so on). These sections are in alphabetical order, and in each, the various pathogens which can cause that type of disease appear in alphabetical order accompanied by details of suitable control measures, and the life-history and structure of the parasite. Chapter 5 is a list of host plants in alphabetical order according to the common name or the Latin name where the author considers this is more helpful in avoiding confusion. Below each host name is a full list of its diseases with short comments about the more important ones. At the end there are a glossary, a selected bibliography and a good index. For the purpose of emphasizing special diseases or important parasites there are about 100 illustrations in the form of photographs or line drawings. The paper and print are of the high quality which we always associate with American publications of this kind.

The book is, in the main, a list of plant diseases, but I think it is more than that. It covers an extensive field, and is a condensation of experience gained by the author over 30 years while travelling about the American continent to give advice on plant disease problems. There is certainly no comparable book in this field in Great Britain. Its scope is vast and its contents are intended for American nurserymen, growers and advisers, but even allowing for plants peculiar to the American continent I consider there is much in its pages which can be of value to horticulturists and plant pathologists in Britain. The diseases of ornamental plants are well catered for, and this branch of pathology is one in which British pathologists are not well versed.

The book claims that without special training, seedsmen and growers will be able to use it as a working guide and reference book in diagnosing and treating plant diseases. I doubt whether such people will appreciate the rather formidable lists of scientific names, and whether laymen in general can achieve accurate diagnoses despite the apparent simplicity of the system here propounded. Nevertheless, I consider that the book can provide valuable assistance to professional horticulturists, advisors and plant pathologists generally. Much of the information has had to be condensed, but it is still an impressive collection of facts skillfully cross-referenced so that most people will find little difficulty in finding quickly the knowledge they seek. It is my opinion that Prof. Whetzel, to whom the book is dedicated and whom I remember with affection, would have been pleased with this contribution by his former pupil.

D. E. Green

CONSTANTS FOR BINARY SYSTEMS

The Physico-chemical Constants of Binary Systems in Concentrated Solutions

By Prof. Jean Timmermans. Vol. 4: Systems with Inorganic + Organic or Inorganic Compounds (Excepting Metallic Derivatives). Pp. xi+1332. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 293*s*.

THIS volume is the last of a series of four prepared by offset printing and contains 900 pages of data on binary mixtures in which at least one of the components is an inorganic compound. The general arrangement follows that adopted in the earlier books and the same properties are recorded. Volume 4 presents the long-awaited indexes of which there are three, one for organic compounds, one for inorganic compounds and one for authors' names. Binary mixtures are not indexed, but under a molecular formula appears the name of one of the components followed by an unclassified list of all pages where the properties of mixtures containing that compound appear. This is a very unsatisfactory arrangement. Suppose, for example, that it was required to find the densities of acetic acid-benzene mixtures, then 166 pages under the title acetic acid would have to be searched. The author index is simply an alphabetical bibliography, so while it is possible to take a page of the main text and find the source of the work cited, it is not possible to look up an author's name and find where his work is included in these volumes.

A pair of names of compounds printed boldly at the head of each page affords assistance in searching the book, but it is a pity that somebody conversant with the peculiarities of the English language was not consulted regarding these titles so that names such as sulphur anhydride and carbon oxide could have been avoided. Some errors were noted in this volume, which contains four pages of errata applicable to Volumes 1–3. However, most of the errors previously pointed out by myself and other reviewers have not been listed. The book ends rather quaintly with a short list of papers which the author has not been able to find.

In spite of the imperfections of the indexing of these volumes this work will be invaluable to many workers as a first reference.

E. F. G. Herington

PHYSICAL TECHNIQUES IN METALLURGY

The Physical Examination of Metals

Edited by Prof. Bruce Chalmers and Prof. A. G. Quarrell. Second edition. Pp. vii+917. (London: Edward Arnold (Publishers), Ltd., 1960.) 168*s*. net.

IN the present edition of this book one large volume replaces the two volumes of the first edition. Further, the original authors have become editors for a team of contributors who provide coverage of what is relatively broad subject-matter. This change, of course, represents a current trend, and although the method has disadvantages, it is well suited to the subject in question. The book is thus a completely new one.

It deals with numerous methods of examination, ranging from those providing information on the atomic scale to those concerned with the detection of flaws. Somewhere in between come techniques for the measurement of basic electrical, thermal, and magnetic properties. Apart from the more obvious topics, the book includes details of many not necessarily expected, such as the damping capacity of metals, measurements of elasticity using mechanical waves, the use of radioactive isotopes, and vacuum techniques. A relevant method not included is that of radiography, but presumably the editors think that this is more than adequately covered in other books. However, in this connexion it should be pointed out that other X-ray techniques, such as X-ray diffraction and microradiography, as well as autoradiography are described.

For each subject the underlying physical principles are well described in clear and satisfactory physical language. The actual experimental methods are discussed, and the treatment is illustrated by typical results. No doubt there are omissions, particularly in detail, and the approach in some sections may place most emphasis on the physics of the subject. However, even if this is true, the book unquestionably achieves its aim, that is to bring together for metallurgists the principles of a wide range of physical techniques.

The volume is attractively produced, well printed and illustrated, and the text is clearly presented. All concerned with its production should derive satisfaction from the final result.

A. R. Bailey

Annual Review of Microbiology

Vol. 14. Edited by Charles E. Clifton, in association with Sidney Raffel and Mortimer P. Starr. Pp. vii+391. (Palo Alto, California: Annual Reviews, Inc., 1960.) 7 dollars.

OVER a period of thirteen years, microbiologists have become confident of finding in the *Annual Review of Microbiology* concise and critical surveys of literature covering a wide range of topics. Volume 14 comes up to the standard set by its predecessors.

This year, out of a total of seventeen articles, four will be of direct interest to virologists. Recent observations relating to the fine structure of virus-infected cells have been ably reviewed by Rose and Morgan, although it is, perhaps, to be regretted that this article is not illustrated. D. L. Walker, discussing *in vitro* cell-virus relationships resulting in death of the cell, provides a valuable survey of the links between cell degeneration and virus-induced metabolic alterations. Intramural spread of bacteria and viruses in human populations is discussed by Williams, and Kissling reviews ecological aspects of arthropod-borne viruses of man and other animals.

Scherbaum's contribution provides an overdue comparison of observations on synchronously dividing micro-organisms with established knowledge of normal cellular metabolism. In discussing the problem of protein synthesis, Novelli has focused his attention on the sequence of enzymic steps between free amino-acids and the formation of peptide bonds, as observed in cell-free systems.

Other topics reviewed include: microbial nutrition; nutrition, metabolism and pathogenicity of mycoplasmas; variation in phytopathogenic fungi; genetics and cytology of *Chlamydomonas*; gene action, a review of the gene-to-protein relationship; immunological speculations; interpretation of immuno-diffusion tests; energy metabolism of chemolithotrophic bacteria; industrial fermentations; intracellular symbiosis in insects and enzyme localization in bacteria.

This volume also includes a cumulative index of authors and chapter titles for Volumes 1–14, an addition which will be of inestimable value to all faced with the task of reviewing the literature.

B. A. Newton

Non-Benzenoid Aromatic Compounds

Edited by David Ginsburg. Pp. xii+543. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1959.) 135*s*.

THE editor was clearly faced with a dilemma in devising a terse yet fully descriptive title for this collection of review articles. As Sir Robert Robinson points out in the foreword, the word 'aromatic' in its ordinary sense was applied to benzene and its derivatives; later it began to imply a measure of stability associated with a benzenoid structure. This brings in the pyridine group, and there is a remarkable similarity in the physical properties of analogous compounds in the thiophen and the benzene series. At the other end of the scale, it was stated at the Chemical Society Symposia, Bristol, 1958 (Special Publication No. 12) and reiterated herein that cyclo-octatetraene shows no aromatic character and behaves as a typical polyolefin.

Appropriately, therefore, the first chapter discusses 'aromaticity' from the theoretical side for both benzenoid and non-benzenoid compounds, with particular reference to the π-electron concept. The chapter on the cyclobutadiene group, which includes diphenylene, is a considerably extended version of matter presented at Bristol. The chemistry of ferrocene and related compounds forms the major part of the section on cyclopentadiene derivatives: pentalene and heptalene follow. Azulenes were considered at Bristol, but are here discussed in great detail in two chapters dealing respectively with the theoretical and preparative aspects; the latter includes most of the recent work of Hafner. The seven-membered systems, tropones and tropolones, form another major topic, a condensed form of more than five hundred papers; this group includes colchicine. The final two chapters discuss shortly cyclo-octatetraene and higher cyclopolyolefins.

To the main texts of most chapters, notes of later publications have been added at the proof stage, their number indicating the lively interest being taken in these subjects. We thus have a timely, authoritative, well-documented and up-to-date series of monographs on an interesting group of substances.

B. A. Ellis

Investigations on Norwegian Cirque Glaciers

Edited by W. V. Lewis. (Royal Geographical Society Research Series, No. 4.) Pp. iv+104+19 plates. (London: Royal Geographical Society and John Murray (Publishers), Ltd., 1960.) 22*s*. 6*d*.

THIS volume gathers together many valuable observations which are mainly concerned with two small cirque glaciers in the Jotunheim, Norway. These particular glaciers have been studied in great detail by a number of specialists, each of whom reports his or her own observations in the book. The geology of the area, as it affects glacial action, is usefully discussed by Dr. M. H. Battey, while Mrs. J. M. Grove supplies very detailed observations of the ice banding of both glaciers, from which she deduces their régime and movement. A detailed physical analysis of the ice-fabric of Vesl-Skautbreen is contributed by Dr. R. J. Adie, and survey results are briefly discussed by J. E. Jackson and Miss E. Thomas.

The major contribution is the valuable analysis by Dr. J. G. McCall of observations made in two tunnels cut through to the corrie back-wall. These results, which required extremely accurate survey work, have given a detailed picture of the mechanics of flow of a small and fairly simple type of corrie glacier. They show that although rotation is important it is not the only type of movement. The observations made of the ice–rock contact at the base of the glacier are incorporated in a discussion of the processes of corrie formation. W. R. B. Battle's contribution discusses techniques and results of recording changes in temperature in bergschrunds, mainly at the Jungfraujoch, Switzerland, and in Norway, with the view of obtaining quantitative evidence of the part played by thaw-freeze in corrie formation. The first and last chapters are contributed by the editor, W. V. Lewis, in which he introduces the authors and sums up the results by applying them to the formation of a typical Lake District corrie, Blea Water.

Although much of the work reported here was done nearly a decade ago and some results have already been published, this very well-produced and illustrated volume is a worth-while contribution to glaciological literature. C. A. M. King

THE AMINO-ACID SEQUENCE OF SPERM WHALE MYOGLOBIN

Chemical Studies

By A. B. EDMUNDSON

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APPLICATION of the method of isomorphous replacement has enabled Kendrew and his colleagues to determine the structural configuration of sperm whale myoglobin first to a resolution of 6 Å. (ref. 1) and more recently to a resolution of 2 Å. (ref. 2) by X-ray analysis of type-*A* crystals of the protein. At the suggestion of Dr. Kendrew, a detailed chemical study of the primary structure of the protein was initiated several years ago, and is being continued by one of us (A. B. E.) at Cambridge. Some of the results obtained thus far in the chemical approach are summarized in the present article. Recently, Kendrew *et al.* (see accompanying article) succeeded in deriving an almost complete amino-acid sequence from the electron density distribution at 2 Å., and the correlation of their proposed sequence with the chemical results is presented in their article.

Our investigation has been concerned with three problems: (1) the derivation of an empirical formula for sperm whale myoglobin; (2) the fission of the single peptide chain in the protein by tryptic hydrolysis as a preliminary step in the elucidation of the amino-acid sequence; and (3) the identification of the tryptic peptides derived from the ends of the peptide chain by end-group methods and sequence determinations.

Amino-acid Composition of Sperm Whale Myoglobin

Since the earlier work of Bodo and Kendrew (personal communication) had demonstrated that crystalline sperm whale myoglobin could be resolved chromatographically into several components, an inquiry into the nature of the structural variations responsible for the inhomogeneity became of considerable importance in connexion with more refined X-ray studies, as well as with the projected, detailed chemical work.

Crystalline sperm whale myoglobin (samples kindly provided by Dr. J. C. Kendrew) was fractionated by the method of Bodo (personal communication; cf. Boardman and Partridge[3]). The protein was chromatographed as the carboxy derivative (COMb) at 4° on the sodium form of 'Amberlite *IRC*–50' at *p*H 5·86 and *p*H 5·88, with citrate buffer, 0·34 *N* in sodium ion concentration, saturated with carbon monoxide, as eluent. Resolution of the protein into five hæm-containing components, and approximately 2 per cent of colourless proteins was achieved. Its chromatographic behaviour in the *p*H range 5·82–5·92 was studied with the view of the definition of optimal conditions for the preparation of each of the components in a purified form.

Fractions containing components I, II, III, IV + V, and V as the predominant (> 90 per cent) constituents were isolated. Components I, II and III accounted for 2–3, 7–10 and 20 per cent, respectively, of the hæm-containing protein in crystalline sperm whale myoglobin; components IV and V together accounted for the remainder.

After acid hydrolysis, each of the fractions was subjected to quantitative amino-acid analysis by the procedure of Spackman *et al.*[4]. The results are summarized in Table 1, and clearly show that, within the experimental errors, the amino-acid composition of the hydrolysate of fraction I, which is known to be contaminated by colourless protein(s), exhibits a significantly different composition. The constancy of the amino-acid composition of the acid hydrolysates of fractions II–V indicates that components II–V, and probably component I as well, are separate species of myoglobin molecules, which we propose to designate as myoglobins II–V.

Table 1. SUMMARY OF THE AMINO-ACID ANALYSES OF CHROMATOGRAPHIC COMPONENTS OF MYOGLOBIN

The composition of each component is presented in terms of the molar ratios of the constituent amino-acids

Amino-acid	Molar ratios of amino-acid residues Component I	Component II	Component III	Components IV + V	Component V
Aspartic acid	9·08	8·18	8·07	8·04	7·95
Glutamic acid	17·6	18·8	19·4	18·7	18·8
Glycine	11·0	11·2	11·0	11·1	11·0
Alanine	14·9	16·7	17·1	17·2	16·6
Valine	7·71	7·96	7·83	8·36	8·01
Leucine	15·8	17·9	17·9	18·1	18·2
*Iso*leucine	7·89	9·22	8·98	8·94	9·09
Serine	6·78	6·13	6·27	5·90	5·74
Threonine	5·45	4·87	5·15	5·18	5·03
Methionine	1·87	1·95	1·90	1·97	1·98
Proline	5·19	4·06	4·19	3·90	4·03
Phenylalanine	5·85	6·15	5·98	5·96	6·12
Tyrosine	3·70	2·94	2·94	2·79	2·92
Histidine	10·1	11·9	11·6	11·6	11·8
Lysine	16·7	19·2	18·8	18·9	19·1
Arginine	4·15	4·15	3·93	4·01	4·05
Tryptophan				1·86	
Amide ammonia.				(7·2)	(7·4)
Integral No. of residues	145*	151*	151*	153	151*

* The totals do not include values for tryptophan. Since the crystalline myoglobin from which these components were separated had a tryptophan content equivalent to 1·58 residues per mole, it is probable that all the components contain two residues of tryptophan.

Rumen[5,6] has recently resolved the structurally similar myoglobin of seals (cf. Scouloudi[7]) into several components by chromatography on *CM*-cellulose. The seal myoglobin components differed electrophoretically (cf. finback whale myoglobin[8]), and it is, therefore, possible that variations in amide content are responsible for the chromatographic inhomogeneity.

Since myoglobin IV and myoglobin V represent the major constituents of crystalline sperm whale myoglobin, the fraction containing these two proteins was selected for structural study. To complete the amino-acid analysis, fraction IV + V from sperm whale myoglobin was subjected to alkaline hydrolysis by the procedure of Drèze[9], and the tryptophan content of the hydrolysate was determined by the starch column method of Moore and Stein[10]. The tryptophan value obtained is given in Table 1, and indicates that myoglobins IV and V both contain two residues of tryptophan per molecule. The amino-acid composition of myoglobins IV and V thus involves 153 residues and approximately 7 amide groups. The molecular weight based on this composition (including the hæm group and the terminal molecule of water) is 17,816.

The chromatographic differences between myoglobins IV and V and the other components remain without explanation. The possibility that the components differ in amide content is at present under investigation.

Peptides obtained on Tryptic Hydrolysis of Myoglobins IV and V

The absence of cystine (cf. Table 1) permitted the direct use of the heat-denatured protein as a starting material for work on the peptides. After the protein was subjected to tryptic hydrolysis at *p*H 8 or at *p*H 9 for 16–20 hr. at 40°, the hydrolysate was separated into a soluble fraction, and an insoluble fraction with which all the hæm was associated. From the soluble fraction 20 peptides and free lysine were isolated in yields of 7–82 per cent by chromatography on 'Amberlite *IRC*–50' and 'Dowex 50–*X*2'. The results obtained on amino-acid analysis of these peptides are summarized in Table 2.

The generally high purity of the peptides revealed by the analyses in Table 2 is noteworthy, and the composition of each peptide is clearly defined by the molar ratios of its constituent amino-acids. The compositions are consistent with the specificity of trypsin, since each peptide, with the exception of the carboxyl terminal peptide, Tryp-16 (which will be considered later), contains at least one residue of lysine or arginine. Peptides Tryp-11, -12 and -19 differ from peptides Tryp-9, -14 and -15 only by the presence of an additional lysine residue. It has therefore been tentatively assumed that free lysine (Tryp-7) was released from each of the three regions of the peptide chain represented by these peptides, although their formation as a consequence of transpeptidation cannot be ruled out on the basis of these results alone. In this connexion a peptide of the composition Arg.(Asp,Ala$_2$,Ileu,Lys).Lys.Tyr was isolated from a *chymotryptic* hydrolysate of the proteins, and since this peptide contains all the amino-acids of peptide Tryp-11, the presence of the latter in tryptic hydrolysates is probably not attributable to transpeptidation.

It should be noted that Tryp-10, isolated in a yield of only 7 per cent (cf. Table 2), is not derived from a unique, unaccounted portion of the amino-acid sequence, since its inclusion as such would necessitate the assignment of a fifth proline residue to the composition of myoglobins IV and V (cf. Table 1). If peptides Tryp-10, -11, -12 and -19 are excluded, the soluble peptides collectively account for 107 residues, including 16 of the 19 residues of lysine and 3 of the 4 residues of arginine.

When the insoluble fraction was exposed to trypsin a second time, small but significant changes were detected in its composition. However, the com-

Table 2. AMINO-ACID COMPOSITION OF SOLUBLE PEPTIDES OBTAINED FROM MYOGLOBINS IV AND V AFTER DEGRADATION WITH TRYPSIN

The composition of each peptide is presented in terms of the molar ratios of the constituent amino-acid residues. The peptides were isolated by ion-exchange chromatography on a series of columns of 'Amberlite *IRC*-50' and 'Dowex 50-*X*2'. The values for the principal components in each peptide are in black type. Values less than 0·01 unit are omitted from the table. The values in parentheses are not included in the totals

Amino-acid	Peptide																					Total No. of residues from peptides Tryp 1–21
	Tryp-1	Tryp-2	Tryp-3	Tryp-4	Tryp-5	Tryp-6	Tryp-7	Tryp-8	Tryp-9	Tryp-10	Tryp-11	Tryp-12	Tryp-13	Tryp-14	Tryp-15	Tryp-16	Tryp-17	Tryp-18	Tryp-19	Tryp-20	Tryp-21	
Aspartic acid	—	**0·99**	—	—	—	—	—	—	0·01	0·03	**0·91**	0·08	—	**0·98**	**0·97**	—	—	**1·93**	**0·98**	0·08	**2·86**	8
Glutamic acid	**0·98**	—	—	—	—	—	—	—	**3·03**	0·22	0·13	**3·00**	—	—	**1·00**	**1·96**	**1·98**	**2·00**	**1·04**	**1·99**	**1·16**	14
Glycine	0·01	—	—	0·03	—	—	—	—	**0·95**	0·06	0·17	**1·14**	0·04	0·06	—	**2·04**	0·02	**2·10**	0·20	0·09	**3·04**	8
Alanine	**1·01**	—	0·06	**1·01**	—	—	—	—	**2·99**	**0·98**	**1·94**	**3·05**	—	**2·00**	**1·03**	—	**1·02**	**2·02**	**1·09**	0·07	**2·85**	14
Valine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**2·11**	—	—	—	2
Leucine	**2·01**	—	—	—	**0·96**	—	—	—	**1·98**	**1·00**	0·06	**2·05**	**1·02**	—	**1·01**	**1·00**	—	**1·02**	**0·98**	**1·01**	0·12	10
*Iso*leucine	—	—	**1·95**	—	—	—	—	—	—	—	**1·02**	0·06	—	**0·97**	—	—	—	**1·98**	0·04	—	0·04	5
Serine	0·03	—	0·06	0·08	0·12	0·04	—	—	**0·98**	0·13	0·21	**0·98**	0·05	0·10	**0·92**	—	—	0·14	**0·96**	**0·97**	0·36	3
Threonine	—	—	—	**1·01**	—	—	—	—	**1·00**	—	0·04	**0·98**	—	—	—	—	**0·98**	—	—	**0·98**	0·03	4
Methionine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**0·89**	—	—	—	**0·96**	2
Proline	—	—	**1·03**	—	—	—	—	—	**1·04**	**0·96**	—	**1·13**	—	—	—	—	—	—	—	**1·03**	**0·99**	4
Phenylalanine	**1·03**	**1·00**	—	—	**0·98**	**0·95**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**0·95**	5
Tyrosine	—	—	—	—	—	—	—	**0·88**	—	—	—	—	—	—	—	**0·96**	—	—	—	—	—	2
Histidine	—	—	—	—	—	—	—	—	**2·97**	—	0·10	**2·94**	**1·00**	—	—	—	—	**0·85**	—	**1·00**	**1·11**	7
Lysine	0·02	—	**1·02**	**0·98**	**1·07**	**1·05**	**3·00**	**1·00**	**2·07**	**1·06**	**2·03**	**2·92**	**0·98**	**1·03**	**0·99**	—	**1·00**	0·06	**1·90**	**1·03**	**1·05**	16
Arginine	**0·96**	**1·01**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**0·94**	—	—	—	3
Total No. of residues	6	3	4	3	3	2	3	2	17	(4)	(6)	(18)	3	5	6	6	6	15	(7)	8	15	107
Yield in per cent	50	42	15	13	42	82	33	45	35	7	20	10	49	62	33	50	72	9	10	56	17	

position was not markedly affected by additional exposures to the enzyme. After exhaustive treatment with trypsin, the insoluble fraction ('core') was subjected to amino-acid analysis, from the results of which the following overall composition of the fraction was deduced:

$$\text{Asp}_2,\text{Glu}_6,\text{Gly}_4,\text{Ala}_5,\text{Val}_6,\text{Leu}_7,\text{Ileu}_4,\text{Ser}_3,\text{Thr},\text{Phe},\text{Tyr},\text{Try}_2,\text{His}_4,\text{Lys}_3,\text{Arg}.$$

This composition was not markedly altered when the insoluble fraction was subjected to the action of chymotrypsin.

The combined analytical results obtained from the soluble and insoluble portions of the tryptic hydrolysate indicate that all the amino-acid residues in the protein, with the exception of one histidine and one leucine residue, were identified as constituents of the peptides. Collectively, these peptides contain six additional residues (Asp_2,Glu,Gly,Ala_2) not assigned to the composition of myoglobins IV and V. It is probable that these additional residues are present in sequences accounted for by both the insoluble fraction and one or more soluble peptides.

The hæm group apparently does not shield the insoluble fraction from the action of trypsin. The composition of the insoluble fraction from a tryptic hydrolysate of urea-denatured globin prepared from myoglobins IV and V was found to be almost the same as that of the insoluble fraction from myoglobins IV and V.

End-group Analyses

Prior to further fractionation, the homogeneity of the peptide material represented by the insoluble fraction ('core') was investigated by end-group analysis. Both the insoluble fraction, and for comparison, the intact protein were subjected to dinitrophenylation by Levy's method[11]. After acid hydrolysis of the dinitrophenyl (DNP) derivatives, a single amino-terminal residue of valine was detected in each case by a combination of the following methods: identification of DNP-valine by paper chromatography[12]; quantitative determination of the valine liberated from DNP-valine by alkaline hydrolysis[13]; and a subtractive procedure in which each polypeptide was subjected to quantitative amino-acid analysis[14] before and after dinitrophenylation. The presence of one DNP-valine residue in the insoluble fraction indicates that the insoluble fraction contains either one peptide with a valine terminal group, or several peptides, one of which possesses a valine end-group, whereas the others have end-groups that were not detected by the procedure used. In the accompanying paper Kendrew *et al.* have deduced that the 'core' must consist of at least two peptides.

Since peptide Tryp-18 is the only soluble peptide containing valine, it was subjected to sequential degradation by Edman's method[15], and the results were compared with those obtained when the protein was subjected to the same procedure. The amino-acids cyclized off at each step were identified by a subtractive method[16], alone or in combination with paper chromatographic identification[17] of the corresponding phenylthiohydantoins. The sequence valylalanine (Val.Ala) was found at the amino-end of the protein, whereas in peptide Tryp-18 the corresponding sequence was found to be valylglutaminylalanine (Val.Glu-NH_2.Ala). The valine terminal peptide in the insoluble fraction thus represents the amino-terminal region of the protein.

The identities of the amino-terminal residues in peptides Tryp-9 and Tryp-21, and the amino-acid sequences in peptides Tryp-1, Tryp-2, Tryp-4, Tryp-5, Tryp-13, Tryp-14, Tryp-15, Tryp-16 (the carboxyl-terminal peptide in the chain), Tryp-17, and Tryp-20 were established by Edman's method. The sequences found are shown in Table 3.

Table 3

Peptide	Sequence
Tryp-1	Ala.Leu.Glu.Leu.Phe.Arg
Tryp-2	Phe.Asp.Arg
Tryp-4	Ala.Thr.Lys
Tryp-5	Leu.Phe.Lys
Tryp-9	N-terminus: Gly
Tryp-13	His.Leu.Lys
Tryp-14	Asp.Ileu.Ala.Ala.Lys
Tryp-15	Ala.Ser.Glu.Leu.Asp.Lys
Tryp-16	Glu.Leu.Gly.Tyr.Gly.Glu-NH_2
Tryp-17	Thr.Glu.Ala.Glu.Met.Lys
Tryp-20	Ser.His.Pro.Glu.Thr.Leu.Glu.Lys
Tryp-21	N-terminus: His

Glutamine, glycine and tyrosine were the principal products liberated from both Tryp-16 and the globin derived from the intact protein by the action of carboxypeptidase *A*, whereas leucine and glutamic acid, as well as alanine, *iso*leucine, threonine and aspartic acid, were released in smaller quantities from the protein. The quantitative similarity in the pattern of release of the principal products liberated in each case substantiates the conclusion that peptide Tryp-16, the only peptide devoid of lysine and arginine, represents the carboxyl-terminal region of the protein.

Work is now in progress on the peptides liberated by the action of chymotrypsin from myoglobins IV and V in order to obtain the information necessary for the connexion, in sequence, of the tryptic peptides along the polypeptide chain.

This work was initiated at the Rockefeller Institute in the laboratories of Dr. S. Moore and Dr. W. H. Stein, to whom we are indebted for their advice and encouragement. Research at Brookhaven National Laboratory was performed under the auspices of the United States Atomic Energy Commission. The work of one of us (A. B. E.) at Cambridge has been facilitated by grants from the United States Public Health Service for a fellowship and for provision of an amino-acid analyser (grant Nos. *A*–3032 and *A*–3032(S1)). We thank Dr. J. C. Kendrew for his continued support of this research and Mrs. Margaret Ross for her skilled assistance.

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A Partial Determination by X-ray Methods, and its Correlation with Chemical Data

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An earlier article in *Nature*[1] described the general features of the 2-Å. Fourier synthesis of sperm whale myoglobin, and showed that the molecule consists of a number of segments of right-handed α-helix separated by irregular regions. In that article doubts were expressed about the possibility of identifying side-chains in a Fourier synthesis calculated at 2-Å. resolution, at which neighbouring covalently bonded atoms are generally not separately resolved. Interpretative techniques have now been improved, however, and it has, in fact, proved possible to identify many side-chains with assurance, and in many others to narrow the choice to two or three. To determine completely the amino-acid sequence of a protein by X-ray methods alone, it will clearly be necessary to work at higher resolution than 2-Å., as we intend to do in future studies of myoglobin; but in the meantime it has been possible, by correlating the present X-ray identifications with the preliminary chemical results, described in the preceding article by Edmundson and Hirs, to arrive at a tentative amino-acid sequence which, though it is incomplete and contains ambiguities and no doubt some errors, cannot be very far from the truth.

Methods of Identifying Side-chains

The Fourier synthesis is normally represented as a stack of contoured 'Perspex' sections representing the electron density in a series of parallel planes cut through the molecule. The previous article described another method of representation, more convenient for model-building, in which the main features of the density at a grid of points in the structure are represented by coloured clips attached to vertical steel rods; the structure of the molecule can be outlined by building skeleton-type atomic models between the rods in such a way that they follow the density indicated by the clips. With the help of this technique, it was easy to locate all the helical segments of the molecule, and, with somewhat less assurance, to follow the course of the main chain in non-helical regions where it turns corners. Once the main chain has been correctly inserted, the density corresponding to the side-chains can easily be seen emerging from it at the appropriate intervals. It is now often possible to identify side-chains by building atomic models between the rods so as to follow the density, and checking the results by reference to the more detailed information in the contour map itself. We have also made use of special sections cut through the density distribution at any desired angle and position; these sections are prepared by interpolation from the original density function by means of a computer programme, devised for *Edsac* Mk. II by Dr. Joyce Wheeler.

Methods of identification can be summarized as follows:

(1) Once the course of the main chain has been established, the starting point of each side-chain becomes defined, since the position of Cβ is fixed relative to the main chain.

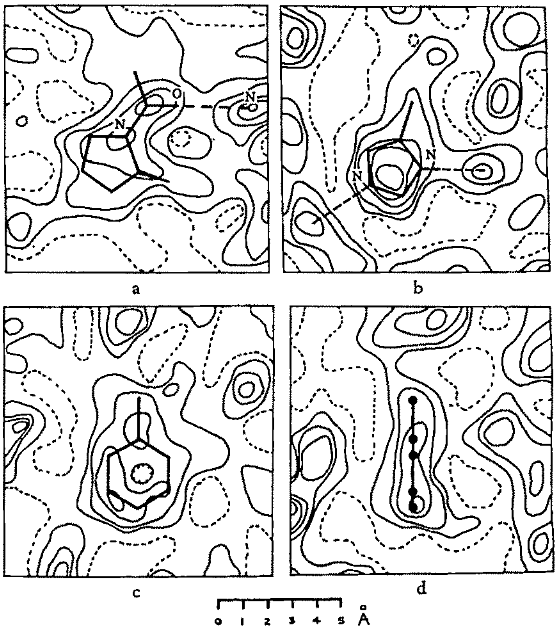

Fig. 1. Sections through parts of the 2-Å. Fourier synthesis showing side-chains. Contour interval 0·5 el./Å.^{3}: atoms not otherwise labelled are carbons. (*a*) Proline, *F*3. The dotted line is a hydrogen bond from the CO group to a NH group along the helix. (*b*) Histidine, *C*1. The dotted lines indicate hydrogen bonds to neighbouring groups, probably ions or water molecules. (*c*) Phenylalanine, *B*14. Note that there is no density peak within Van der Waals distance of the ring atoms. (*d*) Phenylalanine *B*14. Section cut normal to the plane of the ring

(2) Studies of the structures of simpler compounds by X-ray and spectroscopic methods have led to the generalization that, unless strong interactions elsewhere in the molecule impose restraints, the arrangement of the bonds about a singly bonded pair of carbon atoms is always very close to the staggered configuration, the eclipsed configuration being, energetically, relatively unfavourable. A detailed examination of the published structures of amino-acids and peptides (F. H. C. Crick, personal communication) shows that this generalization is applicable to such compounds. We have made the assumption—which our experience here justifies—that, unless there are over-riding reasons to the contrary, the staggering rule is everywhere obeyed within ± 10–15°. It means, for example, that when the Cβ of a side-chain has been correctly placed, only three positions are open to Cγ's, and a search for density can be made in each of these positions in turn.

We have assumed, furthermore, that, in the absence of contrary reasons, such as an interaction with nearby groups, a long side-chain such as lysine is normally in the fully extended (zig-zag) configuration.

(3) Steric considerations are often important in ruling out certain configurations. Thus, in helical

Table 1

1.	*X-ray*	*evidence*		2. *Chemical evidence*	
A	1	Val 4	*a*	Val	▲
	2	Ala 4		Ala	
	3	Gly 5			
	4	Glu 5			
	5	Try 5			
	6	Ser 2, *Ala* 2			
	7	Glu 3, *Ala* 1			
	8	Ileu 4			
	9	Leu 4, *Asp* 1			
	10	Lys 3			
	11	(Not Gly)			
	12	Try 5			
	13	(Not Gly)			
	14	Leu 4			
	15	Leu 4			**Core** ***A***
	16	Glu 4			
AB	1	(Not Gly)			
B	1	Leu 3, *Asp* 1			
	2	Val or *Thr* 5			
	3	Ala 5			
	4	Gly 5			
	5	His 4			
	6	Gly 3, *Ala* 1			
	7	Lys 4			
	8	Leu 3, *Asp* 1			
	9	Thr 3, *Val* 2, *Asp* 1			
	10	Leu 5			
	11	Ileu 5			
	12	Ser 3			
	13	Leu 5			
	14	Phe 5			
	15	*Thr* 3, Lys 2, *Ser* 1			▼
	16	Ser 3, *Thr* 2		Ser	
C	1	His 5		His	
	2	Pro 4		Pro	
	3	Glu.C 5		Glu.C	**20**
	4	Thr 4		Thr	
	5	Leu 4		Leu	
	6	*Ser* 2, *Thr* 2	*x*	Glu	
	7	Lys 3		Lys	
CD	1	Phe 5		Phe	
	2	Asp 2		Asp	**2**
	3	Arg 3	*c*	Arg	
	4	Phe 3		Phe	
	5	Lys 3		Lys	**6**
	6	His 4		His	
	7	Leu 2, *Asp* 2		Leu	**13**
	8	Lys 1		Lys	
D	1	Thr 5		Thr	
	2	Glu 4		Glu.C	
	3	Ala 5		Ala	**17**
	4	Glu 3		Glu.C	
	5	Met 5		Met	
	6	Lys 4		Lys	
	7	Ala 5		Ala	
E	1	Ser 4		Ser	
	2	Glu 2		Glu.C	**15**
	3	*Asp* 4, Leu 1		Leu	
	4	*Leu* 3, Asp 1		Asp.C	
	5	Lys 3		Lys	
	6	Val, *Thr* or *Ileu* 4		*Val*	
	7	*His* 3, Glu 2		*Glu.N*	
	8	*Gly* 5	*x*	*Ala*	
	9	Ileu 3		*Ileu*	
	10	Glu 2, *Ser* 1, *β-f* 1		*Glu*	
	11	Val or *Thr* 5		*Val*	
	12	Asp 3, *Lys* 1		*Asp*	
	13	*Ser* 3, *Thr* 1, *Ala* 1	*x*	*His*	**18**
	14	*Ala* 4, Gly 1		*Gly*	
	15	Leu 3		*Leu*	
	16	Gly 5		*Gly*	
	17	Ala 4		*Ala*	
	18	Ileu, *Val* or *Thre* 5		*Ileu*	
	19	Asp 2, *Leu* 2		*Asp*	
	20	Arg 4, *Lys* 1		*Arg*	
EF	1	Lys 2		*Lys*	
	2	Lys 5		*Lys*	
	3	Gly 2		*Gly*	
	4	Leu or *Asp* 2, *His* 1		*Leu*	
	5	His 5		*His*	
	6	Glu 1, *Ala* 1		*Glu*	
	7	(Not Gly)		*Leu*	
	8	Glu 1		*Glu*	
F	1	Glu or *His* 2		*Glu*	**12**
	2	Ala 2		*Ala*	
	3	Pro 5		*Pro*	
	4	Thr or *Val* 3, *Ser* 1		*Thr*	
	5	Ala 3, *Gly* 2		*Ala*	
	6	His 2, *Asp* 1		*His*	
	7	Ser 4, *Ala* 1		*Ser*	
	8	His 5	*d*	*His*	
	9	Ala 5		*Ala*	
FG	1	(Not Ala, Gly, *β*-f) Lys!?		*Lys*	
	2	(Not Gly)		*Leu*	
	3	Phe 4, *His* 1		*Phe*	**5**
	4	(Not Ala, Gly, *β*-f) Lys?		*Lys*	
	5	Ileu 2		Ileu	
G	1	Pro 4		Pro	**3**
	2	Ileu 4		Ileu	
	3	Lys 5		Lys	
	4	Tyr 5			▲
	5	(Not Ala, Gly, *β*-f)			
	6	Glu 5			
	7	*Phe* 3, His 2			
	8	Leu 3, *Asp* 2			
	9	Ser 4			**Core** ***B***
	10	(Not Gly, Ala)			
	11	Ala 4			
	12	Val or *Thr* 4, Ileu 1			
	13	Ileu 3, *Val* or *Thr* 2			
	14	His 2			
	15	Val or *Thr* 5			
	16	Arg, *Leu*, *Asp*, *Lys* 1			▼
	17	Ala 3, *Asp* 1, *Glu* 1		*Ala*	
	18	*Ala* 3, Thre, *Val*, *Ser* 1		*Thr*	**4**
	19	Lys 3		*Lys*	
GH	1	His 2		His	
	2	*Ala* 4, *Glu* 1	*x*	Asp	
	3	*Gly* 3, Asp 1		Asp	
	4	Glu 4		Glu	
	5	Phe 4		Phe	
	6	Gly 5		Gly	
H	1	Ala 5		Ala	
	2	Pro 4		Pro	
	3	Ala 5		Ala	**21**
	4	Asp 2, *Ser* 2, *β-f* 1		Asp	
	5	Gly 3, *Lys* 1		Gly	
	6	Ala 5		Ala	
	7	Met 5		Met	
	8	Gly 4, *β-f* 1		Gly	
	9	Lys 4		Lys	
	10	Ala 5		Ala	
	11	Leu 5		Leu	
	12	Glu 5		Glu.C	**1**
	13	Leu 5		Leu	
	14	Phe 5		Phe	
	15	Arg 4		Arg	
	16	Lys 4, *Ser* 1		Lys	
	17	Asp 5		Asp.C	
	18	Ileu 5		Ileu	**11**
	19	Ala 5	**14**	Ala	
	20	*Ser* 3, Ala 2		Ala	
	21	Lys 2, *Ileu* 2		Lys	
	22	Tyr 5		Tyr	**8**
	23	(Not Gly, Ala, *β*-f) Lys?		Lys	
	24	Glu 4		Glu.C	
HC	1	Leu 2, *Lys* 2		Leu	
	2	Gly 5		Gly	
	3	Tyr 5		Tyr	**16**
	4	Gly 4		Gly	
	5		*b*	Glu.N	

x, discrepancies between X-ray and chemical data. *a*, terminal amino-group. *b*, terminal carboxyl-group. *c*, bonded to propionic acid side-chain of hæm group. *d*, Attached to iron atom of hæm group.

X-ray data. The degree of confidence in each identification is indicated by a number, with maximum confidence represented by 5. Where a choice of residues is given, that which agrees with the chemical evidence is printed in Roman type, the others in italic type. '*β-f*' means a residue forked at C*β*, that is, Val, Thr or Ileu.

Residues are identified by a letter, or letters, and a number. A single letter refers to one of the helical segments *A–H*; a double letter to an irregular region between two helices. A residue is counted as part of a helical segment (*a*) at the amino-end, if its CO group is bonded to NH along the helix, and (*b*) at the carboxyl end, if its Cα lies between two NH groups bonded to CO groups along the helix.

Chemical data. The numbers in black type are those used by Edmundson to identify tryptic peptides.
Boxes enclose residues the sequence of which has not been chemically determined.
Tryptic peptides believed (on the basis of chymotryptic digestion) to be linked together are so indicated.
Asp.C and Glu.C refer to the acids and Asp.N, Glu.N to the amides.
Peptides the positions of which in the sequence are regarded as doubtful are printed in italics.

The only tryptic peptide isolated by Edmundson and Hirs which is not accounted for in this scheme is **10** (Ala, Leu, Pro) Lys; it is believed on chemical grounds that this residue may be an artefact.

segments, one of the three Cγ positions permitted by the staggering rule is sterically impossible, because an atom in that position would be too close to a carbonyl oxygen atom in the helix. Again, the close-packed internal structure of the molecule means that neighbouring side-chains are generally in contact; often the configuration of one side-chain which has been unambiguously established imposes limitations on nearby side-chains which help in their identification.

(4) Forking of the density in a side-chain can often be clearly seen, and the position of the fork greatly aids identification. Thus a side-chain forked at Cβ must be valine, threonine, or *iso*leucine; a side-chain forked at Cγ must be leucine, aspartic acid or a ring. The shape of the density at the fork is also diagnostic; thus tetrahedral forking at Cγ can only be leucine, while a planar fork must mean aspartic acid or a ring. Ring side-chains (His, Phe, Tyr, Try) can most easily be identified by means of special sections cut in their plane; some examples of these are shown in Fig. 1.

(5) Threonine and *iso*leucine possess a second asymmetric carbon atom, of known absolute configuration. This provides a valuable check on the identification of these two amino-acids.

Some ambiguities remain even when all these methods have been applied. For example, threonine and valine, glutamic acid and glutamine, aspartic acid and asparagine, cannot be distinguished by shape alone; and leucine and aspartic acid, histidine and phenylalanine, are often difficult to distinguish at the present resolution. It is sometimes possible, however, to discriminate between these pairs by looking for interactions with nearby groups. Thus threonine, aspartic acid and histidine can interact with neighbours by means of hydrogen bonds or salt bridges, while valine, leucine and phenylalanine cannot approach nearby groups more closely than is permitted by Van der Waals contacts, which are definitely longer (see, for example, Fig. 1). Another difficulty arises from the fact that a side-chain can sometimes assume two alternative configurations between which it is statistically distributed in different molecules in the crystal. Thus we have found several lysines which can interact with either of two nearby groups, each configuration being correctly staggered; such a lysine appears to be branched and may easily be mistaken for a forked side-chain. Quite apart from these difficulties, the experimental errors inherent in the present Fourier synthesis often make identification difficult; these errors are particularly large near the sites of heavy-atom substitution. Identification is also more difficult in the non-helical parts of the molecule, where the configuration of the main chain is difficult to establish with certainty; and it is difficult to identify side-chains, especially longer ones, which stick out of the molecule into the ambient solution, presumably because they can assume several alternative configurations.

The process of identifying side-chains involves locating precisely, first the main chain atoms to which the side-chains are attached, and secondly, the atoms of the side-chains themselves. These atomic co-ordinates will also be required for structure factor calculations in refining the structure at higher resolution. In all, we have located with reasonable precision (~½ Å.) 634 atoms in helical regions of the main chain and in the hæm group, and 262 atoms in side-chains which have been identified fairly definitely; excluding hydrogens the molecule contains a little more than 1,200 atoms, so, at a conservative estimate, it should be possible to include in the initial structure factor calculations some 75 per cent of the atoms in the structure.

Correlation between X-ray and Chemical Data

Column 1 of Table 1 summarizes the results of a detailed survey of the side-chains, our degree of confidence in each identification being indicated numerically. In all, 151 residues have been located, whereas the chemical analysis indicates a total of 153. There are eight segments of α-helix, indicated by the letters *A–H*; these are separated by non-helical regions *AB*, *CD*, *EF*, etc. So far as we have been able to determine, all the α-helical segments are straight and regular with the exception of *E*, which has a slight but definite bend in the middle.

In column 2, all sixteen tryptic peptides identified by Edmundson and Hirs (see previous article) have been placed alongside corresponding regions of the polypeptide chain, four of them with less confidence than the others. In general, the composition of each peptide determined from the model agrees with that found chemically, as does the sequence (wherever it has been established by Edmundson and Hirs), and the preliminary evidence which they have obtained from the chymotryptic digest as to the order in which the tryptic peptides are connected is confirmed by our placing. Once the peptides have been placed, their known chemical compositions can be used to discriminate between alternative X-ray identifications of particular side-chains, but there remain a few absolute discrepancies which are indicated in Table 1; it must be emphasized that both the X-ray and the chemical data are preliminary, so that some discrepancies and errors

Table 2

	Total composition				Core	
	X-ray identification			Chemical	X-ray	Chemical
	(*a*)	(*b*)	(*c*)			
Gly	8	9	11	11	3	3
Ala	13	14	17	17	3	3
Ser	3	4	6	6	3	3
Val	0	1	6	8	4–5	6
Thr	2	2	5	5	1	1
Ileu	4	5	8	9	3–4	4
Leu	8	10	15	18	8	7–8
Asp	2	5	8	8	—	—
Glu	8	11	17	19	4	5
Lys	6	11	18	19	3	3
Arg	2	3	4	4	1	1
Pro	4	4	4	4	—	—
His	5	5	10	12	3	4–5
Phe	6	6	6	6	1	1
Tyr	3	3	3	3	1	1
Try	2	2	2	2	2	2
Met	2	2	2	2	—	—
Unallocated			9		4	
Totals	78	97	151	153	45	44–46

(*a*) Identified with confidence 5 or 4 (see notes to Table 1) from X-ray data alone.

(*b*) *a*+residues identified from X-ray evidence with help from composition of tryptic peptides which can be placed with confidence (ordinary type in Table 1).

(*c*) Best allocation of residues, taking into account overall chemical composition as well as chemical evidence included under *b*.

Notes. (i) In the allocations in columns *b* and *c*, there are: 16 residues where chemical evidence has been used to select from two equally probable X-ray identifications; 8 residues where chemical evidence has been used to select a less probable from two or more X-ray identifications; 6 residues allocated purely on the basis of chemical evidence (no identification possible from X-ray evidence); 4 absolute discrepancies between X-ray and chemical evidence (see Table 1).

(ii) The figures in columns (*a*) and (*b*) are often reduced by difficulties in distinguishing particular pairs; thus if Val and Thr are taken together, the figures are *a* 8, *b* 11, *c* 11 (chemical analysis, 13).

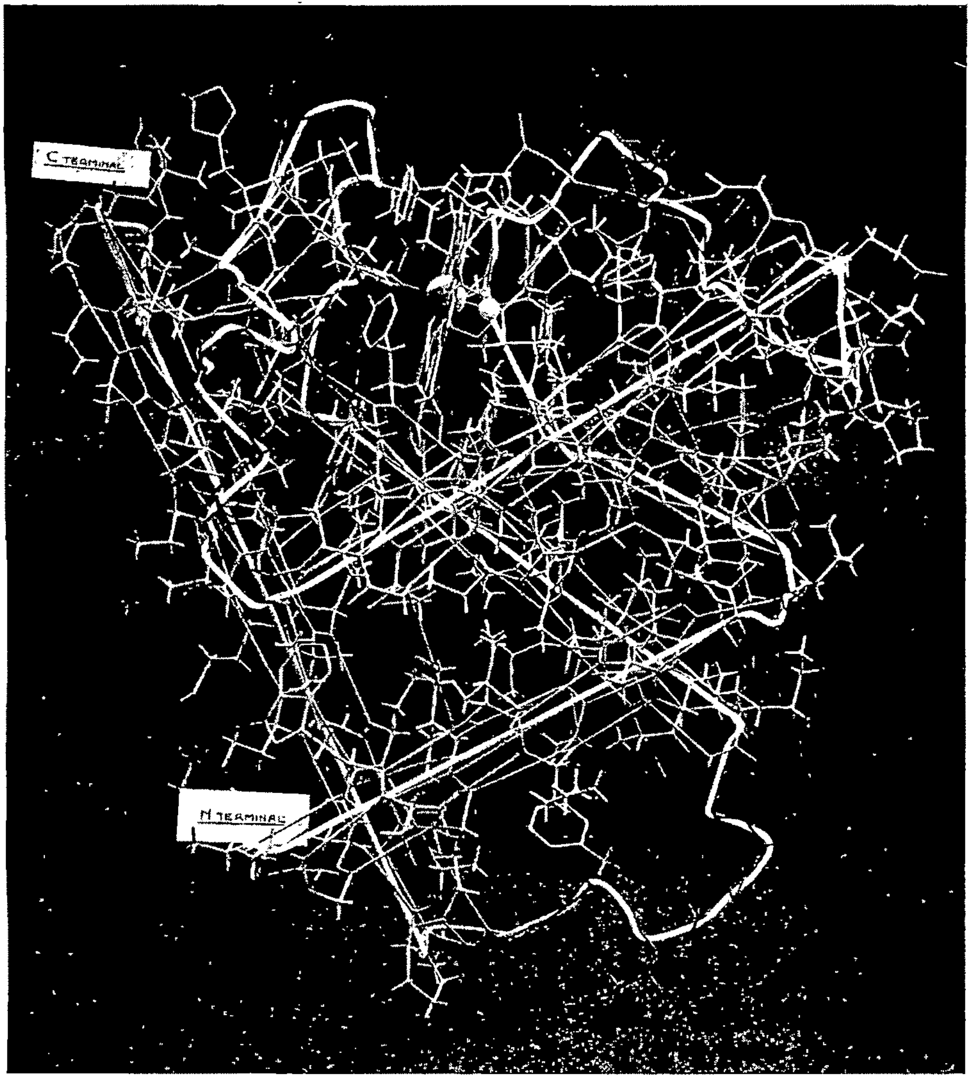

Fig. 2. Model of the myoglobin molecule, including side-chains. The course of the main chain is indicated by a white cord, the N-terminal and C-terminal residues being marked. The iron atom is shown as a grey sphere; the nearby white sphere is a presumed water molecule co-ordinated to it

must be expected. The overall agreement between the X-ray and chemical data as regards total amino-acid composition is summarized in Table 2.

In many cases our assignment of tryptic peptides to particular regions of the sequence was made in advance of more detailed chemical evidence obtained later by Edmundson and Hirs, which in all cases confirmed the assignment. Thus, the sequence of the residues within peptides 11, 15, 17 and 20 was determined chemically after these peptides had been placed as indicated in Table 1; again, the evidence from chymotryptic digestion suggesting the peptide sequences 1–11–8–16 and (part of core)–20–2–6 was not known to us at the time these peptides were similarly arranged on the basis of X-ray evidence.

One major problem concerns the placing of the so-called core, which we have tentatively assigned to the regions indicated in Table 1. As shown in the preceding article by Edmundson and Hirs, tryptic digestion leaves an insoluble core of 44–46 residues. By the usual tests the core possesses a single terminal amino-group which corresponds to that of the intact molecule; accordingly we tried to fit it to the first 44–46 residues (from *A*1 to about *CD*5) at the amino-end of the chain. This arrangement led to several difficulties, however. Thus, the overall composition of this region, determined from the model, does not correspond to that of the core; again, no satisfactory places can be found for peptides 20, 2 or 6; and finally, residue *C*2, which is only 35 places from the end of the chain, is almost certainly proline, an amino-acid which the core is believed to lack. These difficulties could be resolved if it was assumed that the core consisted of two parts, one comprising residues *A*1–*B*15, and the other residues *G*4–*G*16. The total composition of these regions agrees rather well with that of the core (see Table 2); peptides 20, 2 and 6 can be placed naturally from *B*16 to *CD*5 inclusive, and the sequence (part of core)–20–2–6 agrees with Edmundson and Hirs's deductions from the chymotryptic digest; on the other hand, we meet a new and still unresolved difficulty, namely, as yet there is no chemical evidence of a second amino end-group in the core. We may also ask why, if the core indeed consists of two fragments, these should remain stuck together during prolonged digestion. It may be noted that in the intact molecule the two regions assigned to the core pass close to one another, and it may be that some unusual type of chemical bond is involved (we have in fact observed a continuous bridge of high electron density between residues *A*11 to *G*16 which might conceivably include such a bond).

Some Features of the Structure

Fig. 2 is a photograph of a model of the molecule, including side-chains and viewed from about the same angle as Fig. 5 of the previous article[1].

Corner turning. The molecule contains eight non-helical regions, one of which consists of four residues at the carboxyl end of the chain, while the others form corners between lengths of helix. Each corner is different from every other, and the numbers of residues contained in them varies from 0 to 8. It is well known that proline cannot be inserted into an α-helix except at its amino-end, so it is not surprising to find that all four prolines in the molecule are in different corners at the last turn of a helix; one of these corners (*BC*) is very elegant, in that it contains no non-helical residues—every residue forms part of either helix *B* or helix *C*. (Another corner which contains no non-helical residues is *DE*, but there is no proline in this region.) Two other types of interaction, which are evidently important in determining the configuration of a corner, have been observed: (1) the hydroxyl group of serine (*E*1) or threonine (*D*1) hydrogen-bonding to a free peptide imino-group at the amino-end of a helix, and (2) a lysine (*B*7), which originates from quite a different part of the molecule, bonding to a free peptide carbonyl group in corner *DE*. It is by no means obvious, however, what interactions are primarily responsible for the existence of several of the corners. The three-dimensional configuration of a protein is presumably determined by the position and nature of the corners, and eventually one might hope to be able to deduce these from a study of the amino-acid sequence. The variety and complexity of the non-helical regions in myoglobin suggest, however, that this will be by no means a simple matter.

Environment of the hæm group. It is nearly certain that the group which links the iron atom to the protein is histidine (*F*8). On the distal side of the iron atom is a single peak, presumably a water molecule; beyond this is a side-chain (*E*7) which is probably histidine or perhaps glutamic acid (or glutamine). As might be expected, the polar propionic acid groups lie on the outside of the molecule, one probably linked to arginine (*CD*3); on the other hand, the non-polar vinyl groups are buried deep in the interior. The whole inner part of the hæm group is surrounded by non-polar side-chains, and it is noteworthy that there is a large concentration of aromatic side-chains in the general neighbourhood of the hæm group.

General distribution of side-chains and liquid. There is a tendency, perhaps less marked than might have been expected, for polar side-chains to project outwards from the molecule and for non-polar ones to be buried within it; 30 per cent of the polar and 45 per cent of the non-polar residues are buried. This tendency is much more obvious in certain side-chains, especially larger ones; thus all the phenylalanines and methionines are internal, and almost all the lysines, arginines and glutamic acids are external. On the other hand, most of the non-polar alanines project outwards.

Nearly all polar groups, including peptide carbonyl and imide groups, interact either with nearby groups or else with isolated peaks which must be water molecules or ions. Most of these isolated peaks are external; there are, apparently, a few water molecules within the protein molecule, but for the most part the protein is close-packed with little internal free space. The ambient fluid is 4 *M* ammonium sulphate; it is probable that the lattice contains 20–30 ammonium sulphate molecules per myoglobin molecule. Ammonium ions would be indistinguishable from water molecules at the present resolution; on the other hand, sulphates would appear as dense peaks if they occupied defined positions in the lattice. We have actually observed only two or three peaks on the surface of the molecule which are dense enough to be certainly sulphate ions; the fact that the other sulphate ions are not observed suggests that most of them, together with the water in which they are dissolved, are distributed at random.

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Comparison Between the Amino-Acid Sequences of Sperm Whale Myoglobin and of Human Hæmoglobin

By Drs. H. C. WATSON and J. C. KENDREW, F.R.S.

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PERUTZ *et al.* have shown that the tertiary structures of the α- and β-chains of horse hæmoglobin closely resemble those of sperm whale myoglobin[1]. One might predict that there would be correspondences between those side-chains, the specific interactions of which are responsible for the tertiary structures of the three types of chain, and for the closely similar reactions of the two proteins with oxygen and other ligands, even though there are substantial differences between their overall amino-acid compositions. Unfortunately, the amino-acid sequence of horse hæmoglobin has not yet been determined, but most of that of human hæmoglobin has recently been established by Braunitzer *et al.*[2,4],

Table 1

	Hæmoglobin		Myoglobin	
	α-	β-		
1		Val		
2	Val	His		
3	*Leu*	*Leu*		
4	Ser	Thr	Val	*A*1
5	**Pro**	**Pro**	Ala	2
6	Ala	Glu (*e*) (*f*)	Gly	3
7	Asp	*Glu* (*g*)	*Glu*	4
8	*Lys*	*Lys*	Try	5
9	Thr	*Ser* (*l*)	*Ser*	6
10	Asp	Ala	Glu	7
11	*Val*	*Val*	Ileu	8
12	Lys	Thr (*m*)	Leu	9
13	*Ala*	*Ala*	Lys	10
14	Ala	Leu	?	11
15	*Try*	*Try*	*Try*	12
16	*Gly*	*Gly*	?	13
17	*Lys* (*a*)	*Lys*	Leu	14
18	*Val*	*Val*	Leu	15
19	Gly	Asp	Glu	16
20	Ala	│	?	*AB*1
21	His	│	Leu	*B*1
22	Ala	*Val*	*Val*	2
23	Gly	Asp	Ala	3
24	*Glu*	*Glu* (*n*)	Gly	4
25	Tyr	Val	His	5
26	*Gly*	*Gly*	*Gly*	6
27	Glu	Gly	Lys	7
28	Ala	Glu (*h*)	Leu	8
29	*Ala*	*Ala*	Thr	9
30	*Leu*	*Leu*	*Leu*	10
31	Glu	Gly	Ileu	11
32	*Arg*	*Arg*	Ser	12
33	Met	*Leu*	*Leu*	13
34	*Phe*	Leu	*Phe*	14
35	Leu	Val	Lys	15
36	*Ser*	Val	*Ser*	16
37	Phe	Tyr	His	*C*1
38	**Pro**	**Pro**	**Pro**	2
39	Thr	Glu	Glu	3
40	*Thr*	Thr	*Thr*	4
41	Lys	Try	Leu	5
42	Thr	Arg	Glu	6
43	Tyr	Phe	Lys	7
44	Phe	*Phe*	*Phe*	*CD*1
45	**Pro**	Glu	Asp	2
46	His	Ser	Arg	3
47	Phe	*Phe*	*Phe*	4
48	Gly	Gly	Lys	5
49	His	Asp	His	6
50	Leu	*Leu*	*Leu*	7
51	Ser	Ser	Lys	8
52	Ser	*Thr* (*o*)	*Thr*	*D*1
53	│	**Pro**	Glu	2
54	│	Asp	Ala	3
55	│	Ala	Glu	4
56	│	Met	Met	5
57	│	Val	Lys	6
58	│	Gly	Ala	7
59	Asp	Asp	Ser	*E*1
60	Ala	**Pro**	Glu	2
61	Glu	Lys	Asp	3
62	*Val*	*Val*	Leu	4
63	*Lys*	*Lys*	*Lys*	5
64	Gly (*b*)	Ala	Val	6
65	*His* (*c*)	*His* (*i*)	(*His*, Glu)	7
66	*Gly*	*Gly*	*Gly*	8
67	*Lys*	*Lys*	Ileu	9
68	*Lys*	*Lys*	Glu	10
69	*Val*	*Val* (*j*)	*Val*	11
70	Ala	Leu	Asp	12
71	Asp	Gly	(Glu, His)	13
72	Ala	*Ala*	*Ala*	14
73	*Leu*	Phe	*Leu*	15
74	Thr	Ser	Gly	16
75	Asp (*d*)	Asp	Ala	17
76	Ala	Gly	Ileu	18
77	Leu	Leu	Asp	19
78	Ala	Ala	Arg	20
79	His	His	│	
80	Leu	Leu	│	
81	Asp	Asp	│	
82	Asp	Asp	│	
83	│	Leu	Lys	*EF*1
84	│	*Lys*	*Lys*	2
85	│	*Gly*	*Gly*	3
86	│	Thr	Leu	4
87	Met	Phe	His	5
88	Asp	Glu	Glu	6
89	Leu	Leu	Leu	7
90	Asp	Asp	Glu	8
91	Ser	Glu	Glu	*F*1
92	Ala	Ala	Ala	2
93	**Pro**	**Pro**	**Pro**	3
94	Val	Thr	Thr	4
95	Ala	Ala	Ala	5
96	Val	Val	His	6
97	Ser	Ser	Ser	7
98	*His*	His	*His*	8
99	*Ala*	CysH	*Ala*	9
100	His	His	│	
101	*Lys*	Lys	*Lys*	*FG*1
102	*Leu*	Leu	*Leu*	2
103	Arg	Leu	Phe	3
104	Val	Val	Lys	4
105	Asp	Asp	Ileu	5
106	**Pro**	**Pro**	**Pro**	*G*1
107	Val	Leu	Ileu	2
108	Asp	Asp	Lys	3
109	*Phe*	*Phe*	Tyr	4
110	Lys	Arg	?	5
111	*Leu*	*Leu*	Glu	6
112	Leu	Asp	His	7
113	Leu	Leu	Leu	8
114	Ser	Gly	Ser	9
115	Leu	Leu	?	10
116	Ala	Val	Ala	11
117	Val	Val	Val	12
118	Leu	Leu	Ileu	13
119	His	His	His	14
120	Val	Val	Val	15
121	Ala	Phe	Arg	16
122	Ala	Ala	Ala	17
123	Thr	Gly	Thr	18
124	CysH	CysH	Lys	19
125	His	His	His	*GH*1
126	│	│	Asp	2
127	His	Lys	Asp	3
128	Glu	*Glu* (*k*)	*Glu*	4
129	Phe	*Phe*	*Phe*	5
130	Thr	Thr	Gly	6
131	**Pro**	**Pro**	Ala	*H*1
132	**Pro**	**Pro**	**Pro**	2
133	Ala	Val	Ala	3
134	Leu	Glu	Asp	4
135	Ala	Ala	Gly	5
136	Ser	*Ala*	*Ala*	6
137	Leu	Tyr	Met	7
138	Asp	Glu	Gly	8
139	*Lys*	*Lys*	*Lys*	9
140	Phe	Val	Ala	10
141	Val	Val	Leu	11
142	Val	Val	Glu	12
143	Ala	Ala	Leu	13
144	Thr	Ala	Phe	14
145	Ser	Gly	Arg	15
146	Ser	Ala	Lys	16
147	Leu	Asp	Asp	17
148	Leu	Leu	Ileu	18
149	Thr	*Ala*	*Ala*	19
150	Ser	His	Ala	20
151	*Lys*	*Lys*	*Lys*	21
152	*Tyr*	*Tyr*	*Tyr*	22
153	Arg	His	Lys	23
154			Glu	24
155			Leu	*HC*1
156			Gly	2
157			Tyr	3
158			Gly	4
159			Glu	5

Notes. The amino-acid sequence given for myoglobin is that which is considered to be the most probable on the basis of X-ray and chemical evidence.

Non-ordered regions of the hæmoglobin chains are enclosed in boxes.

Corresponding residues in the ordered regions of the chains are italicized; but all prolines are printed in heavy type.

Substitutions: in the α-chain, hæmoglobin *I*: Asp at (*a*), ref. 6; Norfolk: Asp at (*b*), ref. 7; *M* (Boston): Tyr at (*c*) (Gerald, P. S., Efron M. L., Jones, jun., S. H., and Pisciotta, A. V., personal communication); *G* (Philadelphia): Lys at (*d*), ref. 8. In the β-chain, hæmoglobin *S*: Val at (*e*), ref. 6; *C*: Lys at (*f*), ref. 6; *G* (San José): Gly at (*g*), ref. 6; *E*: Lys at (*h*), ref. 6; *M* (Emory): Tyr at (*i*) (Gerald, P. S., Efron, M. L., Jones, jun., S. H., and Pisciotta, A. V., personal communication); *M* (Milwaukee-1): Glu at (*j*) (Gerald, P. S., Efron, M. L., Jones, jun., S. H., and Pisciotta, A. V., personal communication); *D* (Punjab) (= *D*): Glu.N at (*k*) (Baglioni, C., personal communication). In the δ-chain: Thr at (*l*), ref. 9; Asp at (*m*), ref. 9; Ala at (*n*), ref. 9; Ser at (*o*), ref. 9.

by Hill and Konigsberg[5], and by Schroeder and Jones, and it is reasonable to expect correspondences here, too, in view of the accumulating crystallographic evidence that the tertiary structures of hæmoglobins and of myoglobins of different species are very similar.

In Table 1 the human hæmoglobin chains have been placed alongside that of myoglobin (see preceding article by Kendrew *et al.*) in what seems to us the most plausible manner. The chains differ in length (myoglobin 151–153 residues, hæmoglobin α-chain 141 and β-chain 150 residues), and in comparing them we have tentatively assumed that interpolations or deletions occur only in or near inter-helical regions, since even a single change of this kind in the middle of a helix would completely alter the environments of all the side-chains on at least one side of the change. There are substantial regions in both hæmoglobin chains where the sequence of residues has yet to be determined; in each non-ordered region, we have arranged residues so that, where possible, amino-acids coincide with identical residues in the other hæmoglobin chain; where that cannot be done, or where corresponding regions in both hæmoglobin chains are non-ordered, the hæmoglobin residues have been arranged so as to correspond as nearly as possible to residues in the myoglobin chain. The fourth column of the table indicates those regions of the myoglobin chain which are α-helical (for terminology see Table 1 of the preceding article); the first column contains a numbering system which will be used for reference in the present article.

The tentative scheme in Table 1 leads to the following conclusions:

(1) All the prolines in the α- and β-chains can be placed so as to lie at the open end of a helix or in a non-helical region; wherever proline occurs in myoglobin, a proline can be correspondingly placed in both α- and β-chains. Sharp corners (that is, with few or without non-helical residues) do not, however, require the presence of proline; corner *AB* is without proline in any of the three chains, and *DE* has no proline in myoglobin, although it apparently has the same shape in the β-chain[1], which has proline at this position.

(2) Side-chains, the identities of which in myoglobin appear to be determined by specific interactions or steric features, often have counterparts in the hæmoglobin chains. Thus, at residues 26 and 66, helices *B* and *E* approach one another so closely that no side-chain larger than glycine would be possible; glycine is found in each position in all three chains. Residue 65 in myoglobin is probably histidine, and if so is within hydrogen-bonding distance of a water molecule attached to the sixth co-ordination position of the iron atom; histidine appears at the same position in both hæmoglobin chains. (The hæm-linked residue in myoglobin is also histidine, residue 98. Unfortunately, the corresponding region of one of the two hæmoglobin chains is still non-ordered; it is, however, possible to place histidines in position 98 in both α- and β-chains.) In myoglobin, the tyrosine at residue 152 forms a hydrogen bond with the main-chain carbonyl group of residue 105, presumably acting as a stabilizing bridge between the ends of the long helices *G* and *H*; tyrosines occur in the corresponding places in the α- and β-chains.

(3) The two hæmoglobin chains, unlike that of myoglobin, each has a small non-helical tail (2 residues in the α-chain, 3 in β-) at the amino-end, helix *A* being terminated at the proline in position 5. On the other hand, the non-helical tail at the carboxyl end of the myoglobin chain is absent from both hæmoglobin chains. Furthermore, in the α-chain of hæmoglobin (but not in the β-) six residues are missing between residues 42 and 59, that is, in the region near the short helix *D*. Inspection of Perutz's 5·5-Å. model of horse hæmoglobin confirms that in the α-chain the loop between 42 and 59 is shorter than in myoglobin or in the β-chain; this model also confirms that a 'tail' is present at the amino-end, and absent at the carboxyl-end, of each chain (Perutz, personal communication).

(4) Several of the substitutions which have been located in the δ-chain of hæmoglobin A_2 and in the abnormal hæmoglobins are indicated in Table 1. In nearly all cases these residues stick outwards from the molecule, so substitutions would not be expected to affect the tertiary structure. The only exceptions are the change from Thr to Ser at residue 52 in the δ-chain and the very interesting hæmoglobin *M* substitutions. The former residue is important in terminating helix *D* (see preceding communication), and is Ser in α and δ, and Thr in β and myoglobin. The characteristic abnormality of hæmoglobin *M* is an unusual difficulty in reducing the ferric to the ferrous form; in two variants we find that His has been converted to Tyr, in one case on the α-chain and in the other on the β-chain, at residue 65, which is located close to the iron atom opposite the hæm-linked histidine (see (2) above). In the third variant, Val at residue 69 becomes Glu; this residue is also very close to the iron atom.

If non-ordered residues in the hæmoglobin chains are disregarded, there are 50 possibilities for triple correspondences (identical residues in corresponding positions in all three chains)—only 10 (20 per cent) are observed. There are 54 possibilities of double correspondences between α- and β-, of which 26 (48 per cent) are observed; on the other hand, of 70 possible double correspondences between α- and myoglobin, only 20 (28 per cent) are found, and of 91 between β- and myoglobin only 25 (28 per cent). The main impression is that in spite of presumed close resemblances in tertiary structure, the correspondences are remarkably few. It must be concluded from this and the preceding article by Kendrew *et al.* that the crucial interactions which determine the tertiary structure of a protein are very complicated and are not confined to the corners between helices; detailed investigations, perhaps including comparisons between related proteins, will be necessary before general principles become apparent.

We thank Dr. M. F. Perutz for valuable discussions; also Drs. V. M. Ingram, Park S. Gerald and W. Konigsberg for a discussion of the (unpublished) proceedings of the Hæmoglobin Structure Workshop at Endicott House on December 14–16, 1960.

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THE ENGINEER IN COMMONWEALTH DEVELOPMENT

A LITTLE reflexion might suggest that it is perhaps a strange way to honour the achievements of distinguished men by inviting them to accept a presidency which involves a presidential address before a distinguished, but highly critical, audience or to give a named lecture which, because of the special circumstances, likewise attracts wide publicity. Many a famous scientist has emerged from such an ordeal with an impaired rather than an enhanced reputation; the specialist venturing outside the sphere in which he is an acknowledged master is too often apt to be trite or banal. On the other hand, such an occasion affords a great opportunity to present to an influential audience a considered synthesis which may well bear important fruit.

H.R.H. the Duke of Edinburgh is one who rises magnificently to such occasions. No one who heard his presidential address to the British Association at Edinburgh in 1951 could doubt his ability to take the broad, balanced view, based with meticulous care on carefully collected and assimilated facts, and to present his material in such a way as to shame the professional lecturer. This year he was invited jointly by the three great engineering institutions—the Civil, Mechanical and Electrical—to give the seventh Graham Clark Lecture on April 13. The first plan was a survey of "Resources, Human and Material, of the Commonwealth". The basic material was assembled and has been published as a series of statistical tables separately printed as appendixes to the lecture. A wide range of standard sources—in each case the best available—has been used and combined with the results of a number of specific inquiries (especially through the Commonwealth Economic Committee). Throughout these tables the emphasis is on a comparison between the Commonwealth (with countries separately named but including at this date the Union of South Africa) and the world as a whole. Population, labour force, land use, value of agricultural produce, energy production and consumption, primary production, mineral output, mineral reserves, transport and education are included in turn. The whole affords a simple yet effective view of how the Commonwealth stands in relation to the world as a whole.

But, as the Duke so rightly says, "resources are not very interesting until you start doing things to them". Since engineers play a dominant part in this, "I have tried to outline the engineer's part in the various fields of development and some of the opportunities and problems" of the profession. "The engineer is in fact the means by which the people are able to enjoy the fruits of science, whether in building new projects, or in maintaining and keeping up to date what is already in existence."

It is rumoured that the presidential address to the British Association was composed—and that is the right word—on the backs of naval signal forms during moments of hard-earned leisure in the chart room or on the bridge of a ship of the Royal Navy on active service. Incredible as it may seem that there were in fact moments of leisure during the recent royal tour to India and Pakistan, by his own confession the Graham Clark Lecture was similarly composed. One result is a particularly felicitous blend of library research and field observations. It may truthfully be said that no living person is better equipped to produce such a survey. No one has travelled the Commonwealth, from Antarctic to tropics, more widely yet always on duty, his hosts ever anxious to show everything worth while, their guest, always alert to note and record, not so much the detail, as the overall picture.

How does the Commonwealth stand in relation to the rest of the world? With 23 per cent of the land surface and 24 per cent of the population, the existence of 44 per cent of the land unused is a challenge since in food production the figures are not impressive. Only rice (24 per cent), barley (20 per cent) and mutton (23 per cent) are produced in proportion to land and population. It is freely admitted that the picture of the Commonwealth as a whole is distorted by the extreme contrasts between its constituent parts such as Britain and India, and the fact that India–Pakistan have 68 per cent of the total people. Nevertheless, the conclusion seems inevitable that the Commonwealth is at present not producing according to potential. 20 per cent of the world's rain falls on Commonwealth lands; Kariba and Volta are projects now in hand but the Hamilton Falls are unused and, taking energy as a whole—coal, oil, water-power—the Commonwealth's share in output is less than 12 per cent.

His Royal Highness comments, somewhat ruefully, that he has to bear criticism rather more frequently than most, and suggests that a little mild and well-intentioned criticism should be treated as a sign-post rather than as a slander. His comments, though forthright, are scarcely criticisms and are so obviously sincere and well-founded as to command respect. There is the old argument involving 'pure' and 'applied' science—by implication the latter also 'impure'—and it is still true that academic honours, for example, the fellowship of the Royal Society, are more hardly won by the applied scientist. Yet who deserves the greater credit: he who conceives an idea in a laboratory or he who with infinite pains adapts and refines it to the benefit of countless numbers of mankind? "We are apt to forget that much scientific research is only possible with the aid of very advanced engineering; computers, electron microscopes . . . are no longer bits of apparatus that can be knocked up in the lab." It is refreshing, too, to be reminded that our united aim should be to benefit mankind. "Unless large-scale projects and modernization plans are based upon a practical humanity they will contribute very little to man's progress." Or again, "it is becoming increasingly popular to look upon industry merely as a convenient way of giving employment . . . it has to satisfy the needs and desires of the individual". It is clear the Duke would not happily agree with those who go so far as to declare that the possession of resources is of little importance compared with the possession of capital with which to develop them. Such a view is economic colonialism at its worst and, by not referring to either political or economic aspects of development, he makes the obvious assumption that mankind should be the master and not the slave of its own social and economic systems.

Coming from a world traveller, we have timely reminders of the importance of communications and of scale so difficult to judge in small countries such as Britain—five times as far across mainland Canada and twice as far from Bombay to Calcutta as from John o'Groats to Land's End.

Seeing the Duke's interest in youth, it is not surprising that he is far from happy regarding the adequacy of higher education in technical fields. The appeal for forward thinking is well timed and, like every other aspects of life brought into this stimulating address, merits the closest attention.

L. DUDLEY STAMP

THE U.S. ACADEMY OF SCIENCES

OFFICERS, MEDAL AWARDS AND NEW MEMBERS

DR. J. A. STRATTON, president of Massachusetts Institute of Technology, has been elected to a four-year term as vice-president of the U.S. National Academy of Sciences. Dr. G. B. Kistiakowsky, Abbott and James Lawrence professor of chemistry, Harvard University, and Dr. Kenneth B. Raper, professor of bacteriology and botany, University of Wisconsin, have been appointed members of Council for a term of three years.

The following medal awards have been made: the *Henry Draper Medal*, for original investigation in astronomical physics, to Dr. Martin Schwarzschild, Princeton University Observatory; the *Daniel Giraud Elliot Medal*, for meritorious work in zoology or palæontology published each year, to Philip J. Darlington, jun., Museum of Comparative Zoology, Harvard University; the *Kimber Genetics Medal*, for achievement in the science of genetics, to Prof. J. B. S. Haldane, (recently) Indian Statistical Institute, Calcutta; the *Jessie Stevenson Kovalenko Medal*, for contributions to medical science, to Dr. K. F. Meyer, George Williams Hooper Foundation, University of California; the *Mary Clark Thompson Medal*, for services to geology and palæontology, to Dr. Norman D. Newell, American Museum of Natural History; the *James Craig Watson Medal*, for noteworthy discoveries or research in astronomy, to Prof. Otto Heckmann, Hamburg-Bergedorf Observatory.

The following new members have been elected: Prof. D. I. Arnon, professor of plant physiology, University of California; Dr. W. O. Baker, vice-president in charge of research, Bell Telephone Laboratories; Prof. S. Benzer, professor of biophysics, Purdue University; Dr. H. A. Borthwick, principal plant physiologist, Agricultural Research Service, U.S. Department of Agriculture; Prof. R. H. Burris, professor of biochemistry, University of Wisconsin; Prof. Shiing-Shen Chern, professor of mathematics, University of California at Berkeley; Dr. P. E. Cloud, jun., chief, Paleontology and Stratigraphy Branch, U.S. Geological Survey; Prof. J. H. Comroe, jun., director of the Cardiovascular Research Institute and professor of physiology, University of California Medical Center; Prof. D. J. Cram, professor of chemistry, University of California; Prof. J. F. Crow, professor of zoology and genetics, University of Wisconsin; Dr. L. S. Darken, associate director, research laboratory, United States Steel Corporation; Prof. C. Djerassi, professor of chemistry, Stanford University; Prof. W. Von Eggers Doering, professor of chemistry, Yale University; Prof. R. Dulbecco, professor of biology, California Institute of Technology; Prof. A. I. Hallowell, professor of anthropology, University of Pennsylvania; Prof. B. L. Horecker, professor of microbiology, New York University College of Medicine; Prof. R. D. Hotchkiss, member, and professor of cellular physiology, Rockefeller Institute; Dr. Libbie H. Hyman, research associate, American Museum of Natural History; Prof. M. G. Inghram, professor of physics, University of Chicago; Prof. W. N. Lipscomb, jun., professor of chemistry, Harvard University; Dr. H. F. Mark, director, Polymer Research Institute, Polytechnic Institute of Brooklyn; Prof. H. Neurath, professor of biochemistry, University of Washington; Prof. G. E. Palade, member, and professor of cytology, Rockefeller Institute; Prof. R. V. Pound, professor of physics, Harvard University; Prof. L. A. Riggs, professor of physiological psychology, Brown University; Dr. R. B. Roberts, chairman, Biophysics Section, Department of Terrestrial Magnetism, Carnegie Institution of Washington; Prof. Per F. Scholander, professor of physiology, Scripps Institution of Oceanography; Dr. C. D. Shane, astronomer, Lick Observatory; Prof. D. C. Spencer, professor of mathematics, Princeton University; Mr. H. M. Stommel, research associate in physical oceanography, Woods Hole Oceanographic Institution; Prof. Leo Szilard, professor of biophysics, University of Chicago; Prof. J. W. Tukey, professor of mathematics, Princeton University; Prof. F. T. Wall, research professor of physical chemistry, University of Illinois; Dr. A. M. Weinberg, director, Oak Ridge National Laboratory; Prof. J. H. Williams, professor of physics, University of Minnesota.

OBITUARIES

Sir William Savage

THE death of Sir William Savage in his eighty-ninth year on April 6, 1961, means the passing of the last survivor of that distinguished company of medical officers of health who contributed so largely to the progress of public health in the late nineteenth and early part of the present century.

William George Savage, the son of John H. Savage, was born in Jamaica in 1872. He was educated privately, at University College, London, and University College Hospital. He took the B.Sc.(Lond.)

in 1894, the M.B.Lond. with honours in medicine and the gold medal in forensic medicine in 1896, the diploma in public health in 1897 and the M.D.(Lond.) in 1898. After a resident post at the Royal Sussex Hospital, he became assistant professor of pathology in University College, London. From 1900–03 he was lecturer in pathology and public health at University College, Cardiff, and during 1903–09 medical officer of health and public analyst, Borough of Colchester. In 1909 he was appointed the first county medical officer of Somerset. Here he did the work that was to make him famous.

Savage was remarkable in that he continued to pursue bacteriological researches which gave him an international reputation, and at the same time administered the public health of his county with conspicuous success.

The organisms present in food poisoning early engaged Savage's attention, and he did research work for the Medical Research Council, partly in collaboration with Bruce White, into the serology of the *Salmonella* groups and their relation to food poisoning (*M.R.C. Reports*, Nos. 91 and 92, 1925). He also showed that domestic animals and vermin were the main reservoirs of these organisms. He made further fundamental studies into food poisoning. In 1940, in a discussion at the Royal Society of Medicine, he gave an account of some original work on *Salmonella dublin*, pointing out that it was responsible for human infection in a number of cases; and as late as 1956 he published a paper with evidence on problems of *Salmonella* food poisoning, and directed attention to the marked increase of food poisoning in Great Britain since the end of the Second World War (*Brit. Med. J.*, ii, 317; 1956).

Savage strongly advocated the pasteurization of milk; he wrote a report on milk and the public health in 1912, and published the following books: *The Bacteriological Examination of Food and Water* (1906), *Rural Housing* (1915), *Food and the Public Health* (1919) and *Food Poisoning and Food Infections* (1920). He was the author of a number of special reports to the Local Government Board, of papers on the prevention of human tuberculosis of bovine origin, on canned foods in relation to health, and on many other scientific subjects, the majority of which were based on his special investigations. He was Milroy Lecturer and Mitchell Lecturer at the Royal College of Physicians, president of the Society of Medical Officers of Health in 1935, examiner in public health for the Universities of London, Wales and Bristol, and received a knighthood for his distinguished services in 1938; in the same year the honorary degree of M.D.(Bristol) was conferred on him.

In 1937, Savage retired from the post of county medical officer but returned to assist in public health work in Somerset during the Second World War. Thereafter he did important work as chairman of the Ministry of Food's Catering Trade Working Party, while his advice and wisdom continued to be sought by other Government departments and public health bodies.

Savage was a strong man both physically and mentally. His interests were many. He played golf and lawn tennis up to an advanced age, was a daring swimmer and a keen gardener. Interested also in history and archæology, in retirement at Winchester, he published an important book, entitled *The Making of Our Towns*, in 1952 based on extensive research, and had been president of the Somerset Archæological Society in 1945. He was a staunch friend, kind and generous to many. Sir William married Mary A. M. Wilson in 1900, and they had a son and daughter. His son, Dr. S. W. Savage, is county medical officer of Warwickshire.

ARTHUR S. MACNALTY

Prof. Gideon Mer, O.B.E.

PROF. GIDEON GERONIMO MER, professor of epidemiology and director of the Malaria Research Station of the Hebrew University of Jerusalem, died on March 23, at the age of sixty-six, in Haifa. To malariologists all over the world he will be remembered for his work on the transmission of quartan malaria, for his method of determination of the age of mosquitoes—a technique now universally adopted in epidemiological studies of insect-borne diseases for a series of lucid papers on the bionomics of *A. elutus*, and for his studies of insecticides in recent years.

Prof. Mer was born in 1894 in Poniewez, Lithuania (at that time part of Czarist Russia). Both his parents were physicians. After graduating from the Real Gymnasium he went, in 1912, to study medicine in Heidelberg. He remained there only two years. At the call of the idealistic youth movement he interrupted his studies and went to Palestine.

At the outbreak of the First World War he was exiled by the Turkish authorities to Egypt. He joined the British Forces, fought in Gallipoli and elsewhere, and later returned to Palestine with the armies of Allenby. After demobilization he directed the first reclamation works in the malaria-infested valleys of Ezdraelon and Jordan.

In 1924, after a brief return to Lithuania, and following a happy marriage, Mer went back to his medical studies. He chose Italy, for he wanted to gain first-hand experience from the famous Italian school of malariologists of Grassi, Marchiafava and Celli. He graduated with honours in 1928, and after spending a year in postgraduate studies at the schools of tropical medicine in Amsterdam and Paris, he returned in 1929 to Palestine to direct the Malaria Research Station of the Hebrew University at Rosh-Pinah-Galilee, where he established free polyclinics for the Arab population of the region and soon was known as 'Hakim el Kabir', beloved and honoured by all.

Mer's research work was done under most difficult and exacting conditions. The experimental transmission of quartan malaria he carried out on himself and on his wife (volunteer Z. M.). The first trials on suppressive treatment of malaria with 'Chinoplasmin' were done in times of strife at the risk of his own life. During these pre-war years he also found time to serve as medical officer to British troops stationed in Galilee, to many of whom his home in the hills became well known.

With the outbreak of the Second World War, Mer volunteered, together with three of his assistants, to H.M. British Forces and commanded a malaria field laboratory in Iran, Iraq and Burma. He was made O.B.E. for his distinguished services and leadership.

After the War, he returned to Israel to use his newly acquired knowledge of modern insecticides for the reclamation of malarious areas. With the establishment of the State of Israel, he served with distinction on many health missions, as head of the Department of Preventive Medicine of the Armed Forces during the war of independence, and for a brief period as director-general of the Medical Services.

MEIR YOELI

NEWS and VIEWS

Assyriology in London :

Prof. C. J. Gadd, C.B.E., F.B.A.

PROF. C. J. GADD, who until his retirement in 1960 held the chair of Ancient Semitic languages and civilizations at the School of Oriental and African Studies, University of London, is certainly the leading Assyriologist in Britain, and one who is very highly esteemed by his colleagues abroad. His work in the field of Sumerian and Babylonian history and literature, as well as in Mesopotamian archæology, has earned him a world-wide—and fully justified—reputation. Born at Bath in 1893, Gadd was educated at King Edward VI School, Bath, and at Brasenose College, Oxford, served in the First World War, and joined the British Museum in 1919 as an assistant keeper in the Department of Egyptian and Assyrian Antiquities—now split into two independent departments, Western Asiatic Antiquities and Egyptian Antiquities. In 1948 he succeeded Sidney Smith as keeper of the Department, a post he held with great distinction until the year 1955, in which he was appointed to his chair at the University, again as the successor of his former colleague, Sidney Smith.

Besides numerous articles in scholarly journals and five volumes in the well-known series, *Cuneiform Texts from Babylonian Tablets in the British Museum*, Prof. Gadd has published several important works, including *A Sumerian Reading Book* (1924), now unfortunately out of print, and *The Stones of Assyria* (1936), a recognized standard book on the history of the first discoveries of Assyrian Palaces. Prof. Gadd has served on the staff of British archæological expeditions in Iraq (Ur, 1923–24; Nimrud, 1952) and Syria (Alalakh-Atshana, 1946). He was chairman of the Assyriological Section of the Twenty-third International Congress of Orientalists when it met at Cambridge in 1954. He was elected a Fellow of the British Academy in 1940, and was made C.B.E. in 1955. Two years earlier, Oxford conferred upon him the honorary degree of doctor of letters, and his former College elected him to an honorary fellowship.

New Chair of Assyriology

A CHAIR of assyriology, as recently established in the University of London, has been long overdue in Britain. 'Assyriology' now includes, by convention, a group of studies concerning ancient western Asia, from the beginnings of civilization in those parts down to about the time of Alexander's conquests. Thus it comprises not only an assortment of languages, by no means all Semitic, but also a diversity of cultures over a span of three thousand years, their bond of union being, in general, the cuneiform script. At their outset these studies owed perhaps most of all to a few men of genius of British birth; yet a chair of assyriology by name has never hitherto existed. The new foundation replaces the former chair of ancient Semitic languages and civilizations.

Prof. D. J. Wiseman

MR. D. J. WISEMAN, the professor-designate, has already a notable career in the learned world. While assistant-keeper in the British Museum he has made his mark as an interpreter of cuneiform texts. From the later period of Babylon and Assyria he has published two sets of historical documents, *Chronicles of Chaldaean Kings* and *The Vassal-Treaties of Esarhaddon*, both with universal acceptance as definitive. From an earlier age and a provincial *milieu* he has made known *The Alalakh Tablets*, a patch of new ground which he opened with great success. Also to his credit are two widely circulated books upon Western Asiatic archæology. All who are interested in the peoples of the Ancient Orient will wish him, with confidence, a tenure of the chair as brilliant as it promises to be long.

Geophysical Research in the Meteorological Office

DR. FRANK STACEY has returned to the United Kingdom from the Research School of Physical Sciences in the Australian National University at Canberra to take up one of the two new Gassiot research fellowships in the Meteorological Office. He has written extensively on geomagnetism and will conduct his future researches from Edinburgh, where he will enjoy the advantages of close association with scientists of allied interests at the University and at Eskdalemuir Observatory near Dumfries. The latter has, for many years, been the centre of geomagnetic work in the Meteorological Office. Dr. H. M. Iyer has been appointed to the other fellowship. Dr. Iyer is at present working at the University of California and will take up his new appointment in the autumn, when he will work from Kew Observatory on the increasingly important subject of seismology.

C.S.I.R.O. Wildlife Survey Section :

Mr. H. J. Frith

MR. H. J. FRITH has been appointed officer-in-charge of the Wildlife Survey Section of the Commonwealth Scientific and Industrial Research Organization (Australia). He succeeds Mr. F. N. Ratcliffe, who resigned last year to become assistant chief of the Organization's Division of Entomology. Mr. Frith graduated in agricultural science from Sydney in 1941. During the rest of the War he served in the Middle East and New Guinea and won a commission. He joined the Organization after his demobilization in 1946 as an assistant research officer at the Irrigation Research Station, Griffith, New South Wales. He remained at Griffith for five years, engaged on horticultural research on citrus problems. He transferred to the Wildlife Survey Section in July 1951. He was originally seconded to the Section to take part in the rabbit surveys associated with the release of the first myxomatosis-infected animals. From 1952 onwards he was able to develop his special interests in birds. He has worked on a number of Australian species, including parrots, pigeons and finches, but his chief subjects have been wild ducks and mallee fowl.

First World Meteorological Day

THE wide scope of the application of meteorological knowledge to agriculture, aviation, shipping, flood protection, water resource questions of many kinds, industry and the activities of the general public is little realized. Still less are the potentialities of warnings issued by the meteorological services of the world appreciated. These warnings are specifically designed to advise aviators, farmers, fishermen, sailors and all those whose work is affected by the weather. The World Meteorological Organization has therefore established the anniversary of the implementation of the Convention of the World Meteorological Organization, March 23, 1950, as World Meteorological Day. The first of these days was celebrated on March 23, 1961. The object is to attract world public interest to the work of the meteorological services and to the assistance they can give. It is also designed to show the need for continuous interchange of meteorological information on a world-wide basis and for meteorological collaboration in other ways, thus demonstrating that the provision of meteorological services in each country is one of the tangible results of day-to-day co-operation between nations. The 109 members of the World Meteorological Organization have been invited to celebrate the World Meteorological Day annually in their country by such means as exhibitions, lectures, visits to national forecasting offices, television and radio coverage.

Experiments on Animals

A BILL, *S*3570, providing for the control of experiments on animals, has recently been introduced in the U.S. Senate. As an aid to its sponsors the secretary-general of the Universities Federation for Animal Welfare, Major C. W. Hume, has submitted a report on the present practice in Great Britain (*Opinions of British Scientists of Home Office Control of Experiments on Animals*. Pp. 8. London: Universities Federation for Animal Welfare, 1961). The report has been based on questions addressed to British scientists who use animals in their experimental work and, with one exception, show that men of science are well content with the present system. They are satisfied that the present system of control and experiment administered by the Home Office does not in any way frustrate experimental work of the highest quality; and they wish to retain Home Office control so that persons without scientific training may not be permitted to experiment on animals without supervision.

Welfare and Care of Laboratory Animals

THE March issue of the *Bulletin of the International Committee on Laboratory Animals* is considerably bigger than some of its predecessors, because it contains, in addition to its usual international news items, a list of corresponding members, notes on the visit of the secretary-general, Dr. W. Lane-Petter, to Norway, the Near East and the U.S.S.R., and a long bibliography of papers on various aspects of research, classified according to the experimental animals used, including invertebrates. The Committee is organizing a symposium on "The Problems of Laboratory Animal Disease", which will be held in Czechoslovakia early in September. The Proceedings of the symposium on "Living Animal Material for Biological Research" have now become available. Full information about the many activities of the Laboratory Animals Centre is given in the *LAC News Letter*, published free in January and July and obtainable, as the other publications mentioned above also are, from the director, Dr. W. Lane-Petter, Laboratory Animals' Centre, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey.

History of Science

THIS new annual review of literature, research and teaching in the history of science is edited by A. C. Crombie (Oxford) and M. A. Hoskin (Cambridge), and published at Cambridge by W. Heffer and Sons, Ltd. There is a board of advisory editors consisting of G. Buchdahl, Herbert Butterfield, F. N. L. Poynter and W. P. D. Wightman. The review will contain critical essays surveying recent work on special topics, and others directing attention to problems for research. There will also be sections devoted to book reviews, surveys of articles of special interest published in other journals, and information about research in progress, teaching and other matters relating to the professional development of the subject. The first number will be published early in 1962.

Queen Victoria Museum and Art Gallery, Launceston, Tasmania

THE recent issues of the *Records* and the annual reports for 1958–59 and 1959–60 of the Queen Victoria Museum and Art Gallery are complementary to each other. They indicate that museums, in addition to a policy for the conservation of objects, are also centres of scholarly research and do their share in the elucidation of scientific, historical and æsthetic problems. The reports are a record of the vigorous activity of the staff in spite of the usual lack of good exhibition and storage space. In accordance with the general policy of widening the contacts of the Museum with the public, display space has been obtained in the central shopping area of the city. This provides accommodation for a series of fortnightly exhibits of special objects as well as the display of notices regarding the activities of the main Museum. The *Records* are concerned with the geology of western and northern Tasmania and deal with both the topography and stratigraphy of the area (New Series. No. 12 : Comments on the Cainozoid History of Western Tasmania. By B. Scott. Pp. 10. No. 13: Erosion Surfaces in Western Tasmania. By B. Scott. Pp. 11. Launceston: Queen Victoria Museum and Art Gallery, 1959 and 1960).

Work in the Antarctic during I.G.Y.

RUSSIAN explorers have been interested in the Antarctic since their first expedition led by F. G. Bellingshausen and M. P. Lazarev during 1819–21. During the International Geophysical Year, when the Antarctic region was studied by a combined effort of twelve nations, the Soviet expedition, centred on the settlement of Mirny, carried out considerable work in this part of the Antarctic continent. In a series of articles published in *Priroda* certain results of this work are given. Thus B. V. Dubovsky (*Priroda*, 7, 45 ; 1960) provides a note on two newly discovered valleys, one called 'The Valley of I.G.Y.' and the other 'Lazarev's trough'. V. D. Novikov (*Priroda*, 8, 43 ; 1960) provides an account of the construction of the scientific base Mirny, which was completed during February 1956, and later of eight additional bases. All these bases provided means of systematic and detailed geological, glaciological and meteorological studies, all of which are briefly described by Novikov. In two articles A. G. Dralkin (*Priroda*, 9, 48 ; 10, 27 ; 1960) describes the Soviet expedition to the South Pole in 1959 and their meeting of the American contingent of the Polar Amundsen-Scott station. The results of the study of the ice sheet and of the

Earth's crust in the Antarctic continent are presented by G. E. Lazarev and S. A. Ushakov (*Priroda*, 12, 17; 1960). These results show that in the eastern Antarctic the average thickness of the ice sheet is not less than 2,000 m. and its maximum thickness 4,000 m. The level of the rock surface below the ice sheet varies from 1,000 m. above sea-level to 800 m. below sea-level. The thickness of the Earth's crust (above 'Moho') is 5–10 km. for the oceanic type, and 25–70 km. for the continental type. The average density of the crust is 2·8. The result of the gravity work is also discussed and it is suggested that the ice load produces a sag in the crust of the order of 600–700 m.

Singing Sand

As the result of deepening the channel of the River Dnieper below the town of Kremenchug a small artificial island of sand was formed. This sand, as described by G. G. Velikii (*Priroda*, 6, 82; 1960), when trodden on emits a singing sound. Neither very dry nor very wet sand will 'sing'. As a rule the sand emits a high pitch in the morning and a lower pitch towards the evening. The 'singing sand' from the Dnieper may thus be compared with the 'musical sand' from the Isle of Eigg in Scotland and other localities.

Rabies in India

THE prevalence of rabies in India is indicated in the report of the Director of the Pasteur Institute of Southern India, Coonoor, for 1959 (Pp. i+116. Coonoor: Pasteur Institute, 1960). During the year, 34,239 courses of anti-rabies vaccine were issued. Of the 35,403 patients treated at the Institute and subsidiary treatment centres during the year, case cards were returned for 15,447 patients. Of these, 9,007 received a complete course of treatment. During the year 980 patients received a complete course of anti-rabies treatment at the Institute itself (968 Asiatics and 12 Europeans). An incomplete course of treatment was also given to 184 patients (Asiatics) of whom 52 were absolved from further treatment after the possibility of rabies in the biting animals had been excluded. One death was recorded among the 1,164 patients who received complete or incomplete courses of treatment giving a mortality-rate of 0·08 per cent. During the period 1907–59, the total number of patients treated at the Institute was 47,197, among whom 417 later died of rabies giving a mortality-rate of 0·88 per cent. Equally encouraging figures are shown by the subsidiary centres where, in 1959, the number of deaths among 8,027 patients who received a complete course of treatment had fallen to 12 (0·15 per cent). Anti-rabies treatment was also made available for the prophylactic treatment of animals. During the 37-year period from 1923 to 1959, 51,382 animals have received anti-rabies treatment, among which 473 deaths have been reported, giving a mortality-rate of 0·92 per cent. The report also describes the routine work and fundamental research into the nature of other diseases carried out at the Pasteur Institute.

Isolation of Malignolipin from Malignant Tumours

KOSAKI and his co-workers isolated a new phospholipid, malignolipin, from human malignant tumours on the basis of its affinity for porphyrin, and found that it was composed of choline, spermine, phosphoric acid and fatty acid; was specific to malignant tumours; and that it promoted intraperitoneal growth of Ehrlich's ascites cancer. A large amount of malignolipin is needed to establish the structure of this compound, and to reveal its biological and, especially, pathophysiological significance. The method previously described was not suitable for extracting malignolipin in large quantities and, recently, Kosaki and his colleagues have described how the substance can be produced in considerably increased quantities (*Mie Med. J.*, 10, No. 2; August 1960). The investigation makes clear that malignolipin is a phospholipid which has not previously been isolated. Two other articles in the same journal also deal with malignolipin. One indicates a method for detecting and measuring the substance in various tissues including blood and ascites and the other describes the column chromatographic isolation of malignolipin.

Trapping of Plasma by Red Cells

AN accurate method for dividing red-blood cell columns has been devised by Peggy Clark and B. J. Walsh of the New South Wales Red Cross Blood Transfusion Service, Sydney (*Austral. J. Exp. Biol. and Med. Sci.*, 38, Part 6; Dec. 1960). Blood is placed in polyvinyl chloride tubing which is sealed at one end with a glass bead. This tubing is inserted into a piece of glass tube before centrifugation, and later the contents can be frozen before the tubing is divided. The method was used to measure the amount of plasma trapped with the red blood cells. From these measurements the ratio of the true to the observed hæmatocrit value was found to be 0·94 at centrifugal forces greater than 1,430*g*. The ratio decreased with decreasing relative centrifugal force, but variations in the hæmatocrit value did not alter the correction factor. The amount of plasma trapped in the centrifuged and sedimented normal human blood decreased from top to bottom of the red-cell columns, but the amount in different sections of the columns became uniform as the time of centrifugation or of sedimentation increased. A blood sample with a high sedimentation-rate showed a different pattern on sedimentation.

Leverhulme Research Awards

THE following fellowships and research grants tenable for periods up to two years have been announced: *Fellowships*, Dr. A. M. Dunn, lecturer in veterinary parasitology, University of Glasgow, for work on the parasitic helminth fauna of wild ruminants in Britain; P. Haggett, University demonstrator in geography, University of Cambridge, for work on secular changes in the forested area of the Serra do Mar, Brazil, 1814–1962; Dr. R. P. A. Sneeden, lecturer in chemistry, University of Glasgow, for investigations on the structures of large-ring antibiotics; Dr. L. E. Sutton, demonstrator in physical chemistry, University of Oxford, for studies on the significance of molecular shapes and sizes. *Research grants*, N. S. Angus, chief metallurgist, the Production Engineering Research Association, Melton Mowbray, for a metallurgical and chemical examination of samples of early iron and steel; E. G. Bowen, professor of geography and anthropology, University of Wales, Aberystwyth, for studies on the historical geography of the early Christian period in Celtic Europe; J. Heath, of the Nature Conservancy, Grange-over-Sands, for taxonomic and biological studies of the Micropterygidae and Eriocraniidae;

R. F. Jessup, official of the Midland Bank, Ltd., for a survey of Roman barrows in Britain and northern Europe; M. Kaufman, senior lecturer in plastics technology, National College of Rubber Technology, for his studies on the history and development of the plastics industry; W. A. Sands, senior scientific officer, H.M. Overseas Civil Service (Research Branch), for a revision of the termite subfamily Nasutitermitinae from the Ethiopian zoogeographical region; Dr. E. C. Southward, independent research worker, Marine Biological Association, Plymouth, for studies on the biology of *Pogonophora*.

Fishery Research Training Grants

The Ministry of Agriculture, Fisheries and Food and the Department of Agriculture and Fisheries for Scotland are offering a number of one-year postgraduate training grants in fishery research tenable from October 1. These grants are intended to enable selected candidates to undergo a specified course of training to fit them for the investigation of problems in marine or freshwater science. Applications will be considered from candidates for permission to register for a higher degree in circumstances where this seems likely to be consistent with the requirements of the approved fishery research training programme. The number of training grants to be awarded during 1961 will probably not exceed three. Both men and women are eligible to apply. Candidates must be British subjects and graduates with honours in science of a university or possess equivalent qualifications. The value of the training grant will depend on the centre at which training is undertaken, and on whether the student is able to live at home, but will not normally be less than £270 nor exceed £420 per annum. For candidates with two years postgraduate experience, however, a grant of up to £470 per annum may be awarded. Further information can be obtained from the Secretary, Development Commission, 3 Dean's Yard, Westminster, London, S.W.1.

The Association of Applied Biologists

The following honorary officers were elected for 1961–62 at the recent annual general meeting: *President*, Dr. P. W. Brian; *Vice-President*, Dr. M. A. Watson; *President-Elect*, Prof. H. G. H. Kearns; *Treasurer*, Dr. F. Raw; *General Secretary*, Dr. L. Broadbent; *Programme Secretary*, M. J. Way; *Editor*, R. W. Marsh; *Assistant Editor*, Dr. I. Thomas. New members of Council elected were: Dr. F. T. Last, F. G. H. Lupton, Dr. D. Rudd Jones and D. W. Wright.

American Society for Engineering Education

The following have been elected officers of the American Society for Engineering Education: *President*, Dr. Robert W. Van Houten, president of the Newark College of Engineering and director of the Newark Technical School; *Vice-Presidents*, Dr. George A. Marston, dean of Engineering at the University of Massachusetts, and Dr. Curtis L. Wilson, Dean of the Missouri School of Mines and Metallurgy; *Treasurer*, Wendel W. Burton, of the Minnesota Mining and Manufacturing Co.

The International Society for Research on the Reticuloendothelial System

The following have been elected officers and council members of the International Society for Research on the Reticuloendothelial System: *President*, Dr. Albert S. Gordon (United States); *Vice-President (Europe)*, Dr. Bernard N. Halpern (France); *Vice-President (Western Hemisphere)*, Dr. Ignacio Gonzalez-Guzman (Mexico); *Vice-President (Asia)*, Dr. Kaneyoshi Akazaki (Japan); *Secretary-Treasurer*, Dr. John H. Heller (United States); *Council*, Dr. Shigeyasu Amano (Japan), Dr. Baruj Benacerraf (United States), Dr. Charles A. Doan (United States), Dr. Thomas F. Dougherty (United States), Dr. Tede Eston (Brazil), Dr. John F. Loutit (United Kingdom), Dr. Alberto M. Marmont (Italy), Dr. A. Ashley Miles (United Kingdom), Dr. John W. Rebuck (United States), Dr. Jorge Da Silva Horta (Portugal), Dr. Bo Thorell (Sweden), and Dr. Guy Voisin (France).

The International Union of Crystallography

The International Union of Crystallography has announced three forthcoming symposia: a symposium on "Electron and Neutron Diffraction", to be held at Kyoto, Japan, during September 25–30 (further information can be obtained from Prof. S. Miyake, Science Council of Japan, Ueno Park, Tokyo); a symposium in commemoration of the fiftieth anniversary of the discovery by Prof. Max von Laue of the diffraction of X-rays by crystals, to be held at Munich during July 25–28, 1962 (further information can be obtained from Prof. F. Bopp, Institut für Theoretische Physik der Universität, Geschw. Scholl-Platz 1, Munich 22); sixth general assembly and international congress of the Union, to be held in Rome during September 9–18, 1963 (further information can be obtained from Dr. W. H. Taylor, Crystallographic Laboratory, Cavendish Laboratory, Cambridge).

Rutherford Jubilee International Conference

A conference is to be sponsored by the International Union of Pure and Applied Physics, the Royal Society, the Institute of Physics and the Physical Society, and the University of Manchester, to commemorate the discoveries of Rutherford at Manchester, and in particular the fiftieth anniversary of the Rutherford scattering law. The conference will be held at the Physics Department, University of Manchester, during September 4–8. The programme has been divided into nine sessions: *September* 4 (1) high-energy investigations of nuclei (morning), (2) collective motion in nuclei (afternoon): *September* 5 (3) the nuclear ground-state (morning), (4) Rutherford at Manchester (afternoon); *September* 7 (5 and 6) direct interactions (morning and afternoon); *September* 8 (7) weak interactions (morning), (8) open session (afternoon), (9) conference summary. Further information can be obtained from Dr. L. J. B. Goldfarb, Rutherford Jubilee International Conference, Physics Department, The University, Manchester, England.

University News: Belfast

Dr. R. Common has been appointed to a lectureship in geography, and Mr. M. Cohen has been appointed to a lectureship in applied mathematics.

Birmingham

Dr. W. F. Vinen, university demonstrator in physics, University of Cambridge, has been appointed to a vacant established chair of physics within the Department of Physics from October 1, 1961. The title of professor of taxonomic botany has been conferred on Dr. J. G. Hawkes, at present senior lecturer in taxonomy in the Department of Botany, from May 1, 1961.

The following appointments have also been announced. *Senior lectureships*: Dr. G. V. Chester (mathematical physics); Dr. A. S. Jones (chemistry); Dr. L. W. Poel (botany); Dr. J. W. Railly (mechanical engineering); Mr. J. G. Henderson (electrical engineering); Sir Francis Knowles (anatomy); Mr. A. A. Walters (econometrics and social statistics); Dr. W. E. Cavenagh (social study). *Lectureships*: Dr. D. E. Cohen (pure mathematics); Mr. H. P. Rogosinski (pure mathematics); Dr. R. H. Jones (civil engineering); Dr. M. H. Jeynes (bacteriology); W. R. H. Batty (chemistry); Dr. J. P. Simons (chemistry); Prof. E. N. Corlett (engineering production); Dr. A. J. Miller (highway and traffic engineering); T. Brereton (mining and minerals engineering); J. R. Rawling (mining and minerals engineering). *Research fellowships*: Dr. J. Lehmann, to be Imperial Chemical Industries Research Fellow in the Department of Chemistry; P. Johnson, in the Department of Medical Biochemistry and Pharmacology; R. Joy, in the Department of Medical Biochemistry and Pharmacology; Dr. B. Sakita, in the Department of Mathematical Physics.

The University has received and accepted during the period December 22, 1960–April 12 gifts and grants totalling £271,113 for research purposes. Included in this figure are: a grant of £157,143 from the Ford Foundation to support a long-term programme of research in the Department of Anatomy into the reproductive physiology of monkeys; a grant of £42,300 over five years from the Department of Scientific and Industrial Research for research on organic fluorine compounds in the Department of Chemistry.

Edinburgh

The following appointments were recently announced: *Senior Lecturers*, Dr. E. A. Smith (public health and social medicine), Dr. Allan Campbell (zoology); *Lecturers*, G. O. Goudie (engineering), Dr. Tao-Ching Hsu (engineering), A. L. Florence (engineering).

Glasgow

Dr. C. H. Wilson, vice-chancellor of the University of Leicester, has been appointed principal of the University of Glasgow, in succession to Sir Hector Hetherington, who retires on September 30.

London

Dr. W. B. Whalley, reader in organic chemistry in the University of Liverpool, has been appointed to the University chair of chemistry tenable at the School of Pharmacy.

Dr. I. C. Percival has been appointed to the University readership in applied mathematics tenable at Queen Mary College, and Dr. S. J. Pirt, principal scientific officer at the Microbiological Research Establishment, Porton, has been appointed to the University readership in microbiology tenable at Queen Elizabeth College.

Dr. P. Armitage, a member of the Statistical Research Unit of the Medical Research Council at the London School of Hygiene and Tropical Medicine, has been appointed to the University chair of medical statistics tenable at that school. Dr. Dennis Lacy, lecturer at St. Bartholomew's Hospital Medical College, has been appointed to the readership in zoology and comparative anatomy tenable at that college.

Dr. J. A. W. Dalziel of the Imperial College of Science and Technology has been appointed reader in inorganic and analytical chemistry in the Chelsea College of Science and Technology. Dr. J. D. Donaldson of the University of Aberdeen has also been appointed as a lecturer in the Department of Chemistry of the College.

Manchester

Dr. F. J. Ursell, at present lecturer in mathematics at the University of Cambridge, has been appointed Beyer professor of applied mathematics in succession to Prof. M. J. Lighthill on a date to be arranged.

Announcements

Sir John Cockcroft, master-designate of Churchill College, Cambridge, has been appointed chancellor of the Australian National University, Canberra, in succession to Lord Bruce; this appointment will not affect Sir John's position at Cambridge.

Prof. J. R. Partington has been given the Dexter Award of the American Chemical Society for his work in the history of chemistry. Prof. Partington is emeritus professor of chemistry in the University of London, but for some years has been resident in Cambridge. The Dexter Award consists of a money prize and a plaque, and it is expected that the Award will be made at a meeting of the Chemical Society in the autumn by Dr. Edelstein, president of the Dexter Chemical Corporation of New York.

Dr. G. Malcolm Dyson of Loughborough, Leicester, has been given the Austin M. Patterson Award of the American Chemical Society for his work on the storage and retrieval of scientific data by computer techniques. The award is made every two years in honour of the late Dr. Austin M. Patterson in recognition of contributions in the field of chemical literature and especially in the documentation of chemistry. This is the first time that the award has been made to a non-American.

Dr. E. Eastwood, chief of research of Marconi's Wireless Telegraph Co., Ltd., Chelmsford, Essex, has been appointed director of research responsible to the managing director for the Company's research policy.

Prof. George Temple, professor of natural philosophy, University of Oxford, has been appointed chairman of the Aeronautical Research Council, in succession to the late Prof. W. J. Duncan.

The thirteenth British Electrical Power Convention of the Electrical Development Association is to be held at the Winter Garden, Eastbourne, during June 12–15. The theme of the Convention will be "Electricity in the Prosperity and Welfare of the Nation". Further information can be obtained from J. I. Bernard, Electrical Development Association, 2 Savoy Hill, London, W.C.2.

A summer school in health physics (radiation protection) arranged by Dr. Hugh D. Evans, lecturer in radiation hazards and health physics, will be held in the Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, during July 3–14. Further information can be obtained from Dr. H. D. Evans, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

THE SECOND U.K. *SCOUT* SATELLITE

THE British National Committee on Space Research, the U.K. Steering Group on Space Research and the U.S. National Aeronautics and Space Administration have agreed on the scientific instrument payload for the second U.K. *Scout* satellite to be launched by the U.S. National Aeronautics and Space Administration. The scientific instruments, as in the first British *Scout* satellite, will be designed and provided by British scientists.

The experiments are designed to measure radio emissions from the Galaxy at wave-lengths too long to penetrate the Earth's atmosphere; to measure the vertical distribution of ozone in the regions of the atmosphere in which it is being formed; and to measure the number and size of fine dust particles encountered by the satellite. In addition, the galactic radio equipment is designed to gain information on the electrical condition of the high atmosphere.

It is expected that the satellite will be launched in about two years time. The orbit will probably pass over the United Kingdom and the United States, and it should be possible to receive radio information both in these countries and at stations in the British Commonwealth.

As for the first British *Scout* satellite, this one will be launched by a four-stage rocket system using solid propellants. It is expected that the satellite orbit will range between 300 and 2,000 km. from the Earth. The instrumentation is being so arranged that the satellite will supply information on Galactic noise, atmospheric ozone and micrometeorite flux.

Galactic Noise

A project to measure the galactic noise in the frequency-range 0·75–3 Mc./s., and the exploration of the upper ionosphere, is to be carried out by Dr. F. Graham Smith, Mullard Radio Astronomy Observatory, Cavendish Laboratory, University of Cambridge.

Dr. Smith will have three objectives in mind: (*a*) to measure the intensity of the galactic background at as low a frequency as possible under known conditions of receiver sensitivity and under known conditions of ionospheric effects on propagation and aerial impedance; (*b*) to watch for temporal or spatial variations of galactic radiation; (*c*) to explore the electron density in the upper *F* region.

Atmospheric Ozone

Measurement of atmospheric ozone is under the direction of Dr. R. Frith and Dr. K. H. Stewart (Air Ministry, Meteorological Office, London). This will be carried out by two methods: (*a*) a spectrum scanning method; (*b*) a method in which a broad band of the spectrum is observed.

Dr. Frith's experiment will have as its main objective the measurement of the vertical distribution of ozone in the Earth's atmosphere as often and in as many places as possible. These measurements should add to our knowledge of the processes forming and destroying ozone, of the air motions which distribute it and of the effects of ozone on the thermal equilibrium of the upper atmosphere. Measurements will be made of the intensity of the radiation received from the Sun at selected wave-lengths in the ozone absorption region in the ultra-violet at times when the satellite is entering or leaving the Earth's shadow and the solar rays have to pass through the Earth's atmosphere to reach the satellite.

Micrometeorite Flux

The measurement of the micrometeorite flux is is being organized by Dr. R. C. Jennison (Nuffield Radio Astronomy Laboratories, University of Manchester, Jodrell Bank, Cheshire).

Dr. Jennison's experiment has as its objective the detection and measurement of micrometeorites encountered by the satellite. He will use a technique in which the holes formed in a thin metallic film by the impact of micrometeorites will be detected by an optical method. Sunlight passing through the holes will fall on a photosensitive strip of solar cells and give a signal from each hole in turn from which the number and size of the holes, and hence of the micrometeorites, can be deduced. The sensitivity is such that the holes formed by the particles, 1μ in diameter or greater, may be detected.

THE BRITISH AGRICULTURAL HISTORY SOCIETY

THE annual conference of the British Agricultural History Society was held during April 5–7 at Seale Hayne Agricultural College. On the first evening members heard papers from Mr. Peter Holmes, the County agricultural officer, and Dr. W. G. Hoskins, of Oxford. Mr. Holmes described the farming regions of Devon and did his best to disabuse his audience of the idea that Devonshire was entirely composed of the good red soils associated with the 'Glorious Devon' of the travel posters. He emphasized the importance of the hill-farming economy and explained also that there were large areas of indifferent shale soil in the north and north-east which were difficult to farm and not immensely productive.

Dr. Hoskins has recently been engaged in a study of the types of farmhouse on Dartmoor and made an appeal for more field work in the area for, as he said, "The history of the moor is as yet largely unwritten". He outlined briefly what is known of the earlier settlement of the moor and discussed its recolonization at the end of the Middle Ages. There are, he explained, still to be found many sixteenth- and seventeenth-century 'long houses' or buildings of the type that had both human dwelling and cattle stalls under one roof with a common doorway and central dividing passage.

The following day Dr. Hoskins led an excursion over the moor and showed the conference examples of this type of house. The party also visited a deserted village site below Hounds Tor which has recently been discovered by members of the Ordnance Survey. About twelve house sites, thought to be identified with seven or eight farms, are clearly visible from the rubble remains, but nothing is yet known of the date of settlement or of desertion; excavation on the site will start this summer.

On the second evening of the conference members heard a paper from Prof. Slicher van Bath, head of the Department of Rural History at Wageningen in the Netherlands. Prof. Slicher van Bath discussed the use of farm accounts as material for investigating farm management and agricultural output between the Middle Ages and the eighteenth century. He outlined the results of a study he had made of extant accounts in several countries, including those of the English early seventeenth-century farmer, Robert Loder of Harwell (Camden Third Series, **53**, 1936).

On the last morning of the conference there were papers from Mr. J. Z. Titow of Cambridge and Prof. Beresford of Leeds. Mr. Titow discussed his recent work on the types of manors to be found on the Winchester estates in the thirteenth century. It had, he said, been assumed that the descent of villein property was mainly from father to son, but he produced substantial evidence to support a plea for considering more seriously the position of widows. On many of the Winchester manors it was clear that holdings were often left to the widow and that a kind of 'widow cycle of inheritance' was thus set up. A young man in search of a holding would therefore seek a widow with one. As a result of this a widow might remarry and leave the holding on her death to her second husband who in his turn remarried, leaving it on his death to his widow who might then marry again; direct descent to the eldest son appeared to be relatively uncommon.

Prof. Beresford set out some provisional ideas on the development of the unusual settlement pattern in fourteenth-century Cornwall. He explained that a great deal more work was required on Cornish settlement patterns, and that potential workers would be encouraged to know that from the material in the Duchy of Cornwall archives it was possible to trace the occupation of Duchy holdings from the fourteenth century to the early nineteenth. Dr. Hoskins, in his concluding remarks as chairman for Prof. Beresford, said he hoped members, as a result of the conference, would be more conscious of the need for work in south-west England and the rich material that awaited them. He was glad, too, that the Society had met in the highland zone, for, in his opinion, the British highlands had been neglected by agrarian historians.

J. W. Y. HIGGS

DISEASE TRANSMISSION BY TICKS

THE papers presented at the recent sixth symposium of the Zoological Society of London on "Some Aspects of Disease Transmission by Ticks", held in London on March 8, revealed how some of the pieces of this complex jig-saw puzzle are gradually being filled in, but at the same time it was indicated that there are major deficiencies in our knowledge. Dr. J. MacLeod, by reference to a very interesting mechanical analogue of the forces at work, showed that the disease associations of ticks represent meshes in the food web of a biotic community which includes the pathogens, their tick vectors, the vertebrate hosts and the vegetational environment which supports and/or shelters them all. In this connexion it is worth noting that the physical relationships of tick distribution in Britain to the type of ground-cover are known for only one of the nineteen species of ticks; the biotic relationships, with two exceptions, are practically unknown. So far as British ticks are concerned, *Ixodes ricinus* has been the species most thoroughly investigated, principally because of its known disease relations. One of these diseases, Scottish louping ill, like other viruses of tick encephalitides, appears to have a casual rather than an intimate association with its hosts, which are principally sheep in Britain. Although the ease with which this disease can be transmitted by non-British ticks, for example, *Rhipicephalus appendiculatus*, suggests that any of the mammal-infesting British ticks would be capable of transmitting it to any British mammal; the infection has been demonstrated in *I. ricinus* from sheep in Nature, both by inoculation and infection.

Clinically, louping ill resembles poliomyelitis of man, and the virus multiplies primarily outside the central nervous system and during the fever, which precedes clinical signs, the pathogenic micro-organism can be detected in the blood. Dr. W. S. Gordon's experimental results indicate that most sheep on a louping ill farm eventually become infected. Many develop only a fever, recover and are afterwards immune, but a small proportion develop clinical signs of infection of the central nervous system. In these cases lesions of an encephalomyelitis can be demonstrated and virus recovered from the brain and the spinal cord. On a world scale, according to Dr. C. E. Gordon Smith, there are thirteen known tick-borne animal viruses transmitted by five genera of hard ticks and one of soft ticks. All these viruses, except one, cause disease in man or domestic animals which are usually incidental hosts of the viruses. Their long-term natural maintenance depends on the geographical and behavioural association between vector tick and ground-frequenting mammal, and bird species, in the blood streams of which the virus inoculated by tick-bite can circulate adequately to infect further ticks. Because tick populations are adequate only where suitable vegetation provides the appropriate microclimate, infections occur in restricted foci. Wild vertebrates do not usually show the disease, but can be infected and circulate virus only once because of antibody formation, and accordingly their population turnovers must supply enough new susceptibles annually. Movement of these viruses to new areas probably depends on the carriage of infected ticks by large mammals or migrant birds. A stable enzootic disease changes to epizootic or epidemic mainly because of population-changes in ticks or vertebrates, or introductions by man of domestic animals or introduction of virus to new areas.

A second febrile disease of sheep and cattle in Britain is tick-borne fever, which was discovered during the investigation of louping ill by Gordon *et al.* Hitherto tick-borne fever has been classified as a *Rickettsia*, but Dr. A. Foggie indicated that apart from the presence of an arthropod vector it has little in common with the true rickettsiae, such as those causing typhus and Rocky Mountain spotted fever. The forms seen in parasitized phagocytes have some resemblance to large viruses, and, under the phase-contrast microscope, the elementary bodies in morulæ have been seen in rapid Brownian movement, a phenomenon paralleled in colonies of psittacosis virus. While the organism had been provisionally named *Rickettsia phagocytophilia*, Dr. Foggie was of the opinion that it should now be included in the genus *Cytoecetes*, in so far as it morphologically resembles the type species *C. microti*, a tick-borne parasite of the polymorphs of voles in Massachusetts. Tick-borne fever is widespread, and an important point, made by Dr. MacLeod, was that in relation to 'inefficient' links in the biocœnose through self de-ticking, as in birds and some carnivores, transmission can occur by the second day after attachment of nymphs. Sheep are a reservoir of infection for ticks for at least several weeks after subsidence of the reaction. Cattle and goats are susceptible, and cross-infectivity and cross-immunity tests by Dr. Foggie suggest that there may be specific differences between the bovine and ovine strains of the organism; a recently isolated strain from red deer was of similar infectivity to the sheep strain. The harmful effects of louping ill are aggravated by the presence of the tick-borne fever microorganism, as shown by Dr. Gordon's experiments in exposing susceptible sheep to natural infection with louping ill and tick-borne fever.

In the wider field of rickettsial infections and ticks, Prof. D. S. Bertram outlined the characteristics of rickettsial organisms as deduced by various workers from studies, including some by electron microscopy, on species of *Rickettsia* pathogenic to man. The significance of ticks in human tick-borne rickettsiosis was considered as well as comments on tick-borne *Rickettsia*-like organisms of veterinary interest. Prof. Bertram then illustrated that differentiation within the controversial assemblage of pathogenic and symbiotic organisms may benefit from electron microscopy, and that this technique presents an opportunity for fruitful re-examination of the fate of the different rickettsiae in their tick hosts.

Although tick pyæmia is constantly associated with an infestation of *Ixodes ricinus*, the tick does not appear to be a vector of the causal *Staphylococcus*. Dr. Foggie's observations show that *Staphylococci* of the same bacteriophage types as those found in tick pyæmia cases were found to be common commensals on the skin of lambs in both tick-free and tick-infested areas. Lambs in the neutropænic phase of tick-borne fever were shown to be a hundred-fold more susceptible to the intravenous inoculation of *Staphylococci* than healthy lambs. The subcutaneous or intradermal inoculation of *Staphylococci*, either with or without an extract of tick salivary gland, or the feeding of ticks on heavily contaminated skin areas, failed to reproduce the disease in both normal and neutropænic lambs.

Piroplasmosis (redwater fever) in cattle in Britain is due to a piroplasm now believed to be distinct from *Babesia bovis*, and for which resuscitation of the earlier name of *B. divergens* is proposed. Dr. H. E. Shortt made the point that piroplasmosis is one of the major causes of morbidity and mortality in cattle, and as such has a serious effect in limiting the potential food supplies of man. So far as is known, ticks are the chief and very likely the only vector of this condition, and, although Africa is probably the most seriously affected continent, all parts of the world harbour both piroplasms and their tick-vectors. Our knowledge of the life-history of the piroplasms in the tick is still inadequate and Dr. Shortt, in describing the life-cycle of *Babesia canis* in *Rhipicephalus*, has made notable contributions to filling in many of the blanks. The possibility of the introduction into Britain of equine and canine piroplasmosis, transmitted by *Dermacentor reticulatus* on the continent, was made by Dr. MacLeod. The suggested occurrence of *B. caballi* in Britain cannot be ignored, and with the apparent establishment of *D. reticulatus* in south-west England, due probably to similar ecological requirements to those of *I. ricinus*, the wild and free-range ponies of Devon and Cornwall, and in the New Forest, may well be ecologically susceptible to devastation by one or both of these disease agents if they were introduced. Their susceptibility is more probable from the apparent catholicity of the vector-range of these piroplasms, as the latter have been transmitted experimentally by several tick species.

Man is normally insusceptible to infection, but as ticks are almost as ubiquitous as mosquitoes and in infested regions man is constantly exposed to their bites, the possibility of the tick acting as a reservoir of occult or inapparent parasites of man cannot be ignored. Prof. P. C. C. Garnham stated in this connexion that the removal of the spleen in man abolishes immunity and the occult parasites multiply and a fatal infection may ensue. The point was made that thousands, if not millions, of people living under rural conditions, and in contact with pastures, may harbour inapparent infections of *Babesia*, other piroplasms, or even certain tick-borne viruses. Whether such a condition is harmless, even in people possessing a spleen, is a subject being investigated further by Prof. Garnham.

Relapsing fever, a tropical disease associated with soft ticks, is transmitted by the bite and/or contamination of the bite wound by the coxal fluid which is produced by some species during feeding. Dr. G. A. Walton, in discussing human relapsing fever epidemiology in Eastern Africa, described the morphological, biological and distributional peculiarities of four species of Argasid ticks previously included under the name *Ornithodoros moubata* (Murray, 1877). The part played by each of them in the transmission of human relapsing fever was outlined with reference to the evolution of the East African domestic subspecies, and the decisive effect on local transmission of *Borrelia duttoni* by the feeding preferences of its races on one hand and the distribution and social behaviour of the native human races on the other. The existence and implications of immunity to the infection in man were illustrated in relation to minor and major human population movements and to other factors producing changes in the territorial incidence of the disease. Dr. M. G. R. Varma directed attention to the fact that, in an infected tick, spirochætes may be found in practically every organ; but the organs are not necessarily infected with the same frequency or to the same degree. Moreover, two species of spirochætes may behave differently towards the same tick organ. Both the frequency and the degree of infection are connected with the method of transmission of spirochætes. Thus it has been shown that the salivary glands of nymphs of *Ornithodoros moubata* are more heavily infected with *Borrelia duttoni* than are those of adults. There is also some evidence to show that infection of the salivary glands may die out in the course of time, so that old adults, apparently as a result of repeated feeds, rarely transmit the infection by bite.

Infection of the coxal glands in those species of ticks which do not produce any coxal fluid while feeding can have no importance in the transmission of spirochætes and such ticks transmit solely by the bite. Coxal fluid from such ticks has been shown to be non-infective although the glands themselves may be infected.

Continued experimental passage of the spirochætes through the vertebrate hosts may result in their failure to infect ticks successfully. However, by inoculating spirochætes directly into the hæmocœle, they are able to infiltrate the salivary glands and coxal glands and transmit the infection, suggesting that the gut may act as a partial barrier to the entry of spirochætes into the hæmocœle.

Some knowledge of the nature of the food imbibed by ticks and the methods of acquiring it may be of significance in assessing the timing of the intake and introduction of blood pathogens from and to the host. Examination of sections of larval and nymphal ticks feeding on rat skin by Miss Evelyn Sutton and Dr. D. R. Arthur shows evidence that a process involving the intake and digestion of non-blood tissue occurs. Moreover, field observations of adult *Ixodes ricinus* feeding on cattle revealed that ticks of the same degree of engorgement range in colour from light yellow to greyish, especially in the earlier stages. These colour differences are a reflexion of the colour of the gut contents visible through the integument, and, as afterwards confirmed by spectrophotometric analysis, the darker coloured gut contains a high proportion of blood and the lighter coloured gut a predominance of non-blood fluids. The ingestion of blood-inhabiting pathogens by the tick may accordingly be delayed for some days and in some cases may not occur at all. In certain larvæ, particularly those with short penetrant ability, it may well be that the diet is exclusively one of non-blood digested tissues, in which event the ingestion and passage of blood-inhabiting pathogens are negligible.

One of the significant features in ticks and mites is the ability of their pathogens to pass transovarially from one generation to the next. Prof. Bertram discussed how this may happen in rickettsiae, Dr. Varma showed how it occurred in ticks infected with *Borrelia duttoni* and Col. Shortt mentioned the passage of *B. canis* through the ovum of *Rhipicephalus*. Transovarian transmission in ticks and mites may be either obligatory or incidental. Prof. Garnham stated that obligate transovarial transmission occurs in *Karyolysus* and in *Rickettsia tsutsumagushi*, whereas *B. canis* for example, as Shortt described, may either progress from adult to egg to larva (that is, transovarial) or from stage to stage. Viruses sometimes pass through the egg, and spirochætes often are transmitted in this way, although the organism tends to become less virulent to the vertebrate host after such passage, and, if this is repeated for several generations, the progeny is eventually freed of infection. It has been suggested that ease of transovarial transmission in ticks may be related to the fact that only a single membrane separates the egg from the hæmocœle, whereas in insects greater insulation against the entry of viruses and pathogens is afforded by the presence of three membranes.

The proceedings of the symposium will be published in full by the Zoological Society of London.

D. R. ARTHUR

QUEEN ELIZABETH COLLEGE, LONDON

SIR JOHN ATKINS LABORATORIES

QUEEN ELIZABETH COLLEGE (University of London) was again honoured by a visit from H.M. Queen Elizabeth, the Queen Mother, Chancellor of the University of London, when, on May 9, she opened the new building on Campden Hill, the Sir John Atkins Laboratories. Her Majesty addressed members of the College and distinguished guests assembled outside the Laboratories, and then made a tour of the building, where she talked to a number of the staff and students who work there.

The building, designed by Messrs. Adams, Holden and Pearson, and erected by Messrs. Holland and Hannen and Cubitts (G.B.), Ltd., is six storeys of brown Buckinghamshire facing bricks, with panels of cream bricks between the lines of windows, and reconstructed Portland stone dressings. It houses the Departments of Biology, Microbiology, and Nutrition, which moved from the main College building last autumn, and released much-needed space there for the expansion of other departments. On part of the top floor there is a self-contained Isotope Unit for the use of any department needing to work with radioactive materials.

The Nutrition Department was founded in 1954, following the establishment by the University of London of the degree of B.Sc. (Nutrition), still the only degree of its kind in the world. For the first six years it worked in very restricted quarters, in part of two prefabricated huts. Now it has an area of more than 12,000 sq. ft., and is the first university department in Britain to have been specially designed for teaching of and research in nutrition. In addition to a large laboratory for forty-two undergraduates and a smaller teaching laboratory for twelve advanced students, there is accommodation for twenty-two research workers, with ten animal rooms for the rats and mice used in studying nutritional problems. Already, with the aid of grants from the research councils, from research foundations and from various industrial organizations, it has been possible to add eight graduate research workers to the staff of the Department. From this beginning the College will go on advancing by research the science of nutrition, a science which has assumed a new importance in face of the imminent danger of food production and food distribution failing to keep pace with the upsurge of world population; and by training a steadily increasing body of nutritionists, qualified in all the aspects of the subject—scientific, social and economic—which now make up the full study of nutrition, the College will provide the workers needed not only to help in the fight against hunger and malnutrition, but also to deal with the different nutritional problems of the well-fed countries.

The Department of Microbiology, already known for its research, but hitherto greatly hampered by lack of space, now has fully air-conditioned research laboratories and is probably one of the best equipped non-medical microbiology departments in any university in Britain. At present its undergraduate teaching is confined to ancillary courses, but if the University decides to introduce a B.Sc. (Special) degree in microbiology, Queen Elizabeth College will be among the first to provide courses for it. The large teaching laboratory, with about forty student places, is admirably fitted for the purpose. The University has just instituted a readership in microbiology tenable at the College, and from October 1961 the reader will be head of the Department.

The Department of Biology has the whole of the ground and first floors and part of the lower ground floor of the new building for zoological and botanical teaching and research. In its old premises it had little room for research workers. Now it has accommodation for twenty, including the teaching staff, and already six postgraduate research workers have been added to the Department, including one post-doctoral Research Fellow. The two teaching laboratories each have sixty places for undergraduates, and the College, which already had one of the biggest entries in the University for the B.Sc. (General) degree, hopes to be able to increase the number still further. Britain needs specialists, but it also needs many scientists with a broad and balanced education for industry, for teaching, and for many other important walks of life. This need is well filled by graduates with a London B.Sc. (General) degree, and the College aims at seeing that this degree is accorded the importance it deserves.

It is most fitting that this new building should be named after Sir John Atkins, the first Fellow of the College. Sir John was largely responsible for the foundation of the College. As a young doctor he felt so strongly the need for a college where domestic subjects could be taught with a proper scientific background that in 1911, by his own efforts, he raised the necessary funds for the erection of the main buildings in Campden Hill Road. He joined the Governing Body in 1912 and still serves on it. Through all these years he has watched over the interests of the College, and cherished its aims. For thirty-six years, as chairman of the Council, he guided it through the most crucial stages of its development. The Sir John Atkins Laboratories will be a permanent reminder of all that past, present and future generations of students owe to him.

DAMAGE BY *RHIZINA UNDULATA* IN CONIFEROUS PLANTATIONS

THOSE who attempt to establish, or re-establish, forests artificially on land on which there was never any natural forest, or from which every remnant of a natural forest soil has long since disappeared, are always liable to encounter fresh trouble. This fact is brought home forcibly in a recent publication by the Forestry Commission which is concerned with a fungous disease which, significantly, has been observed to be serious mainly in plantations recently established on non-forest soils*.

The title, *Group Dying of Conifers*, is unfortunate. Groups of trees may die for a variety of reasons as, for example, from strikes by lightning, from deposition of toxic materials, like manure heaps, within the stand, from attacks by fungi other than *Rhizina*, and so on, and why this forest record is not simply entitled *Rhizina undulata* with an appropriate equivalent in English is misleading and difficult to understand. A suitable name would appear to be the 'fire-site fungus'.

Rhizina undulata Fr. ex Fr. is described as 'colonizing' the sites of fires lit during forestry operations in pole-sized stands. From these sites it spreads outwards over a limited period of years, with the result that groups of dead trees occur within the stand. In some cases these breaks in the canopy give access to strong winds and form starting points of what may develop into serious windthrow. *Rhizina* has been recognized as a parasite since 1894, and has been described from both eastern and western States in the United States as well as from Europe. Significantly, it does not appear to attack leaf-tree species, only conifers. The first record of so-called 'group-dying' in Great Britain seems to have been in 1936, but it is only since 1953 that *Rhizina* has been frequently observed associated with groups of dead trees in the British Isles.

Messrs. Murray and Young, on the basis of a study of dead groups of trees in 58 forests or estates in Britain, 50 of which are situated along the western seaboard, have clearly confirmed that the fungus becomes recognizable on fire sites, and a very large proportion of the dead groups were associated with fires. This has led to the prohibition of all fires, even those lit for the brewing of tea, in or near the plantations of the Forestry Commission. This means of control can be strongly recommended to other forest owners, although it is often difficult to overcome the passion for tidiness which seems normally to be associated with artificial plantations.

The general history of the disease and its behaviour are described together with a more detailed survey of two infested forest areas in Scotland. There is an excellent description of the fungus itself; but in discussing the nature of the attacks and the behaviour of the fungus the authors have obviously been handicapped by inadequate experience of site examination and by the prevailing ignorance of soil organisms, including fungi, and of their relationship to the organic matter in the soil. It is unfortunate that no full description of any of the sites or soils examined is published although it may have been recorded elsewhere. It has been found that Sitka spruce is especially susceptible to attack, and not solely because it happens to occur in no fewer than 38 of the 58 areas examined. A point which is not made clear is whether *Rhizina* exists in the soil before the plantation is established—possibly associated with the organic matter therein—and Sitka spruce is most often planted on soils with a high content of organic matter or on grass- or rush-covered sites. *Rhizina* may exist in close association with grasses, for example, and only when the organic matter is altered or consumed by fire does it virulently attack the feeding roots of the trees. What, for example, happens when *Rhizina* encounters the mycorrhizal fungi usually associated with conifers ? There is a great need for more research into the occurrence and behaviour of all the micro-organisms found in the soil, and it is important for foresters that soil research should be based, where possible, on the soil of the natural forest. While the natural forest is a reality which has stood the test of time, the artificial forest still remains an illusory concept nowhere brought to satisfactory permanency, and liable to be seriously disturbed by organisms, like *Rhizina*, beyond the forester's control.

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* Forestry Commission. Forest Record No. 46: *Group Dying of Conifers*. By J. S. Murray and C. W. T. Young. Pp. 19+12 figures. (London: H.M.S.O., 1961.) 3s. net.

TRENDS IN JUVENILE DELINQUENCY

FOR some years the World Health Organization has taken an interest in the problem of juvenile delinquency. In 1949, at the request of the United Nations, the Organization carried out a study of the psychiatric aspects of the origin, prevention and treatment of juvenile delinquency as a contribution to the United Nations programme for the prevention of crime and treatment of offenders. Since then, representatives of the World Health Organization have taken part in many conferences and have regularly attended meetings of the United Nations Consultative Group on the Prevention of Crime and the Treatment of Offenders; and the Organization has, through its Regional Office for Europe in Copenhagen, organized a seminar on the psychiatric treatment of offenders.

In 1959, again at the request of the United Nations, another study was carried out by a psychiatric consultant, Dr. T. C. N. Gibbens, senior lecturer in forensic psychiatry in the University of London, who was appointed by the World Health Organization to complement the earlier study with a review of present trends in juvenile delinquency, based on his own personal experience, the recent literature and first-hand information obtained during visits to a number of countries—Australia, Denmark, the Federal Republic of Germany, Israel, Lebanon, Poland, Sweden and Yugoslavia. Dr. Gibbens's report, which was submitted to the second United Nations Congress on the Prevention of Crime and Treatment of Offenders, has now been published with some minor amendments*.

The new forms or manifestations of juvenile delinquency consist partly of delinquent acts which are a consequence of new opportunities for crime, but which do not differ in their essential character from more traditional forms, and partly of offences which appear to reveal a fundamental change in behaviour, or the participation of sections of the community which have not previously been involved in crime.

It is with these newer forms that the author is primarily concerned in his review of social changes, changes in family life and in the individual delinquent, special property offences, sexual offences, wayward girls, violent offences, alcoholism and drug addiction, and hooliganism. He also discusses present trends in prevention, paying particular attention to the development of prediction studies designed to identify young children who are in danger of being seriously delinquent in the future, and to the tendency to design preventive programmes in such a way that their results can be scientifically evaluated. A chapter on treatment deals with diagnosis, treatment at liberty and institutional treatment.

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* World Health Organization Public Health Papers. No. 5: Trends in Juvenile Delinquency. By T. C. N. Gibbens. Pp. 56. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollar. (Geneva: World Health Organization; London: H.M.S.O., 1961).

SIMIAN MALARIA

A RECENT issue of the *World Health Organization Chronicle* is concerned with the transmission of malaria parasites between monkey and man*.

The knowledge that such a transmission can take place dates back to 1932, when, in India, Knowles and Das Gupta found that *Plasmodium knowlesi* could cause a febrile infection in man. Knowledge of simian malaria in relation to man was extended in 1948, when *P. rodheini* of African chimpanzees was found to be morphologically and immunologically identical with *P. malariae* of man. Subsequent work showed that human malaria parasites such as *P. vivax*, *P. falciparum* and *P. ovale* could be transmitted to chimpanzees through anopheline mosquitoes although patent parasitæmia appeared only in splenectomized apes.

In certain parts of West Africa the chimpanzee is known to harbour *P. malariae*, and man has been shown to be readily susceptible to the strains harboured. The anopheline vector of the *P. malariae* of these chimpanzees has not been identified. In Sarawak certain strains of human parasites of quartan malaria have been observed to have a peculiar morphology which suggested that the parasite might be of simian origin. In the Amazon region of South America, some *P. vivax* infections in man have been held to be derived from simian malaria. *P. simium*, found in howler monkeys of the forests near Santos, has been suspected of being closely related to *P. ovale*.

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* *World Health Organization Chronicle*, 15, No. 1, January 1961.

In May 1960, came a new development. During research at the U.S. National Institutes of Health, Bethesda, by Eyles, Coatney and Getz on the transmission to rhesus monkeys of *P. cynomolgi bastianelli*, some very large-scale inoculations of sporozoites into monkeys were carried out. Two staff members of the Institutes, neither of whom had had any recent contact with human malaria, were dealing at close quarters with heavily infected mosquitoes; they both developed illness with fever which, after a remittent period, proved to have tertian periodicity. Blood smears, made on new slides and stained with new stain in clean dishes, showed that both had *vivax*-type parasites with enlarged erythrocytes and Schuffner's stippling. Five millilitres of blood from each of the two individuals, injected intravenously into two uninfected rhesus monkeys, produced *cynomolgi*-type infections in 8 days in one monkey and in 10 days in the other. Two human volunteers each received 10 ml. of blood intravenously from one of the individuals; both developed clinical attacks of malaria with *vivax*-type parasites, enlarged erythrocytes, and Schuffner's stippling, but without high parasite densities.

The two individuals in whom the infection was first discovered received chloroquine treatment within 24 hr. of diagnosis. At the time of treatment both showed low levels of parasitæmia. The parasites promptly disappeared with treatment and no relapse occurred.

Another person allowed 30–50 *Anopheles freebornii*, heavily infected with *P. cynomolgi bastianelli*, to feed on him, and experienced headache, malaise and fever eleven and twelve days later. No parasites were found in his blood, but a monkey free of disease, intravenously injected with 10 ml. of his blood, developed an overt infection in 6 days. A fourth person developed a typical malaria paroxysm with *vivax*-type parasites fourteen days after being bitten by 10 *A. freebornii*, heavily infected with *P. cynomolgi bastianelli*.

Cross-immunity studies were undertaken at Bethesda on two monkeys which had recovered spontaneously from infections with *P. cynomolgi bastianelli*. On being re-inoculated with heavy doses of parasites taken from one of the two monkeys which had been infected by intravenous injection of the blood of one of the two accidentally infected staff members, one monkey showed a transient parasitæmia indicating almost complete immunity. The other had an attack of malaria that was shorter and less severe than the usual *bastianelli* attack. These results supported the view that the human infections were due to *P. cynomolgi bastianelli*.

The significance of these infections of human beings lies in the fact that they were transmitted by mosquitoes which had fed on rhesus monkeys. The theoretical possibility of natural infection of man with monkey malaria had already been suspected; but it was the anthropoid ape, not the monkey, which was formerly suspected as being a possible carrier of human malaria. Monkeys are much more numerous and widespread than apes, and live in closer association with man, and at least seven species of anophelines are known to be able to transmit *P. cynomolgi* to rhesus monkeys. Some of these species are exophilic, and therefore unlikely to be affected by residual insecticides; moreover, the host preferences of anopheline vectors for man and for the monkey are often almost the same. Consequently, experimental proof that man can be infected by monkey malaria opens up a new range of possibilities.

EFFECT OF MASS TRANSFER ON THE VELOCITY OF RISE OF BUBBLES IN WATER

By Dr. J. H. LEONARD and Prof. G. HOUGHTON

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ALTHOUGH Datta, Napier and Newitt[1] were the first to suggest that "mass transfer (from bubbles) may be accompanied by some retarding effect", no definite conclusions were reached in this respect from their experiments on carbon dioxide bubbles rising in water. In the present measurements both velocities of rise and mass transfer-rates for bubbles could be measured separately over short distances in a column of water. The apparatus used was a modification of that[2] in which soluble bubbles were formed in a cup under mercury and allowed to rise up through a completely closed 'Pyrex' glass column (9 cm. internal diameter, 270 cm. long) filled with water; the change in volume of each bubble was recorded by registering the position of a mercury meniscus in a horizontal capillary side-arm, which provided the only opening to the atmosphere. By stretching a fine platinum wire through the capillary and connecting the wire to a bridge circuit it was possible to record the position of the mercury meniscus and hence the bubble volume on one channel of a high-speed Sanborn oscillograph. Although the principle was used earlier[3,4], these earlier experimenters used high-speed photography to determine the volume of the bubble by photographing the position of the liquid meniscus in the capillary. However, the present apparatus also differs from that of Houghton *et al.*[2] in that the rising bubbles interrupt a series of light beams and photoelectric cells placed at intervals of 47·0 cm. up the column. As the bubble passed each light beam, the change in light intensity was recorded on a second channel of the Sanborn oscillograph. In this way it was possible to determine the average bubble velocity and the average mass transfer-rate separately over each 47·0-cm. section of the column together with the corresponding average bubble diameter over the same section. In certain instances the velocities of rise, obtained by the method described, were also confirmed independently in a separate apparatus by forming the bubbles at jets of various sizes, using a micrometer hypodermic syringe, and then timing them over a 110 cm. length of column, using a stop-watch and starting the measurements at a distance of 20 cm. from the jet (cf. ref. 5).

Figs. 1 and 2 show that mass transfer has a profound effect on the velocity of rise of soluble gases in water for bubbles with equivalent spherical diameters in the range 2–6 mm. Bubbles with diameters greater than 7 mm. have not been included in these figures, since mass transfer was found to have no significant effect on the velocities of rise of bubbles in the diameter range 6–20 mm., presumably because the profile drag and inertial effects present in the larger bubbles were much more important than the surface effects. The mass transfer-rates for the gases in Figs. 1 and 2 are in the following descending order: carbon dioxide > nitrous oxide > ethylene ≃ nitrous oxide – nitrogen mixture (50 mole per cent) > nitrogen. The largest velocities of rise are found for insoluble gases represented in Fig. 1 by the data for nitrogen bubbles rising in water saturated with nitrogen; in this system, the bubbles expand as they rise, because of the reduction in the hydrostatic head. The gas with the largest mass transfer-rate is carbon dioxide, which also demonstrates the lowest velocities of rise. The systems with intermediate mass transfer-rates between insoluble nitrogen and carbon dioxide also show intermediate effects on the velocity of rise. For example, Figs. 1 and 2 show that bubbles formed from a 50–50 mixture of nitrous oxide and nitrogen have velocities of rise half-way between those of pure nitrogen and pure nitrous oxide. In fact, the figures show that the effect of mass transfer is to lower the velocity of rise as the mass transfer-rate increases, so that the velocities of rise are in the following order: nitrogen > nitrous oxide – nitrogen mixture (50 mole per cent) ≃ ethylene > nitrous oxide > carbon dioxide.

It is evident that the lowering of the velocity of rise for soluble bubbles cannot be explained simply on the basis of a change in terminal velocity of rise with diameter, since the velocities of rise of insoluble bubbles decrease with increase in diameter in the range 2–6 mm., whereas, when mass transfer occurs, the velocity either increases or does not change much with diameter. Furthermore, in no case did the diameter change more than 5 per cent of its value over any section of the column.

Another possible explanation might be that as a bubble decreases in size, it should accelerate if the velocity of rise follows the curve for insoluble bubbles. The acceleration would then produce an increase in virtual mass of the bubble (cf. ref. 6), which in turn would reduce the buoyancy forces and indirectly affect the velocity of rise. However, the experiments using pure nitrous oxide bubbles in aqueous nitric acid (*p*H 0·7) shown in Fig. 2 tend to eliminate this explanation. The velocities of rise of pure nitrous oxide bubbles in dilute nitric acid were the same as the velocities of rise of insoluble nitrogen bubbles, even though the mass transfer-rate of nitrous oxide in the nitric acid solution was the same as that in pure water. This observation indicates that, although the phenomenon depends on the mass transfer-rate, it is also sensitive to the presence of electrolytes in solution. A simple explanation for all these phenomena that comes to mind is that electrokinetic effects caused by mass transfer might induce an electrically charged layer at the bubble surface, which in turn would retard the bubble motion by increasing the skin friction. This surface potential would be analogous to the zeta-potential, which is known to be sensitive to the presence of acids and alkalis, so that the presence of a zeta-potential would also explain the apparently anomalous case of nitrous oxide bubbles in aqueous nitric acid as well as the dependence of the phenomenon on the rate of mass transfer across the boundary. Furthermore, electrokinetic effects at the surface would explain why the phenomenon is only observed with small bubbles, where the surface effects would be large and inertial and profile effects would be small.

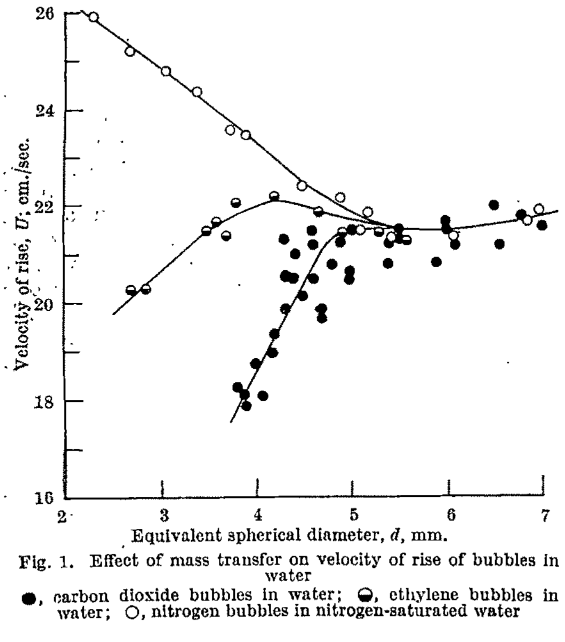

Fig. 1. Effect of mass transfer on velocity of rise of bubbles in water

●, carbon dioxide bubbles in water; ◒, ethylene bubbles in water; ○, nitrogen bubbles in nitrogen-saturated water

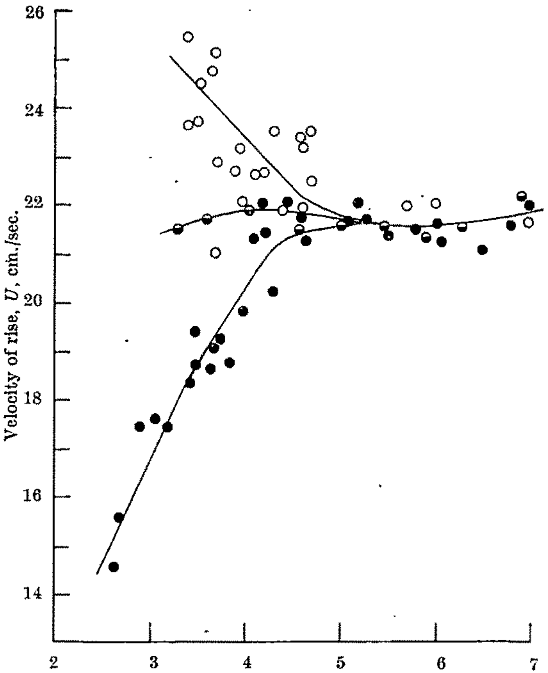

Fig. 2. Effect of mass transfer on velocity of rise of bubbles in water

●, nitrous oxide bubbles in water; ◒, nitrous oxide–nitrogen bubbles (50 mole per cent nitrous oxide) in water; ○, nitrous oxide bubbles in aqueous nitric acid (*p*H 0·7)

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SCHULZE–HARDY RULE AND MASS ACTION LAW

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IN two previous papers of this series[1,2] the equation:

$${}''c(1 - x)/{}'cx = 10^{({}'z - {}''z)a} = {}'D/{}''D = S \qquad (1)$$

was shown to be valid for the equilibrium concentrations ${}''c$ and ${}'c$ and for the adsorbed fractions, x and $1-x$ of two ions of valency ${}''z$ and ${}'z$ in equilibrium with the coagulated silver iodide. a is a constant, S the selectivity coefficient, ${}''D = x\gamma/{}''c$ and ${}'D = (1 - x)\gamma/{}'c$ are their distribution coefficients.

Equation (1) can formally be obtained from the difference of the equations:

$$\log {}'c = -\,{}'za + \log (1 - x)\gamma - \log {}^0D \qquad (2)$$

and

$$\log {}''c = -\,{}''za + \log x\gamma - \log {}^0D \qquad (3)$$

resp.

$$\log {}^0D = \log {}^iD - {}^iza \qquad (4)$$

where $\gamma = \overline{C}\,w/V$ is the maximal adsorbed amount expressed in equivalents per litre, w the amount of the solid in the system of volume V, $\overline{C}$ is the adsorbing capacity of the solid, that is, the amount adsorbed per unit amount of the solid, and ${}^0D$ is the distribution coefficient of a hypothetical particle of zero valency which would be adsorbed as charged particles.

The exchange of adsorbed counter ions can be represented by association processes between counter ions and the adsorbed potential determining ions:

$$(A_{{}^iz}M_{z^-}{}^{i})_{\text{ads.}} \rightleftharpoons ({}^iz\;A^{z^-})_{\text{ads.}} + z^-M^i$$

If chemical thermodynamics is applied to these association reactions the constants in equations (1) to (4) are obtained:

$$a/0{\cdot}4343 = \mu_{A^{z-}_{\text{ads.}}}/z^-RT$$

$$\ln {}^0D = (\mu^0{}_{AM^i_{\text{ads.}}}/z^- - \mu^0{}_{M^i})/RT$$

$$\ln {}'D = \ln a_{AM'_{\text{ads.}}}/a_{M'} = \ln(1-x)\gamma/c'$$

$$\ln {}''D = \ln a_{AM''_{\text{ads.}}}/a_{M''} = \ln x\gamma/c''$$

$$\ln {}^iD = \ln a_{AM^i_{\text{ads.}}}/a_{M^i}$$

for

$$z^- = 1,\ a_{AM'_{\text{ads.}}} = (1-x)\gamma,\ a_{AM''_{\text{ads.}}} = x\gamma,$$

$a_{M'} = {}'c, a_{M''} = {}''c$. $\mu_{A^{z-}_{\text{ads.}}}$ is the chemical potential of the potential determining ion in the double layer. The constancy of a with pI for different z, was established experimentally, therefore $\mu_{A^{z-}_{\text{ads.}}}$ is constant and independent of pI, while the adsorbed quantity of the potential determining ion is inversely proportional to pI (refs. 1–3). $\mu^0{}_{M'} = \mu^0{}_{M''}$ and $\mu^0{}_{AM'_{\text{ads.}}} = \mu^0{}_{AM''_{\text{ads.}}}$ are the standard chemical potentials of the counter ions M' and M'' of valency ${}'z$ and ${}''z$ in the solution and in the adsorbed layer and $a_{M'}$, $a_{M''}$ $a_{AM'_{\text{ads.}}}$ and $a_{AM''_{\text{ads.}}}$ are their activities.

By eliminating a in equations (2), (3) and (4) the following equation is obtained:

$$K_c = {}^0D^{({}'z - {}''z)} = {}'D^{{}''z}/{}''D^{{}'z} = [(1-x)\gamma/{}'c]^{{}''z}({}''c/x\gamma)^{{}'z} \qquad (5)$$

which is identical with the mass action law equation, K_c being its constant.

If ${}'c \gg {}''c + \gamma x$, then $x \ll 1$ and $1-x \doteq 1$ and equation (5) can be written:

$$\log 1/{}''D = {}''z/{}'z \log {}'c - {}''z/{}'z \log \gamma K_c{}^{-1/{}'z} \qquad (6)$$

Equation (6) is identical with Strickland's expression[4] suggested for the determination of ion charges and was used by Kraus and Nelson[5] and others[6] for this purpose.

Consequently, the coagulated silver iodide may be considered as an ideal ion exchanger, the role of the coagulating ions being that of the exchange ions.

Supposing that two ions are exchanging in accordance with coulombic repulsion forces, one of valency ${}'z = 0$, the other of ${}''z = z$, then equation (1) may be written as:

$$\log c/{}^0c = -\,az + \log x/(1-x) \qquad (7)$$

The logarithm of the concentration at which a given constant ratio of two ions is adsorbed (x = constant) is increasing linearly with the decreasing valency of the ion. ${}^0c$ can be considered as a concentration of a hypothetical adsorbable species without charge, which follows from equation (7) for known x, z and c.

It is a general opinion that the Schulze–Hardy rule should be a consequence of the relative adsorption tendencies of the coagulating counter ions of different valencies. The coagulation value $c_{\text{coag.}}$ is an experimentally observed limiting concentration at which the coagulation sets in abruptly, and all terms determining it must be constant. The positions which are in the double layer of the coagulated silver iodide occupied by coagulating counter ions have to be occupied on the stable silver iodide by stabilizing particles. In a simple system like the system AgI—I^-—H_2O—counter ion, the only particles which can be considered as stabilizing are the water molecules. The adsorbed quantity of remaining species, the I^- ions, does not depend on the concentration of the counter ions and cannot be made responsible for coagulation. The coagulation will be induced when the stabilizing water molecules are exchanged by the coagulating counter ions. The reactions representing this process in the double layer can be written:

$$(A_zM_{z^-})_{\text{ads.}} \rightleftharpoons z\,A^{z^-}{}_{\text{ads.}} + z^-M$$

$$(A_2{}^{z^-}.z^-H_2O)_{\text{ads.}} \rightleftharpoons 2\,A^{z^-}{}_{\text{ads.}} + z^-\,H_2O$$

Chemical thermodynamics applied to these association reactions gives the equation (3) for $z^- = 1$ with equal constants, a and ${}^0D$, namely:

$$\log c_{\text{coag.}} = -\,za + \log x_{\text{coag.}}\gamma/{}^0D \qquad (8)$$

for:

$$a = (\mu_{(A_2{}^{z-}z^-H_2O)_{\text{ads.}}}/z^- - \mu_{H_2O})0{\cdot}4343/2RT =$$

$$\mu_{A^{z-}_{\text{ads.}}}\ .\ 0{\cdot}4343/z^-RT$$

$$\ln {}^0D = (\mu^0_{(AM)_{\text{ads.}}}/z^- - \mu^0_M)/RT$$

$\mu_{(A_1{}^{z^-}.x\text{-}H_2O)_{\text{ads.}}}$ is the chemical potential of the hydrated potential-determining ion in the double layer. It is constant because both $\mu_{A^{z-}_{\text{ads.}}}$ and μ_{H_2O}, the chemical potential of water in the liquid phase, are constant. ^{0} stands for the respective standard chemical potentials. Equation (8) should represent the Schulze–Hardy rule and is according to the adsorption theory, thermodynamically justified. Any other non-linear ($\log c_{\text{coag.}} - z$) relationship would contradict the requirements of thermodynamics.

A linear relation between the logarithm of coagulation value and valency was deduced theoretically by Težak[7,8] and was confirmed experimentally[7,9]. On the basis of this linear relationship, Težak *et al.*[9,10] suggested a coagulation value method for the determination of ion charges.

It was shown experimentally[1,2] that the slope of the Schulze–Hardy line in equation (8) and the slope of the adsorption line for $x/(1-x)$ = constant in equation (7), namely, the a values, are equal for the negative silver iodide system. Consequently, Strickland's and Težak's methods can be considered as the consequence of the same adsorption-desorption, respectively, ion exchange equilibria for which the mass action law is valid. The equality of a is thermodynamically justified, since a is proportional to the chemical potential of the adsorbed potential determining ions, which is the same in both cases.

If the coagulation value represents that activity of the coagulating ion in solution at which, in the double layer, its chemical potential becomes equal to the chemical potential of the stabilizing particles, then $x_{\text{coag.}} = 0{\cdot}5$.

Since ${}^0D$ cannot be determined by adsorption experiments on coagulated silver iodide (for $c > c(x = 1)$ ${}^0D$ is not constant) it follows from coagulation experiments:

$$\log {}^0D \doteq -za + \log 0{\cdot}5\,\gamma/c_{\text{coag.}}$$

I wish to express my thanks to Prof. Božo Težak for his interest in my work and for many helpful discussions.

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PHOTOCHEMICAL REACTIONS IN FROZEN SOLUTIONS

By Prof. SHIH YI WANG

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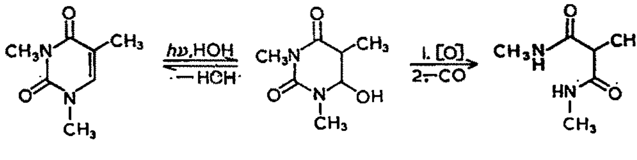
SZENT-GYÖRGYI has shown that photochemical reactions in aqueous solutions, in frozen aqueous solutions and in frozen aqueous solutions containing organic solvents differ from each other[1]. He has recently suggested[2] that a photochemical reaction in frozen solution differs from that in liquid solution because the formation of crystalline ice forces the solute molecules into solid aggregates[3]. In confirmation of this hypothesis, he found that the phosphorescence observed in frozen solution also occurs in a dry film. Similarly, I have obtained chemical evidence that an identical photoproduct was isolated from the irradiation of pyrimidines, either in frozen solutions or as dry solid films. However, the photoproduct obtained from the irradiation of aqueous solution was different.

By extending the hypothesis of molecular aggregate formation in frozen solution, one would expect that the photochemical behaviour of a solute in frozen aqueous solution containing organic solvent would be similar to that of its organic solution; for on freezing, the organic solvent and solute molecules would be excluded from the water crystals and form 'puddles'. That the photochemical reaction actually occurs in such 'puddles' of organic solvent has been adduced from the fact that the methanolic addition product of 1,3-dimethyluracil is formed from the irradiation of 1,3-dimethyluracil either in frozen solution containing 2 per cent methanol or methanolic solution.

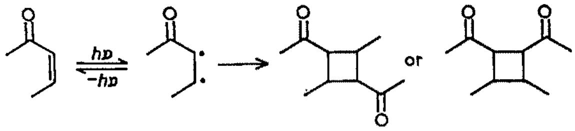
From this work, two novel techniques of great interest for photochemical study are evident: (1) frozen solution irradiation may be used as an efficient method for irradiating solids; (2) formation of 'puddles' may offer a new means for study of low-temperature photochemical reactions.

In an earlier report from this laboratory on the effect of ultra-violet irradiation on 1,3-dimethylthymine (DMT) in aqueous solution, it was suggested that a water addition product is the first intermediate formed. The intermediate is probably unstable at room temperature and reconverted to its starting material by dehydration[4]. It is not unreasonable to expect that this intermediate would become stable at low temperatures and could be isolated. In order to prove the above, an examination of low-temperature irradiation was initiated.



In frozen solution, the absorption maximum of DMT decreased rapidly when irradiated with ultra-violet light. This is to be expected if the hydration product were formed. The original spectrum would then be reconstituted by standing at room temperature. However, the product obtained from frozen solution irradiation was stable even when treated with acid, base or heat. It must be concluded that the assumed product, 6-hydroxy-1,3-dimethylhydrothymine, was not formed. This observation indicated that irradiation with ultra-violet light in aqueous solution and in frozen solution must proceed through two different mechanisms. In order to eliminate the possibility that temperature played a part in the alteration of reaction pathways, the irradiation of DMT was carried out in the aqueous solution and in the frozen solution, both at ~0°. Since this difference in irradiation behaviour still was observed, it can be concluded that temperature alone is not the cause of the difference.

In order to further our understanding of the nature of frozen solution irradiation, the isolation and identification of the product were carried out. From the elementary analysis and molecular weight determinations, the product was readily recognized to be a dimer of DMT[5].



Photodimerization has been widely observed for α,β-unsaturated keto-derivatives[6]. These dimers are generally believed to be formed through the union of the double bonds from two molecules. A diradical intermediate, as shown, is generally favoured as the mechanism for the formation of the cyclobutyl ring. The probability of dimer formation will depend on the distance between the two reacting molecules. Therefore, photodimerizations occur mostly in the solid state rather than in solution. Since highly strained cyclobutyl rings are formed in this process, the photoproducts are known to be quite unstable. It is reported that photodimers are reconverted to their monomers by treatment with either acid[7] or heat[8] or ultra-violet light[9]. DMT-dimer obtained in frozen solution irradiation, as noted above, is stable toward heat, acid or base, but ultra-violet light converted it to the monomer, DMT, in liquid solution.

Since the DMT-dimer is formed by the irradiation of frozen solution, irradiation of frozen solution must resemble irradiation of solid aggregates. Further evidence for this assumption will be presented in the ensuing sections.

(A) Nature of Irradiation of Frozen Solutions

(1) *Heterogeneity of Frozen Aqueous Solutions.* The heterogeneity which serves as the basis for the hypothesis that solid aggregates are formed on freezing aqueous solutions is analysed quantitatively as follows: Frozen solutions of DMT and 1,3-dimethyluracil (DMU) were analysed spectrophotometrically; they showed differences in their concentrations between different layers. Optical density readings for a 0·1 m*M* solution after freezing are given in Table 1.

Table 1

Layer*	Dry-ice mixture		Liq. N_2	Ice-NaCl	Freezer
	DMT	DMU	DMT	DMU	DMT
Outermost	0·66 × 10^3	0·42	0·74	0·49	0·21
Intermediate	1·08	1·10	1·04	1·04	0·82
Core	1·12	1·16	0·96	1·15	1·78

* When a beaker containing solution is frozen, crystals of ice will form first on the surface of the beaker and grow towards the centre. However, when such a beaker is placed instead in a tray, which in turn is placed directly on the metal shelf in a refrigerator, the top layer freezes first. Therefore, the reverse effect was observed (Table 2).

The concentration difference is often 8–10 times between two layers for compounds such as uridine, thymidine, etc.

After irradiation the frozen solutions were also analysed as three separate layers. For example, a thymine solution (0·1 m*M*, $\varepsilon^{H_2O}_{\text{max.}}$ 0·78 × 10^3), gave the readings presented in Table 2.

Table 2

Layer	$\varepsilon^{H_2O}_{\text{max.}}$		Yields (per cent)	
	Before irrad.	After irrad.	Relative	Absolute
Outermost	1·53	0·19	169	88
Intermediate	0·70	0·10	76	86
Core	0·17	0·02	19	88

The relative percentage yields of dimer were calculated assuming homogeneous distribution of solute while the absolute yields were calculated on the basis of the real concentration in each layer. The relative percentage yields were found to be variable. However, when the percentage yields were calculated according to the concentration of each layer, that is, the absolute amount of material present in each layer, then the absolute percentage yields were almost the same throughout. This result suggests that the irradiation is related to the absolute amount of material present.

(2) *Effect of Temperature and Methods of Freezing on Irradiation Reaction.* When individual solutions were frozen under different conditions such as in a 'deep-freeze', or by means of liquid nitrogen or carbon dioxide-2-*n*-butoxyethanol, and then irradiated at identical temperatures in a carbon dioxide-2-*n*-butoxyethanol mixture bath, the results obtained were approximately the same. In addition, when all solutions were frozen in a dry-ice mixture but the irradiation temperature of each solution was varied by means of liquid nitrogen, carbon dioxide mixture or ice-sodium chloride baths, the decrease in absorption maximum was still the same (Table 3).

Table 3

Effect of method of freezing (DMU)

$O.D.^{H_2O}_{\text{max.}}$	In ice-NaCl	In CO_2 mixture	In liquid N_2
Before irradiation	0·973	0·965	0·960
After irradiation	0·703	0·683	0·706

Effect of temperature during irradiation (DMT)

$O.D.^{H_2O}_{\text{max.}}$	– 10°	– 70°	– 190°
Before irradiation	0·838	0·838	0·840
After irradiation	0·390	0·394	0·419

Therefore, neither the temperature during irradiation nor methods of freezing altered the photochemical behaviour of solid aggregates in frozen solutions.

One attempt was made to form a homogeneous frozen solution by dropwise freezing with dry-ice mixture. Each individual drop was added after the previous drop was completely frozen; nevertheless, dimers were formed on irradiation. These findings suggest that within the means of common laboratory facilities, heterogeneity always results when an aqueous solution is frozen.

(3) *Irradiation in Rigid Medium*[10]. In order to demonstrate that no dimerization should occur in the absence of molecular aggregates, glycerol was used as the solvent for the irradiation study. On cooling,

the glycerol solution of DMT became rigid glass rather than crystalline and the individual molecules were on the average 100 Å. apart[10]. On irradiation, the absorption maximum of DMT remained the same as before irradiation, indicating strongly that no photodimerization occurs in a homogeneous solution.

(4) *Formation of Dimers in Frozen Aqueous Solutions and Films of Dry Solid.* (*a*) Irradiation in frozen solution[11–13]. Under my experimental conditions, all the uracil and thymine derivatives tested form photodimers. Therefore, this is a general phenomenon for pyrimidine molecules which have α,β-unsaturated ketone as their predominant electronic configuration. Table 4 gives some of the compounds which have been irradiated as frozen solutions. Also given is the maximum percentage decrease at $\lambda_{max.}$ for each compound and its time for reaching this maximum decrease. Based on these two values, the quantum yields were calculated. The light energy absorbed by the solution is $1 \cdot 24 \times 10^{-6}$ Einstein per minute under this condition.

Table 4

0·1 m*M* solution	Maximum decrease in $\lambda_{max.}$ (per cent)	Minimum time (min.)	Quantum yield* × 10^{-3} (mole/Einstein)
Thymine	86	3	230
1,3-DMT	75	6	100
Thymidine	49	100	4·0
Ca^{++}-thymidylate	41	120	2·8
Uracil	68	10	55
1,3-DMU	25	15	13
Uridine	21	90	1·9
Uridylic acid	40	150	2·2
6-Methyluracil	60	80	6·0
1,3,6-Trimethyluracil	50	45	9·0

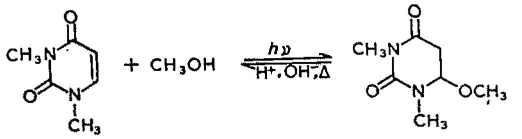
* These values are provided to indicate the order of magnitude of the quantum efficiency of the reactions. The fact that some of the light is reflected from crystalline ice surfaces renders it difficult to assign a 'true' value of light-energy absorbed by the frozen solution.

Evidence for the formation of dimers is based on the isolation and characterization of uracil-dimer (anal. found C, 42·61; H, 3·29; N, 25·16. No absorption maximum between 220–360 mμ), DMT-dimers (Isomer-I: m.p. 258–259°; mol. wt. found 301; anal. found C, 54·56; H, 6·61; N, 18·34. Isomer-II: m.p. 225–226°; mol. wt. found 302; anal. found C, 54·67; H, 6·73; N, 18·28), and thymine-dimer[13] (anal. found, C, 47·47; H, 4·69; N, 22·23. No absorption maximum between 220–360 mμ). Other photodimers were shown by the decrease of their absorption maxima during irradiation with ultra-violet light in frozen solutions. After thawing, the original spectra could be reconstituted by the irradiation of the thawed solution with ultra-violet light at *p*H ~2 (acid *p*H used to prevent the formation of the water addition product during the irradiation of aqueous solution whenever necessary).

(*b*) Irradiation of dry solid films. The question still remains, Does dimerization occur because the solute is present in dry aggregates in a frozen solution, as suggested above, or is there some more subtle reason? If only dry aggregates are required then irradiation of a dry solid film should yield similar results. When a thin layer of solid thymine or uracil was irradiated, thymine-dimer or uracil-dimer was isolated from these irradiation studies; these dimers were characterized by ultra-violet and infra-red spectra, and were found to be identical to those obtained when frozen solutions were irradiated. Not only do the foregoing results support the proposed concept, but they also emphasize that the crystalline structure of water is not necessary for the photochemical behaviour noted for frozen solution. Frozen solution irradiation offers a novel method of solid irradiation which is experimentally advantageous; it is convenient, controllable, involves neither the tedious technique nor the large quantities that are ordinarily necessary for chemical studies of irradiation reactions using dry solid.

(B) Photochemistry in 'Puddles'

Szent-Györgyi showed that the photochemical behaviour of a compound irradiated in a frozen aqueous solution is altered when the frozen solution contains 2 per cent methanol. He stated "... an admixture of 10 per cent glycerol or 2 per cent methanol to the water deprive it of its specific action on excitation because these admixtures destroyed the regularity of the water crystals"[1]. In my frozen-solution experiments, when thymine and DMT were irradiated in the presence of 2 per cent methanol or 10 per cent glycerol, the spectra were the same before and after irradiation, a finding strongly indicating that no reaction has occurred. However, since heterogeneity of the frozen solution was shown to be responsible for the photodimerization, a 'puddle effect', again based on heterogeneity, is proposed to explain the above phenomena. One may hypothesize that, on freezing, the water molecules tend to crystallize, but the solute molecules, together with some of the added methanol or glycerol, are excluded from the water to form puddles. However, 'glycerol puddles' are different from 'methanol puddles'. As noted above, the former when frozen form a rigid medium, and as a result no photodimerization should occur on irradiation. The latter when frozen form a methanolic solution, and therefore a photochemical reaction in this solution should be observed. In order to show the 'puddle effect' of methanol, a compound that fulfils the following criteria must be studied: (*a*) forms a photodimer when irradiated in frozen solution; (*b*) forms a solvent addition product when it is irradiated in solution. A compound with the above characteristics was found in DMU. When DMU dissolved in methanol is irradiated with ultra-violet light, the decreasing absorption maximum of DMU indicates the formation of 6-methoxy-1,3-dimethylhydrouracil



The addition of acid or alkali will cause 6-OCH_3 compound to revert to DMU[14]. When a frozen aqueous solution of DMU containing 2 per cent methanol was irradiated, a decrease in absorption maximum was also observed. Again, addition of acid (*p*H ~2) or alkali (*p*H ~12) caused a total reconstitution to DMU, as shown by the ultra-violet spectrum. This fact indicates that the solvent addition product was formed, which strongly supports the 'puddle effect' hypothesis. Therefore, Szent-Györgyi's observation that irradiation of a frozen solution containing 2 per cent methanol was similar to that of a liquid solution is to be expected[15].

(Irradiation of dilute solutions of 0·1 m*M* rhodamine *B* (Table 5, *B*), 0·1 m*M* flavine mononucleotide

Table 5

State	*B*	FMN	Mixture
Liquid solution	Orange	Yellowish green	Orange
Frozen solution	No light emission	Orange	Bright pink
Frozen solution + 2 per cent MeOH	Orange	Yellowish green	Orange
Solid film	No light emission	Orange	Bright pink

(FMN) and a mixture of 0·1 m*M* rhodamine *B* and 1 m*M* thiamine hydrochloride were used by Szent-Györgyi to demonstrate the dramatic changes in fluorescent colours between the frozen solutions and their liquid counterparts[1]. I have extended these experiments by the comparison of these fluorescent colour changes with solid films. These were prepared by drying 10 m*M* methanolic solutions of *B*, 10 m*M* methanolic solution of the mixture and a saturated methanolic solution of FMN on glass plates. Table 5 shows the changes in colour viewed under an ultra-violet lamp (Mineralight No. *R*. 51, Ultraviolet Prod. Co., San Gabriel, Calif.). The light emission from liquid solutions and frozen solutions with 2 per cent methanol is the same, suggesting that the 'puddle' formation probably occurs in the latter. The light emission from solid films is the same as that of their frozen solutions, suggesting that the fluorescent colours observed with frozen solutions probably is due to the formation of solid aggregates on freezing.)

This 'puddle' formation in frozen solution certainly merits further investigation. This system resembles the non-aqueous environments of living organisms which permit the occurrence of specific reactions, such as electron transport in mitochondria[15] and oxygen exchange in hæmoglobin[16]. By replacing 2 per cent methanol with various organic solvents, one may experiment with the molecular rearrangements, Diels–Alder reaction, etc., of various compounds. The formation of 'puddles' produces an essentially water-free medium at low temperatures for the reactions under investigation, a condition impractical to achieve by merely cooling organic solvents because of water condensation. It is my belief that this 'puddle effect' probably offers a new area for photochemical study.

At the outset of my systematic study of the photochemistry of nucleic acids and related compounds, I considered that photochemical changes occurring in the solid-state may possibly be related to the irradiation-induced changes of deoxyribonucleic acid (DNA) in biological systems. This concept is based on the Watson and Crick[17] and Wilkins *et al.*[18] proposal for the molecular structure of DNA. They suggested that DNA may consist of two polynucleotide chains helically wrapped around each other with 10 base residues per turn. The pitch is 34·6 Å. and the distance between the two layers of base residues is 3·4 Å. Thus, the proximity of the bases may permit the interaction of two adjacent base residues on the same chain to form pyrimidine-pyrimidine, purine-purine or pyrimidine-purine dimers coincident with the breaking of interchain H-bonds. The conditions for the irradiation of solid films, discussed above, for the yield of pyrimidine-dimers were obtained only after an understanding of the nature of the irradiation of frozen solutions was attained. Therefore, if biological systems and compounds such as chloroplasts, proteins and nucleic acids are closely related to the 'pseudocrystalline' state, the irradiation of solids or frozen solutions may yield more important information than irradiation in other media.

Irradiator. A bank of seven ultra-violet lamps (General Electric Germicidal tube *G*15*T*18) was used. The distance between the lights and the solution for irradiation was 33 cm. A voltage regulator was connected to the irradiator and a light intensity of 50 MW./sq. ft. was obtained[19]. For isolation study, the voltage regulator was omitted and a light intensity of 80 MW./sq. ft. was used.

Quantum Determination. This was measured by the method of Forbes and Heidt[20].

Frozen Solutions. Ten ml. of solution in a 50-ml. beaker was frozen by various means and the thickness of the solution was about 1 cm.

Separation of Layers. Fifteen ml. of frozen solution in a 100-ml. beaker was used (1 cm. thickness). It was thawed gradually by placing the beaker in a water bath at room temperature. Some of the thawed liquid was pipetted out until 5 ml. could be obtained. This was referred to as the outermost layer. Then, with further thawing, a second 5 ml. portion, the intermediate layer, was collected. The remainder was called the core layer.

Gross Irradiation for Isolation. One litre of 1 m*M* solution was frozen and then was irradiated in an enamel pan (11·5 × 18 in.) for 30 min. This pan was placed on a dry-ice mixture to keep the bath frozen. The irradiated solution was then thawed and was evaporated to dryness with a flash evaporator for further purification. Usually, the product of several runs was combined.

(*a*) *Uracil-dimer, thymine-dimer.* The crystalline residue was taken up in boiling water. After filtration, the filtrate was concentrated for crystallization. The crystalline product was obtained easily because the dimers are much more insoluble in water than the monomers.

(*b*) *DMT-dimers.* The crystalline residue was taken up in chloroform, and, after filtration, the combined solution from 3 pans was concentrated to 5 ml. To this an adequate volume, 25 ml., of petroleum ether (30–60°) was added to make the solution just appear cloudy. On standing, a crystalline product was obtained, m.p. 210–230°. Every 100 mgm. of the above product was dissolved in 2 ml. of chloroform and then 5 ml. of petroleum ether was added for crystallization. From this 45 mgm. of a product, m.p. 250–255° was obtained. Further crystallization of this product gave a high melting isomer, m.p. 258–259°. From the crystallization of the mother liquor, a low melting isomer, m.p. 225–226°, was obtained.

Irradiation of Solid Film. A 10-m*M* methanolic solution of thymine or uracil was prepared, and 5 ml. of this solution was pipetted on to a 'Pyrex' baking pan (9 × 12 cm.) very slowly and allowed to form an even, dry film over the bottom of the pan. After irradiation for 30 min., the residue was taken up in boiling water and then concentrated for crystallization.

(*a*) *Thymine.* A total of 5 mgm. of thymine-dimer was obtained by combining 5 runs. (*b*) *Uracil.* A total of 7 mgm. of uracil-dimer was obtained by combining 8 runs.

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MECHANISM OF SODIUM TRANSPORT IN CELLULAR MEMBRANES

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THE function of a nerve cell in the process of conducting impulses can easily be described by the behaviour of its membrane potential. At rest, the potential difference, outside minus inside, is about +50 to +100 mV. During activity, when an impulse passes, this potential is rapidly abolished and even to some extent reversed. Afterwards, and within a few milliseconds, the original potential difference is re-established. The wave of changes in potential is termed the action potential.

It has been shown very convincingly that the first, decreasing phase of the action potential is due to the sudden and very rapid transfer of sodium ions from the outside to the inside of the cell membrane[1]. This movement of sodium ions occurs in the direction of the concentration gradient, and thus needs no additional source of energy. The rising phase of the wave is due to an outward movement of potassium, which is again in the direction of the concentration gradient. Ultimately, the repetition of this event would lead to the destruction of the ionic concentration gradients, and thus to the loss of the ability to form an action potential, or in other words, to a block in the excitability and function of the nerve cell. However, the nerve cell membrane possesses a mechanism which is capable of transporting sodium ions from the inside to the outside, against the concentration gradient[1]. Thus this gradient does not disappear, but is eventually re-established to its full extent.

When one tries to understand the basic principles of these processes, two things have first of all to be explained: (1) the ability of the cell membrane to bring about and to maintain the concentration differences of ions on its two sides; (2) the nature of the changes which occur in the membrane permiting sodium ions to enter the cell and thus cause the action potential. As regards the first problem, it must be borne in mind that the different concentrations of sodium ions on the two sides of the membrane are in a dynamic equilibrium. There is a continuous exchange of sodium ions through the membrane, but at rest the concentrations remain constantly the same[1]. The outward movement of sodium ions is dependent on an active transfer mechanism which needs metabolic energy for its function. This energy is supplied in the form of adenosine triphosphate[1], which is in turn generated through the process of oxidative phosphorylation.

The second problem, that of the mechanism of formation of the action potential, is concerned with a sudden increase in the inward movement of sodium ions. The nature of the changes in the membrane which cause this phenomenon is still quite obscure. The discussion which follows is concerned with the mechanism of active transport of sodium ions across the cell membrane. Some new data on the nature of the biochemical mechanism underlying the phenomenon of active transport will also be presented and discussed.

Microsomal Adenosine Triphosphatase as an Indicator of Sodium Transport

Recently, evidence[2-7] has been obtained which bears on the mechanism of sodium transport across the cell membrane. This evidence also provides a plausible explanation for the mode of action of general narcotics such as ethyl alcohol[5] and ether. Some implications concerning present thought on biochemical phenomena in highly organized subcellular structures are also evident.

Microsomes from rat brain, when isolated in the manner described in detail elsewhere[3], exhibit a powerful adenosine triphosphatase activity. This activity is completely dependent on magnesium ions, and shows the property of being stimulated by sodium ions (Table 1). When sodium is present in concentrations of between 30 and 100 m*M*, there is observed an activity of adenosine triphosphatase of some 20–25 per cent higher than the activity in the absence of sodium. Somewhat lower concentrations of sodium show a lower stimulation, and concentrations less than 5 m*M* do not stimulate to a measurable extent. To find an explanation for these observations, attention is directed to the well-known phenomenon of respiratory control in electron-transport-coupled phosphorylation, catalysed by mitochondria. When phosphate and phosphate acceptors are absent, no electron transport occurs. In the brain microsomal system, less dephosphorylation of adenosine triphosphate occurs in the absence of sodium. On the basis of these experiments, it is assumed that microsomes from rat brain catalyse two enzymatic reactions, one of which has adenosine triphosphate as a substrate, the other sodium ions. These two reactions would be coupled to each other in an obligatory manner, just as are electron transport and phosphorylation in the mitochondrial system. When no sodium ions are present, no reaction could occur with adenosine triphosphate, and a control mechanism analogous to the mechanism of respiratory control would therefore be in operation. Obviously the reaction with sodium cannot occur in a sodium-free medium, and the consequence is a lower rate of adenosine triphosphate dephosphorylation. The fact that the latter activity is not completely extinguished in the absence of sodium is capable of explanation when one considers that the microsomes may have suffered damage during the preparation, and that such damage is likely to produce a certain amount of 'uncoupling'. It is also possible that enough sodium is bound to the microsomes to cause the observed rate in the absence of sodium.

Table 1. ACTIVITY OF ADENOSINE TRIPHOSPHATASE OF RAT BRAIN MICROSOMES IN THE PRESENCE AND IN THE ABSENCE OF SODIUM AND POTASSIUM IONS[8]

Exp. No.	Additions	μmoles P_i liberated	Activ. (+) inhib. (−)
1	None	0·63	–
	Na^{+}, 0·1 *M*	0·74	+ 0·11
	Na^{+}, 0·01 *M*	0·65	+ 0·02
2	None	0·44	–
	Na^{+}, 0·1 *M*	0·57	+ 0·13
	Na^{+}, 0·015 *M*	0·54	+ 0·10
	K^{+}, 0·1 *M*	0·43	– 0·01
	K^{+}, 0·015 *M*	0·44	0
	Na^{+}, 0·05 *M* and K^{+}, 0·05 *M*	0·57	+ 0·13

The assay contained : 20 m*M* *tris*-chloride buffer, *p*H 7·5, 5 m*M* magnesium chloride, 5 m*M* *tris*-adenosine triphosphate, 0·1 ml. of enzyme suspension in isotonic sucrose, the indicated amounts of the alkali metal ions as chlorides, and when these would not make the mixture isotonic, the necessary amount of sucrose. Final volume 1 ml., temperature 30° C., incubation time 10 min.

In this connexion, it is interesting to note that potassium ions cannot cause a stimulation in the microsomal adenosine triphosphatase (Table 1). The view has been adopted that the isolated microsomal system represents and contains the sodium-specific mechanism for the transport of ions across cellular membranes. Some evidence from other laboratories can be taken as supporting this view. Some years ago, Skou demonstrated[6] that submicroscopic particles from crab nerve possess a sodium-and-potassium dependent adenosine triphosphatase activity. He also interpreted this as an expression of an active sodium transport mechanism in these particles. Skou's results indicated that the sodium-dependent mechanism was also dependent on small amounts of potassium ions, a finding which is at variance with the results obtained in this work[2,3]. It is quite possible that the microsomal preparation contained enough potassium to mask any existing effects of this ion, and the discrepancy between the findings of Skou[6] and those of this work[3] may therefore not be important. Quite recently, Post *et al.*[7] reported on a sodium-and-potassium dependent adenosine triphosphatase in erythrocyte membrane fragments which very much resembles the enzyme from crab nerves[6] and from brain microsomes[3]. Hanzon and Toschi[8] showed that the microsomes obtained in the fractionation of brain homogenates were derived from membranous structures in the cytoplasm, the endoplasmic reticulum or the α-cytomembranes. These are probably continuous with the outer cell membrane, and probably structurally and functionally identical with it. In addition, Toschi found[9] that the acetylcholinesterase was concentrated in the microsomal fraction, thus suggesting an important functional role for this fraction in the impulse-cycle. On different lines, Hokin and Hokin[10] have postulated a mechanism for sodium transport across cellular membranes. Their views are based on the mechanism for the incorporation of phosphate into phosphatidic acids of brain microsomes. The phosphatidic acids would function as sodium carriers in the internal lipide phase of the cell membrane. The overall process results in a hydrolysis of adenosine triphosphate.

Table 2. INHIBITION OF ACTIVITY OF ADENOSINE TRIPHOSPHATASE BY PYRIDINE ALDOXIME DODECYLIODIDE[4]

Exp. No.	Additions	μmoles P_i liberated			
		in absence of Na^{+}	Act. (+), inh. (−)	in presence of 0·1 *M* Na^{+}	Act. (+), inh. (−)
1	None	0·75	–	0·93	+ 0·18
	PAD, 10^{-4} *M*	0·47	– 0·28	0·47	– 0·46
	PAD, 10^{-4} *M* and Eserine 10^{-4} *M*	0·49	– 0·26	0·49	– 0·44
	Eserine 10^{-4} *M*	0·81	+ 0·06	0·94	+ 0·01
2	None	0·72	–	0·85	+ 0·13
	PAD, 3×10^{-5} *M*	0·71	– 0·01	0·72	– 0·13

The assay conditions were as in Table 1.

Note added in proof. More recent experiments have shown that the stimulation of the microsomal ATPase by sodium ions can be considerably increased by the presence of small amounts of potassium. Nevertheless, potassium alone has no effect. These findings abolish the main discrepancy between Skou's work[6] and mine.

Inhibition of the Adenosine Triphosphatase

The behaviour of the sodium-dependent microsomal adenosine triphosphatase was also studied in the presence of some substances which are known to cause a depolarization of the cell membrane, and which are accordingly of interest in connexion with the problem of active sodium transport and impulse formation. Acetylcholine showed no effect on the adenosine triphosphatase activity, either alone or in the presence of eserine, which would inhibit the acetylcholinesterase. On the other hand, the quaternary nitrogen compound pyridine aldoxime dodecyliodide (PAD) strongly inhibited the activity of adenosine triphosphatase (Table 2). The inhibition showed different characteristics in the absence and in the presence of sodium. If a suitable concentration of about $3 \times 10^{-5} M$ was chosen, only the sodium-stimulated adenosine triphosphatase was inhibited, and the remaining activity still exceeded that obtained in the absence of sodium; the unstimulated activity was not affected at all. If the concentration of PAD was increased, the inhibition also became noticeable in the sodium-free experiments. In each case where the inhibitor concentration was sufficiently high to cause inhibition in the sodium-free experiments, the remaining activity was the same, irrespective of the presence or absence of sodium. Such behaviour suggests that the reaction is sub-maximal in absence of sodium and that PAD specifically inhibits an enzymatic reaction which is involved in the splitting of ATP. As will be seen later, another type of inhibition of the adenosine triphosphatase can also be obtained.

Dettbarn *et al.*[11,12] have shown that PAD depolarizes the nerve cell membrane. They assume that the depolarizing action of PAD is due to the fact that this substance is analogous to acetylcholine in structure. It has indeed been shown[13] that its action on acetylcholinesterase can be explained on the basis of this molecular analogy. The foregoing experiments show that PAD inhibits the microsomal adenosine triphosphatase in a specific manner, suggesting that PAD directly interferes with the mechanism of reaction. According to the views of Nachmansohn *et al.*[14], this would mean that acetylcholine should also cause a similar inhibition. The fact that this is not so is perhaps not too serious an obstacle, if the lipide insolubility of acetylcholine is taken into account. A more serious obstacle, however, is the fact that eserine does not block the inhibition by PAD. In Dettbarn's experiments[12], eserine blocked the effect of PAD, and this effect is one of the main points in his argument for an analogy between PAD and acetylcholine.

Grundfest[15] has suggested that the inhibition of the mechanism of sodium transport is a cause of the action potential. However, such a mechanism does not seem to be sufficient to explain the great speed of the inward sodium flow during the action potential. It has been pointed out by Katz[16] and by Hodgkin and Keynes[1], that a mere inhibition of the active transport mechanism would not provide a quantitatively satisfactory basis for this phenomenon. However, as the experiments described here suggest that the active mechanism of sodium extrusion from the cell is inhibited by acetylcholine analogues, the role of this substance in the formation of the action potential in a manner closely related to that suggested by Grundfest cannot be ruled out completely.

Non-Specific Inhibitions

In addition to PAD, a number of other substances cause a decrease in the rate of hydrolysis of adenosine triphosphate by brain microsomes. Detergents, alcohols, and the anæsthetics ether and chloroform are such substances. The type of inhibition acquired with these compounds is quite different from that obtained by PAD. While the behaviour of the adenosine triphosphatase in the presence of PAD suggested a specific mode of action for this compound, no such specificity seems to be involved in the case of the other substances. Thus the same concentration of an inhibitor, for example, deoxycholate, causes about the same amount of inhibition, irrespective of whether sodium is present or not. The remaining activity is thus always, within certain limits of inhibitor concentration, higher in the presence of sodium than in its absence. It is of particular interest that dodecylsulphate, a detergent with the same lipophilic alkyl group as PAD, also causes this type of non-specific inhibition (Table 3). The role of the dodecyl group as the inhibitory principle in PAD can thus be ruled out, and the quaternary nitrogen assumes a dominant position as the active grouping in this compound. The analogy between PAD and acetylcholine is thus strengthened, and support is given to the possibility of an inhibition of the sodium transport mechanism by acetylcholine.

Table 3. INHIBITION OF ADENOSINE TRIPHOSPHATASE BY SODIUM DODECYL-SULPHATE (DS)[4]

	μmoles P_i liberated			
Additions	in absence of Na^+	Act. (+), inh. (−)	in presence of 1·0 M Na^+	Act. (+), inh. (−)
None	0·75	–	0·93	+ 0·18
DS 10^{-4} M	0·59	− 0·16	0·72	− 0·21

The conditions were as described in Table 1.

Table 4. THE EFFECT OF ETHYL ETHER AND CHLOROFORM ON THE MICROSOMAL ADENOSINE TRIPHOSPHATASE

	μmoles P_i liberated			
Additions	in absence of Na^+	Act. (+), inh. (−)	in presence of 0·1 M Na^+	Act. (+), inh. (−)
None	0·73	–	0·81	+ 0·08
Ether 0·02 M	0·68	− 0·05	0·75	− 0·06
Ether 0·04 M	0·66	− 0·07	0·73	− 0·08
Chloroform 0·01 M	0·62	− 0·11	0·70	− 0·11

The conditions were as described in Table 1.

It is interesting to note that in addition to the above-mentioned detergents, compounds such as alcohols, ether and chloroform cause a non-specific inhibition of the microsomal adenosine triphosphatase (Table 4). The concentrations of these compounds used in the experiments are approximately such as would occur during heavy alcohol intoxication or during anæsthesia. The effects observed with these substances offer a new and interesting possibility of explaining their mechanism of action on a biochemical basis. First, it is known that ethanol causes a decrease in the resting membrane potential of the nerve cell[17]. Secondly, the rate of adenosine triphosphate hydrolysis is diminished by ethanol[4]. This leads to a smaller quantity of adenosine diphosphate being formed, and thus, through the well-known mechanism of respiratory control in mitochondria, to a decrease in the rate of oxygen consumption. This is in very close agreement with findings on the effect of ethanol on the oxygen consumption of the whole brain or brain slices[18]. The biochemical mechanism of the action of alcohol and of anæsthetics thus seems now to be within the reach of studies on a subcellular level.

Mechanism of the Non-Specific Inhibition

It was explained above that the inhibition by PAD of the sodium-dependent adenosine triphosphatase seems to be due to a specific inhibition of the adenosine triphosphatase reaction. The inhibition caused by the other substances mentioned above is of another type, and could not be interpreted as an inhibition in the classical manner. The mechanism of this inhibition therefore remains to be explained. All the substances tested are lipophilic in character, and would have a considerable affinity for the lipide structures forming the major part of the microsomal mass. A certain structural disorganization undoubtedly follows when these structures are exposed to detergents. In mitochondria, such phenomena have been observed and subjected to thorough study. In the present case, it is not possible to delineate the exact mechanism of the inhibition. More extensive data are needed for a complete explanation. One possibility could be that the substances mentioned bring about a disturbance in the spatial arrangement of the enzymes involved, and thus a decreased reaction-rate.

Conclusion

The study of phenomena related to the active, energy-consuming transport of molecules across cellular membranes has generally been possible only with preparations consisting essentially of intact cells. Such a situation is not ideal for a detailed biochemical analysis of the phenomenon. A number of extremely important cell functions are intimately connected with the active transport of various substances across the cell membrane. The secretory mechanisms of glands, the re-absorption of a great number of substances in kidney tubuli, and the electrical activity of nerves and other conducting tissues may be mentioned. From the biochemical point of view all these phenomena are almost entirely unexplained. The possibility of studying phenomena related to the active transport of sodium ions through the nerve cell membrane by the use of a suspension of isolated microsomes from the brain has been indicated above. Such a technique may prove useful in the study of the mechanisms involved in these processes.

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CODING RATIO FOR THE RIBONUCLEIC ACID VIRUSES

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IT is a generally held view that nucleic acids carry the information necessary to specify the primary structure of proteins. As there are only four kinds of nucleotides in any one kind of nucleic acid and 18–20 kinds of amino-acids in protein, it is reasonable that at least a triplet of nucleotides is required to specify a particular amino-acid[1]. Several hypotheses have been advanced to account for the coding of protein primary structure in terms of nucleotide triplets[2,3]. A nucleotide triplet is presumed to be composed of adjacent nucleotides along a single polynucleotide chain (at least in the case of ribonucleic acid (RNA)).

Yčas, however, has recently advanced the interesting hypothesis that, in the case of virus RNA, not a triplet of nucleotides along the RNA chain but rather a single nucleotide corresponds to any given amino-acid. This hypothesis necessitates that the 18–20 amino-acids fall into four groups, one group for each kind of nucleotide[4]. Further, in order to distinguish among amino-acids within any one group, it is necessary to invoke a second source of information not contained in the RNA of the virus (at least in the above sense of coding). This hypothesis, if true, should presumably hold for more than RNA viruses. We might expect to find a coding ratio, n (defined as the number of nucleotides specifying an amino-acid) of unity for most RNA molecules. It is the purpose of the present article to discuss Yčas's novel and important suggestion.

First, let us briefly outline Yčas's approach in arriving at the conclusion that n is unity. This approach is to compare both the protein and nucleic acid composition of six well-characterized RNA viruses, southern bean mosaic (SBM), tomato bushy stunt (TBS), cucumber (CV), tobacco mosaic (TMV), turnip yellow (TY) and polio (PV). To do this the quantity $1/2\Sigma|D_a|$ is defined as "one-half the sum of the absolute values of the differences between the quantities, expressed as moles per cent, of corresponding amino-acids in two proteins". An analogous expression, $1/2\Sigma|D_n|$, is defined for the two corresponding virus RNA's. The ratio of these two, $1/2\Sigma|D_n| \;/\; 1/2\Sigma|D_a|$, is designated r. This and the coding radio, n, are considered for the case of 'non-overlapping' codes only. The author then suggests that in general $r \leqslant 1/n$. However, this conclusion rests on several assumptions, mentioned below.

By comparing the composition of each of the RNA viruses in turn to that of turnip yellow (which has the most extreme compositional differences of all six viruses), Yčas finds the ratio r to be 0·90, SBM; 0·75, TBS; 0·66, CV; 0·83, TMV; and 1·09, PV. Two of these figures, at least, are indistinguishable from unity, and all are greater than 0·6. These high values of r are taken to mean, then, that the coding radio, n, cannot be greater than unity itself, as $r \leqslant 1/n$. Yčas shows it is possible to assign the individual amino-acids to four groups such that for all viruses the sum of the mole fractions of the amino-acids in any group is equal to the mole fraction of a particular nucleotide in the corresponding RNA, in keeping with a coding ratio $n = 1$. The nucleotide groupings are these: adenylate – glutamic acid (glutamine), glycine, leucine, phenylalanine and tryptophan; cytidylate – cysteine, lysine, methionine, proline and threonine; guanylate – alanine, arginine, tyrosine and valine; uridylate – aspartic acid (asparagine), *iso*leucine, histidine and serine. Unknown, X, factors not carried on the RNA distinguish among members of the same group.

Assuming a coding ratio $n = 1$, let us see what effect transformation of one nucleotide into another, or one group of nucleotides into another, has on the ratio r. For this purpose define the nucleotides as

A, C, G and U, and the corresponding amino-acids as $a_1, a_2, \ldots, c_1, c_2, \ldots, \ldots$ There are in all twelve possible kinds of single transformations among nucleotides, when transforming (formally) the composition of one virus into that of another. These are symbolized by the diagram:

$$\begin{tikzcd}
& U \arrow[dl, shift left] \arrow[dr, shift left] \arrow[dd, shift left] & \\
A \arrow[ur, shift left] \arrow[rr, shift left] \arrow[dr, shift left] & & C \arrow[ul, shift left] \arrow[ll, shift left] \arrow[dl, shift left] \\
& G \arrow[ul, shift left] \arrow[ur, shift left] \arrow[uu, shift left] &
\end{tikzcd}$$

All these taken singly have an r value of unity. However, we can distinguish several types of pairs of nucleotide transformations, some of the overall r value of which is less than unity. For example,

$$\begin{matrix} A \to C \\ a_1 \to c_1 \end{matrix} \text{ and } \begin{matrix} C \to A \\ c_1 \to a_1 \end{matrix} \tag{1}$$

this cannot be distinguished from no transformation at all, so all such pairs are eliminated from the diagram as contributing nothing:

$$\begin{matrix} A \to C \\ a_1 \to c_1 \end{matrix} \text{ and } \begin{matrix} C \to A \\ c_2 \to a_2 \end{matrix} \tag{2}$$

this has an overall value $r = 0$;

$$\begin{matrix} A \to G \\ a_1 \to g_1 \end{matrix} \text{ and } \begin{matrix} G \to C \\ g_1 \to c_1 \end{matrix} \tag{3}$$

this is identical with $\begin{matrix} A \to C \\ a_1 \to c_1 \end{matrix}$, and $r = 1$;

$$\begin{matrix} A \to G \\ a_1 \to g_1 \end{matrix} \text{ and } \begin{matrix} G \to C \\ g_2 \to c_2 \end{matrix} \tag{4}$$

this has an overall value $r = 0{\cdot}5$.

Therefore, we see that if we are to maintain the overall r value of unity, we have to eliminate all nucleotide transformations (or pairs, etc., of transformations) which have $r < 1$ associated. Consequently, for a case, say, where two viruses, X and Y, differ in RNA composition by $C_x > C_y$ and A_x, G_x, $U_x < A_y$, G_y, U_y respectively (as is the case for TY and PV), the above diagram for possible nucleotide transformation would be restricted to $\begin{tikzcd} U \arrow[dr] & \\ A \arrow[r] & C \\ G \arrow[ur] & \end{tikzcd}$ in order that r be unity.

In addition to these considerations we must determine what effect transformations in the unknown, X, factors have on the ratio r. If such a transformation occurred, it could cause the following compositional change: $\begin{matrix} A(X) \to A(X') \\ a_1 \to a_2 \end{matrix}$, with an associated r value of 0. However, when X factor transformations can be coupled (formally) to nucleotide transformations as in the following example, the resulting r value for the two taken together is unity.

$$\begin{matrix} A(X) \to A(X') \\ a_1 \to a_2 \end{matrix} \text{ and } \begin{matrix} A \to C \\ a_2 \to c_2 \end{matrix} \text{ or } \begin{matrix} G \to A \\ g_1 \to a_1 \end{matrix}$$

The entire argument above, while it does not militate against the hypothesis of Yčas, does point out that there is no *a priori* reason to suspect that, given a coding ratio $n = 1$, the ratio r should be also unity. In fact, one would *a priori* suspect that r would be less than unity. All these considerations make me feel that the hypothesis of a coding ratio $n = 1$ is somewhat artificial, and that the experimental fact $r \infty 1$ is best explained on some other basis.

Table 1. AMINO-ACID AND NUCLEOTIDE COMPOSITION OF RNA VIRUSES

The data on composition of the viruses is that compiled by Yčas (ref. 4); amino-acid abbreviations are those of the same reference

Amino-acid	TMV	TBS	SBM	TYV	CV	PV	Nucleotide triplet*	
ala	9·2	8·5	7·7	7·4	9·0	7·8	AGU	ala
arg	6·7	5·3	6·4	1·6	7·0	4·7	AGG	arg
asp	11·6	11·1	7·3	5·1	12·9	11·9	AGU	asp
cys	0·6	0·8	0·9	2·5	0·0	0·8	CCU	cys
glu	10·4	5·7	6·8	7·1	5·8	7·7	AUU	glu
gly	3·7	8·6	9·0	4·3	2·6	6·8	AGG	gly
his	0·0	1·2	1·3	1·4	0·0	2·4	CUU	his
ilu	5·5	3·3	6·0	8·2	4·6	4·8	ACU	ilu
leu	7·9	10·9	8·5	8·5	9·4	8·5	CGU	leu
lys	1·2	3·4	3·0	6·4	2·1	4·7	CCG	lys
met	0·0	0·8	2·6	2·0	0·0	1·5	CUU	met
phe	4·8	3·6	8·4	2·7	7·8	4·4	GUU	phe
pro	4·8	3·9	4·7	11·6	6·5	7·2	CCC	pro
ser	11·0	8·6	9·0	8·3	11·7	7·0	AAG	ser
thr	10·4	11·0	12·4	13·6	7·7	9·1	ACC	thr
try	1·2	0·5	—	0·7	0·3	—	CUU	try
tyr	2·4	2·8	4·3	1·5	2·7	3·9	UUU	tyr
val	8·5	10·0	6·8	7·4	10·0	7·2	ACG	val

Nucleotide		TMV	TBS	SBM	TYV	CV	PV
Triplet		30	27	27	24	28	25
Exp.	*A*	29	28	26	23	26	29
Yčas		28	29	28	23	26	27
Triplet		21	22	24	36	21	25
Exp.	*C*	19	21	23	38	19	22
Yčas		17	20	24	36	16	23
Triplet		25	28	26	19	27	25
Exp.	*G*	26	28	26	17	26	24
Yčas		27	27	25	18	29	24
Triplet		25	22	24	21	24	24
Exp.	*U*	27	25	25	22	30	25
Yčas		28	24	24	23	29	26

* Without regard to order within a triplet.

For this reason I have re-investigated Yčas's assertion that coding ratios greater than unity are necessarily ruled out. His proof that $r \leqslant 1/n$ is contingent on the condition that many different kinds of amino-acids contribute to $\Sigma|D_a|$. Also, the exact nature of the code will affect r somewhat. However, in order to disprove the contention that n must be unity for the RNA viruses, it is merely necessary to show that some code for which $n > 1$ is compatible with the published data. That such is the case is shown in Table 1. This triplet code was devised by the inelegant process of trial and error. There is no reason for believing that it is unique, or that one which fits the data better might not be devised. Although the agreement with experiment is not so good as with the singlet code, the code does accomplish the one end for which it was designed: to show that the composition of the six viruses is not incompatible with at least one triplet code. This relieves us of the necessity of having to assume a coding ratio of $n = 1$ and the concomitant postulate of other unidentified carriers of information not residing within the virus nucleic acid.

It will be noticed that the present code is neither a commaless code (that is, it contains UUU and CCC) nor is it a code according to Gamow's formulation (that is, order within the triplet is important). The present triplet code is examined in more detail elsewhere.

I wish to thank Dr. Martynas Yčas for stimulating my interest and for discussing his hypothesis with me.

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A SEQUENTIAL GROWTH RESPONSE TO GIBBERELLIC ACID, KINETIN AND INDOLYL-3-ACETIC ACID IN THE WHEAT COLEOPTILE (*TRITICUM VULGARE* L.)

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IN earlier work[1] the two fundamental growth processes of cell division and cell expansion in relation to the growth and differentiation of the wheat coleoptile were investigated. This was done by estimating the cell number, using the hæmocytometer method of Brown and Rickless[2], and cell volume by dividing the fresh weight of the coleoptile by the respective cell number, making the usual assumption that cell volume is equivalent to the fresh weight of the cell. In these experiments the wheat (var. Eclipse) was grown at 23° C. in darkness. Whole coleoptiles were excised from young seedlings and the enclosed primary leaves teased out under a dissecting microscope. The results are presented in Table 1.

Table 1

Age of coleoptiles (hr.)	Fresh weight per coleoptile (mgm.) (± 2 *s.e.*)	Cell No. per coleoptile × 10^{-3} (± 2 *s.e.*)	Average cell volume × 10^{9} (cm.^{3}) (± 2 *s.e.*)
18	0·515 ± 0·019	40·15 ± 4·04	1·29 ± 0·086
24	0·625 ± 0·028	39·80 ± 3·55	1·55 ± 0·156
30	0·902 ± 0·049	40·35 ± 1·48	2·24 ± 0·11
36	1·57 ± 0·088	58·05 ± 5·64	2·73 ± 0·25
42	3·14 ± 0·25	80·3 ± 4·03	3·93 ± 0·50
48	4·40 ± 0·22	104·0 ± 7·38	4·32 ± 0·32
54	6·93 ± 0·63	118·0 ± 8·66	5·93 ± 0·98
60	10·5 ± 0·18	131·0 ± 3·54	8·01 ± 0·11
66	15·4 ± 0·23	145·0 ± 16·90	10·60 ± 1·16
72	23·5 ± 0·69	149·0 ± 6·76	14·63 ± 1·01
78	—	148·0 ± 12·30	—
84	37·1 ± 1·8		24·9 ± 1·20
96	58·2 ± 3·4		39·1 ± 2·26
108	66·6 ± 1·7		44·7 ± 1·12
120	77·3 ± 8·4		51·9 ± 5·74
144	75·9 ± 10·6		50·9 ± 7·20

From these results the rate of increase in cell volume, cell number and coleoptile fresh weight over 6-hr. periods was calculated and the histograms are depicted in Fig. 1 *a–c*. The histogram representing the average rate of increase in cell volume (Fig. 1*a*) is rather misleading since the method of estimation of cell volume incorporates the negative growth-rate of cells in the process of division (that is, the volume of a cell is halved approximately when it divides), and therefore tends to under-estimate the growth-rate when cell division is taking place. A much better estimate of the average rate of increase in cell volume is given by the histogram of fresh weight for the whole coleoptile, ignoring cell number altogether. Reference to this histogram (Fig. 1c) shows that cell expansion in the coleoptile can be divided into two phases: an initial phase in which the rate of cell expansion increases rapidly and then falls abruptly, and a second slower, almost linear, phase of cell expansion which continues for 30 hr. before declining. A very similar histogram showing these two phases of cell expansion has also been obtained for another variety of wheat (King II).

Since it has been shown that gibberellic acid and kinetin elicit greater elongation in young tissues than in the more mature[3], it was postulated that the early rapid growth-phase in the coleoptile might be regulated by endogenous gibberellin or kinin-like substances, whereas the second, slower, linear phase of cell expansion might be invoked by endogenous auxins.

As a means of testing this hypothesis, the reaction of coleoptiles of different ages to exogenous solutions of gibberellic acid, kinetin and indolyl-3-acetic acid was investigated. This experiment was carried out in two stages. First, dosage-response curves were determined for a series of coleoptile ages for each growth regulator (Fig. 2). The wheat was grown under the conditions already described here. Four replicates of five coleoptiles (with their enclosed primary leaves) were used for each treatment. Each batch of five coleoptiles was placed in a test-tube (3 in. × ½ in.) containing 0·6 ml. of the solution to be tested, and after sealing with 'Parafilm' the tubes were transferred to a klinostat for 24 hr. at 26° C. in red light. The coleoptiles were measured before and after treatment.

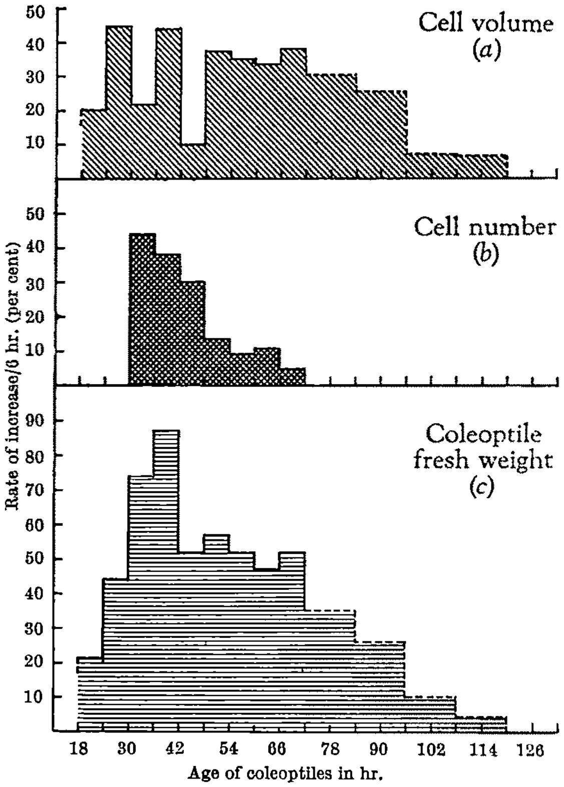

Fig. 1. Histograms illustrating the percentage rates of increase during 6-hr. periods of (*a*) the average cell volume, (*b*) cell number, and (*c*) the coleoptile fresh weight, during the growth and differentiation of the wheat coleoptile

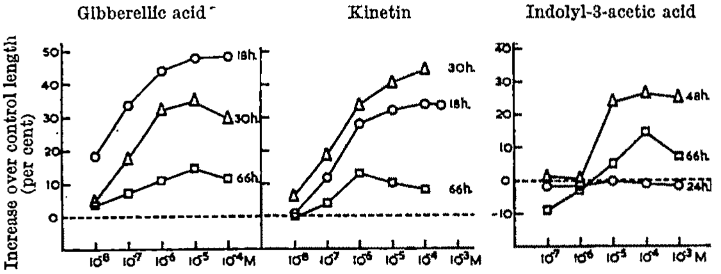

Fig. 2. Dosage-response curves for gibberellic acid, kinetin and indolyl-3-acetic acid given by coleoptiles of different ages (*h* = hours from sowing)

By referring to the dosage-response curves, it was possible to note the concentrations giving the optimal growth-response over a range of coleoptile ages for each growth regulator (Fig. 2). Thus a 10^{-5} *M* solution of gibberellic acid invoked an optimal response for coleoptiles at 18, 30 and 66 hr. after sowing and was therefore chosen to represent gibberellic acid in the final stage of the experiment. Similarly, a 10^{-4} *M* solution was selected for both kinetin and indolyl-3-acetic acid. (There was no significant difference between the growth response of 66-hr. coleoptiles to 10^{-4} *M* and 10^{-6} *M* concentrations of kinetin.)

By using only one concentration for each growth regulator, it was possible to determine the growth response of coleoptiles at 18, 30, 42, 54, 66 and 78 hr. after sowing in one large-scale experiment and thereby eliminate any inter-experimental variation in coleoptile sensitivity. These results are shown in Fig. 3 with twice *S.E.* limits. The wheat coleoptile is most sensitive to exogenously supplied gibberellic acid at or before 18 hr. after sowing, and during growth and development the response declines until at 78 hr. it is only 2 per cent above controls. Kinetin, on the other hand, gives a maximal growth stimulation at 30 hr. after sowing. Lastly, coleoptiles do not give a positive response to indolyl-3-acetic acid until 30–42 hr. after sowing and are most sensitive at about 54 hr. Thus under these experimental conditions it was established that there is a sequential growth response of the coleoptile to the three growth regulators, first to gibberellic acid, then to kinetin and finally to indolyl-3-acetic acid. This result has been obtained on three separate occasions.

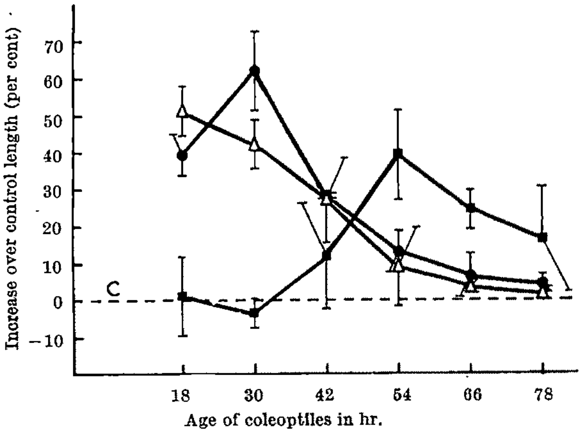

Fig. 3. Sequential growth response of the wheat coleoptile to exogenous solutions of gibberellic acid, kinetin and indolyl-3-acetic acid. The vertical lines indicate the 2 *S.E.* limits. △, Gibberellic acid, 10^{-5} *M*; ●, kinetin, 10^{-4} *M*; ■, indolyl-3-acetic acid, 10^{-4} *M*

The greatest sensitivity to gibberellic acid can be correlated with the period of cell expansion that precedes division (compare Fig. 1 *b* and *c* with Fig. 3), whereas kinetin elicits the greatest response from coleoptiles in which many of the cells are about to divide (compare Fig. 1*b* with Fig. 3). These results suggest that these two growth regulators have separate roles to take in the growth process. Nevertheless, their concerted efforts could account for the initial rapid cell expansion phase as postulated in the hypothesis above. This dual-action concept was supported by: (1) an examination of stained sections of 18-hr. coleoptiles after treatment with gibberellic acid and with kinetin for 24 hr., which showed that the growth response for both substances was due to cell expansion only; and (2) by a correlation between the rapidly falling growth-rate occurring at 42–48 hr. with a corresponding fall in sensitivity of the coleoptile to gibberellic acid, and kinetin (compare Fig. 1c with Fig. 3). Although indolyl-3-acetic acid did not induce cell expansion in very young coleoptiles, this does not mean that it is not required for other physiological functions and, indeed, tissue culture studies suggest that both it and kinetin are necessary for cell division[4,5].

One cannot be certain from these results that the natural growth of the coleoptile is controlled in this way by endogenous growth regulators, but evidence is accumulating in the literature for the presence of gibberellin[6,7] or kinin-like substances[5] in young tissues, and the occurrence of indolyl-3-acetic acid in oat coleoptiles (20–30 mm. long) has been claimed by several workers[8,9].

Perhaps the most plausible explanation of the sequential growth-response is that there is an optimal concentration ratio of these three types of growth regulators (and possibly others) for each stage of cell growth. One might postulate that, in a predetermined order, one concentration ratio induces the early type of cell expansion, another invokes cell division and another the final cell expansion phase. Such a coding system might operate by its action on enzyme activation or synthesis at each stage of growth[10,11]. Thus it is conceivable that cellular growth and differentiation proceed as the result of growth regulators, individually[11], or in certain concentration ratios[4,10] leading to the activation of a particular enzyme spectrum. These enzymes, by their action on their specific substrates, could in turn bring about a gradual change in the concentration pattern of the growth regulators in the cell and thereby initiate the activation of a different enzyme spectrum and so on, until differentiation is complete.

I thank Prof. R. L. Wain for his interest and encouragement in this work. The results will be reported in detail elsewhere.

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EFFECTS OF QUALITY OF LIGHT ON DEVELOPMENT OF ROOTS ON DWARF BEAN HYPOCOTYLS IN PRESENCE AND ABSENCE OF BORON

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SINCE Warington[1] discovered that boron is an element essential for growth of *Vicia faba* and demonstrated[2] that meristems cannot develop normally without it, many theories about its role have been advanced but none is entirely satisfactory. The latest theories are by Gauch and Dugger[3], who consider that boron plays a part in the transport of carbohydrate, and by Winfield[4] and Whittington[5], who postulate that it is concerned in the formation of pectic substances in the cell wall. The results described here suggest that boron metabolism is influenced by the quality of light received by the plant. Hemberg[6] found that cut plants of *Phaseolus vulgaris* with two developed leaves formed roots on their hypocotyls only when the solution bathing them contained boron. This was afterwards confirmed by Gorter[7], and Weiser[8] obtained similar results with *Clematis* cuttings. My work followed a chance observation that the extent to which roots developed on hypocotyls of cut bean plants without boron depended on whether the light supplied was from fluorescent tubes only or these tubes plus incandescent lamps.

Plants of dwarf bean (*Phaseolus vulgaris*; var. Canadian Wonder) were grown from surface-sterilized, weight-graded seed sown in John Innes compost; the plants were in cabinets at a constant ambient temperature of 20° C. and light was provided either by fluorescent tubes alone (3,500° K., white, reflector) or these were supplemented with incandescent tubes ('architectural' strip lights), when the ratio of watts consumed by the fluorescent to incandescent light was 8 : 3. Humidity was not controlled, and the plants were given a daily photoperiod of 8 or 16 hr. The light intensity at the leaf surface was 1,400 ft.-candles.

When the first pair of leaves was fully expanded and the next leaf just beginning to unfold, the hypocotyls were severed 4 cm. below the cotyledonary node, sterilized by agitation for a few seconds in hydrogen peroxide ('10 vol.'), washed in distilled water and then placed in 100 ml. conical flasks covered with black paper. The flasks contained 100 ml. of half-strength Hoagland solution + 1 mgm./l. iron as tartrate, made up in glass-distilled water further purified by passage through a mixed anion and cation exchange resin and which was stored in polythene containers. The solution in half the flasks also included 10 μgm. of boron as boric acid, because preliminary experiments showed 0·1 mgm./l. to be optimal for stimulating root growth. The flasks were of boro-silicate glass and may have supplied some boron to the plants, but, if so, they had little effect compared to added boron. The cut plants with no additional boron are referred to as the low-boron and the others as high-boron plants. While the plants were growing in the compost, before the hypocotyls were cut, they showed no signs of boron deficiency, but when the hypocotyls were severed and placed in solutions without added boron the plants quickly showed symptoms of boron deficiency.

After severing the hypocotyls, half the plants previously grown in fluorescent light were given incandescent light in addition, while the other half was maintained in fluorescent light alone. Similarly, half the plants previously given both fluorescent and incandescent light were maintained in this condition and the other half transferred to fluorescent light alone. Thus, there were four light treatments, and in each the same photoperiod was maintained both before and after severing the hypocotyl. The cut hypocotyls were placed in the different culture solutions topped up daily with the purified distilled water. After 14 days, the roots emerging from the hypocotyls were counted as well as the number of 'root initials'—the number of protrusions at the surface of the hypocotyl which failed to develop further. The total fresh weight of roots of each plant was also determined as well as the dry weight of the 4-cm. length of hypocotyl. The number of roots formed is taken to indicate the number of meristems that developed, whereas fresh weight measures the growth of the meristems. The length of hypocotyl occupied by roots was found to be constant in all treatments. A total of 136 plants was used in the 16-hr. photoperiod and 160 in the 8-hr. photoperiod.

All plants received a treatment with fluorescent light (−). Treatment in which plants received incandescent light before excision is called X, and after Y; thus, a combination of $X + Y$ indicates that the plants received incandescent light throughout the experiment. There was no effect of quality of light in the 8-hr. photoperiod and the following remarks refer mainly to results from a 16-hr. photoperiod. Table 1 shows that incandescent light given before or after excision of the hypocotyl decreased root number and total root fresh weight of low-boron plants. By contrast, the light treatments did not affect high-boron plants. As many roots were formed on high-boron plants in an 8-hr. photoperiod as in a 16-hr. period, but the shorter day-length decreased the number of roots formed by low-boron plants. In addition to affecting root formation, boron alters the response to the type of light. Although estimates of root initials are subjective and those in Table 1 are only approximate, they show that root initials were few with treatments that formed roots freely, and many with others. Thus, root initials usually varied inversely with the number of roots, but roots and root initials together totalled many more with high-boron than low-boron plants. Root fresh weight varied with root number, with the additional effect

Table 1. MEAN NUMBER OF ROOTS AND ROOT INITIALS, ROOT FRESH WEIGHT AND HYPOCOTYL DRY WEIGHT OF BEAN PLANTS SUBJECT TO INCANDESCENT AND FLUORESCENT LIGHT IN LOW- AND HIGH-BORON SOLUTIONS

		8 hr. − boron			8 hr. + boron			16 hr. − boron			16 hr. + boron		
		(−)	X	Mean	(−)	X	Mean	(−)	X	Mean	(−)	X	Mean
No. of roots	(−)	^{a}4·1	8·2	^{b}6·1	^{a}50·4	41·3	^{b}45·9	^{a}30·9	11·2	^{b}21·0	^{a}49·0	50·5	^{b}49·7
	Y	6·5	1·7	4·1	44·1	43·3	43·7	17·3	8·5	12·9	48·4	44·5	46·5
	Mean	^{b}5·3	4·9	5·1	^{b}47·2	42·3	44·8	^{b}24·1	9·8	17·0	^{b}48·7	47·5	48·1
	L.S.D.	a=6·64 b=4·70			a=9·08 b=6·42			a=7·20 b=5·09			a=9·84 b=6·96		
No. of root initials	(−)	^{a}18·5	14·9	^{b}16·7	^{a}2·6	6·6	^{b}4·6	^{a}13·8	16·5	^{b}15·2	^{a}1·0	2·3	^{b}1·6
	Y	17·7	16·0	16·9	3·8	3·4	3·6	12·2	17·1	14·6	3·3	2·7	3·0
	Mean	^{b}18·1	15·4	16·8	^{b}3·2	5·0	4·1	^{b}13·0	16·8	14·9	^{b}2·2	2·5	2·3
	L.S.D.	a=3·68 b=2·61			a=2·24 b=1·58			a=4·00 b=2·80			a=2·12 b=1·51		
Fresh weight roots (mgm.)	(−)	^{a}25	43	^{b}34	^{a}239	196	^{b}217	^{a}335	99	^{b}217	^{a}350	374	^{b}362
	Y	41	11	26	232	202	217	204	89	147	313	295	304
	Mean	^{b}33	27	30	^{b}235	199	217	^{b}270	94	182	^{b}332	334	333
	L.S.D.	a=63·3 b=44·8			a=68·0 b=48·1			a=68·6 b=48·5			a=73·9 b=52·8		
Dry weight hypocotyl (mgm.)	(−)	^{a}66·4	68·7	^{b}67·6	^{a}68·9	65·4	^{b}67·1	^{a}95·1	84·5	^{b}89·8	^{a}101·9	93·9	^{b}97·9
	Y	74·3	65·1	69·7	71·1	64·3	67·7	98·7	87·6	93·2	89·1	92·3	90·7
	Mean	^{b}70·4	66·9	68·6	^{b}70·0	64·8	67·4	^{b}96·9	86·1	91·5	^{b}95·5	93·1	94·3
	L.S.D.	a=7·50 b=5·30			a=8·18 b=5·79			a=8·15 b=5·76			a=8·85 b=6·26		
Fresh weight per root (mgm.)	(−)	6·1	5·2	5·6	4·7	4·7	4·7	10·8	8·8	9·8	7·1	7·4	7·3
	Y	6·3	6·5	6·4	5·3	4·7	5·0	11·8	10·5	11·1	6·5	6·6	6·5
	Mean	6·2	5·8	6·0	5·0	4·7	4·8	11·3	9·6	10·5	6·8	7·0	6·9

(−), fluorescent light. *X*, incandescent light before excision. *Y*, incandescent light after excision.

that incandescent light after excision decreased fresh weight of high-boron plants also. Incandescent light, however, affected low-boron plants more, for without it plants had similar root fresh weights whether or not they were given boron (350 and 335 mgm.). In other words, boron deficiency restricts root growth only with incandescent light. Although boron increased the number of roots, the low-boron plants had greater fresh weight per root than high-boron plants in both photoperiods (Table 1), perhaps because the fewer meristems of low-boron plants suffered less competition for products of photosynthesis. Similarly, the primary leaves of whole bean plants grown in water culture without boron were larger than those grown with boron (boron-deficient plants had a mean midrib length of 70 mm. and those with boron 65 mm., based on a sample of 360 plants), possibly because nutrients which would normally go to the growing point are diverted to the leaves when the growing point aborts in boron-deficient conditions[9].

The growth of the hypocotyl was decreased in low-boron plants by incandescent light given before excision but not after. By contrast, incandescent light decreased hypocotyl dry weight in high-boron plants after excision. Thus, boron had little effect on root formation in plants illuminated only with fluorescent light; but with incandescent light, root formation depended on added boron.

The leaves were fully illuminated but little radiation reached the submerged hypocotyl, because covers on the flasks excluded lateral illumination and direct illumination was confined to the narrow opening of the flask partly obscured by the primary leaves. The action of light on root formation can therefore be assumed to be indirect. Incandescent light inhibited root formation only in the 16-hr. photoperiod, perhaps only because too few roots formed on low-boron plants in an 8-hr. photoperiod to show differences. Any inhibition presumably arises in the leaf and persists after the hypocotyl is severed. How inhibition by incandescent light is linked with boron deficiency is uncertain, but there are several possibilities. For example, boron may protect a root-promoting substance from destruction by incandescent light or perhaps incandescent light affects mobility of boron in the plant.

The site where the light stimulus interacts with boron depends on the mobility of boron in the cut plants. Published evidence that boron is rather immobile is confirmed in my experiments. The soil contained enough boron for growth of the plants, but as soon as the hypocotyl was severed, more was necessary for meristems to develop in both root and shoot, indicating that boron already in the plant could not serve for this. Scholz[10], in experiments using single tobacco leaves with a divided root system, found that boron was not transferred from roots supplied with boron to those without it, but accumulated in the leaf. He concluded that boron moves only in the direction from root to shoot, and Albert[11] came to a similar conclusion. When I treated leaves of boron-deficient plants with boron solution, boron was evidently translocated to the hypocotyl, because meristem development was stimulated, and in the experiments described above boron was transferred from the solution bathing the hypocotyl to the shoot, because apical meristem development continued in these plants. Evidently under these conditions boron is translocated either upwards or downwards, but perhaps this applies only to inorganic boron, and conditions in intact plants may be different. The evidence does not show where boron and the light stimulus interact.

In some respects boron resembles indoleacetic acid in stimulating root formation on hypocotyls; but indoleacetic acid and boron differ in that the former is necessary to stimulate meristem activity, whereas, as Hemberg (*loc. cit.*) points out, the latter does not affect the formation but only the growth of roots; hypocotyls grown in media deficient in boron develop wart-like elevations (called 'root initials' here), which do not develop into roots. It seems unlikely, therefore, that the present phenomenon is concerned with indoleacetic acid metabolism. My experiment suggests a connexion between radiation in the red or far-red part of the spectrum that inhibits root-growth when boron is a limiting factor, but this has yet to be investigated fully. That light and boron metabolism may be connected is suggested by other work. Symptoms of boron deficiency develop sooner and more severely in long than short days (Gauch and Dugger[3]) and Stegemann[12] concluded that the action of radiation is regulated by boron. Albert[11] suggested that some factor, other than carbohydrate, present in the top of the plant, is affected by light and may influence the response of tomato plants to boron deficiency. Skok[13] found that sunflower leaves were more radiosensitive with than without boron.

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AN ESTIMATE OF THE AROMATICITY OF THE ORGANIC MATTER OF A PODZOL SOIL

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IN our work on the chemical structure of the organic matter of the A_0 and B_h horizons of a podzol we were interested in a practical method for estimating the aromaticity of these materials. Such a method has recently been described by Mazumdar *et al.*[1] and Chakrabartty *et al.*[2] for determining the aromaticity of lignite, bituminous and higher-rank coals. Since soil organic matter resembles lignites and brown coals in many respects, we thought application of this procedure to such material might yield valuable information with regard to its aromaticity. As a check on our technique, we included a sample of lignite in this investigation because values for the aromaticity of this material are available in the literature.

According to Mazumdar *et al.*[1], heating of coal in air at 170° C. for approximately 500 hr. causes the complete oxidation of the 'disordered' (aliphatic and alicyclic) carbon while leaving the aromatic network intact. The oxidation of the 'disordered' carbon results in the formation of oxygen-containing functional groups, mainly carboxyls. Therefore, the only aliphatic carbon and hydrogen in the final oxidized product is that in carboxyl groups. Thus the total carbon and hydrogen in the final oxidized product, corrected for the carboxylic carbon and hydrogen, gives the total aromatic carbon and hydrogen present in the original material. In addition, in the case of hydrogen, it is necessary to correct for hydrogen replaced by quinone groups[2].

The soil samples and methods of extraction of organic matter were the same as those described previously[3] except that all extractions were made under nitrogen and the extracts were dried under reduced pressure at room temperature. The lignite sample originated from the Souris, Manitoba, area. Carboxyl groups were determined by reaction with calcium acetate[4], phenolic hydroxyls by reaction with barium hydroxide under nitrogen and correcting for carboxyls[5]. Carbonyls were determined by the method of Fritz *et al.*[6] and ultimate analyses by dry combustion.

1-gm. samples, spread in thin layers in aluminium dishes, were heated in an oven at 170° C. for various lengths of time up to and including 600 hr. A current of air was passed through the oven at a constant rate. Samples were withdrawn every 100 hr., weighed and analysed for functional groups and ultimate composition. Under these heating conditions, Mazumdar and Lahiri[7] reported that lignite and higher-rank coals reached constant weight after 300–400 hr. With our samples the A_0 material reached constant weight at approximately 500 hr. while the lignite and B_h preparation still continued to lose weight although the rate of loss was low (1·8 and 1·6 per cent in the 500–600 hr. period). In view of these findings we adopted a heating period of 600 hr. for all samples.

All results shown in Tables 1–4 under "after oxidation" refer to 600 hr. of heating.

Heating caused an increase in the carbon contents of the A_0 and B_h materials and a decrease in that of lignite (Table 1). All materials reached a constant carbon content between 200 and 300 hr. Most of the hydrogen was lost during the first 100 hr. This is in agreement with the findings of Chakrabartty *et al.*[8] that dehydrogenation appears to be the main reaction in the initial stages of coal oxidation. After 600 hr. of heating, 100, 93 and 87 per cent of the original nitrogen still remained in the oxidized residues of the lignite, B_h and A_0 materials respectively.

The carboxyl contents of the original lignite, A_0 and B_h materials were 2·0, 6·7 and 40·9 per cent respectively. In the case of lignite the carboxyl content increased to a constant value after about 400 hr. of oxidation. On the other hand, the carboxyl contents of the A_0 and B_h materials decreased during the initial stages of the oxidation. This was followed by a slight rise with constant values being obtained after 400 hr.

The values for carbon aromaticity (Table 2) represent aromatic carbon as a fraction of total carbon. The values in brackets represent aromatic carbon as a fraction of total carbon less carboxyl carbon. These values are shown in order to give an estimate of the aromaticity of the carbon skeleton. As shown in Table 2, the two values for lignite are identical because this material and higher-rank coals contain few or no carboxyl groups prior to oxidation in contrast to soil organic matter preparations. The value of 0·56 for the aromaticity of lignite agrees well with that reported by Chakrabartty *et al.*[2]. The hydrogen aromaticity, similar to the carbon aromaticity, was calculated on the basis of both total hydrogen and total hydrogen minus carboxyl hydrogen (Table 3). Hydrogen aromaticity of the lignite analysed by Chakrabartty *et al.*[2] was 0·29.

Infra-red spectra of the A_0 and B_h materials taken at various stages of the oxidation showed a gradual decrease and finally complete disappearance of the 2,920 and 2,850 cm.$^{-1}$ bands (aliphatic C—H). After 50 hr. of heating the infra-red spectra of the B_h material showed new bands at 1,840 and 1,775 cm.$^{-1}$ which, together with the band already present at

Table 1. Analytical Characteristics Before and After Oxidation
(all data on dry ash-free basis)

Sample	Loss in weight (per cent)	C (per cent)	H (per cent)	Atomic ratio (H/C)	N (per cent)	Functional group analysis			Functional Group 0
						O_{COOH} (per cent)	O_{OH} (phenolic) (per cent)	O_{CO} (per cent)	Total 0
A_0									
Before oxidation		56·72	5·21	1·10	2·37	4·7	6·6	1·5	0·38
After oxidation	50·2	58·79	3·81	0·78	4·17	4·0	7·3	3·0	0·45
B_h									
Before oxidation		50·92	3·34	0·79	0·74	29·1	5·3	5·0	0·90
After oxidation	40·8	56·21	2·08	0·44	1·16	23·7	10·0	8·1	1·03
Lignite									
Before oxidation		65·13	4·30	0·79	0·92	1·3	6·6	5·0	0·46
After oxidation	34·2	61·58	2·60	0·51	1·44	11·2	10·1	1·9	0·73

Table 2. Carbon Aromaticity
(based on the oxidation of 100 gm. of dry ash-free material)

Sample	Input* C (gm.)	Output* C (gm.)	C_{COOH} in oxidized residue* (gm.)	Aromatic C (gm.)	Non-aromatic C (gm.)	Aromaticity fa
A_0						
Before oxidation	56·72 (54·92)†			27·78	28·94	0·49 (0·51)†
After oxidation		29·28	1·50			
B_h						
Before oxidation	50·92 (40·00)†			24·41	26·51	0·48 (0·61)†
After oxidation		33·28	8·87			
Lignite						
Before oxidation	65·13 (64·60)†			36·32	28·81	0·56 (0·56)†
After oxidation		40·52	4·20			

* See Table 1.
† Corrected for C_{COOH} in original material.

Table 3. Hydrogen Aromaticity
(based on the oxidation of 100 gm. of dry ash-free material)

Sample	Input* H (gm.)	Output* H (gm.)	Correction for		Aromatic H (gm.)	Non-aromatic H (gm.)	$\frac{\text{H arom.}}{\text{H}}$	$\frac{\text{H non-arom.}}{\text{H}}$	Atomic ratio $\frac{\text{H arom.}}{\text{C}}$	Atomic ratio $\frac{\text{H non-arom.}}{\text{C}}$
			H_{COOH} (gm.)*	Aromatic H (gm.)* (H replaced by quinone)						
A_0	5·21 (5·06)†	1·89	− 0·13	+ 0·10	1·86	3·35 (3·20)†	0·36 (0·37)†	0·64 (0·63)†	0·39 (0·41)†	0·71 (0·70)†
B_h	3·34 (2·42)†	1·23	− 0·74	+ 0·20	0·69	2·65 (1·73)†	0·21 (0·29)†	0·79 (0·71)†	0·16 (0·21)†	0·63 (0·52)†
Lignite	4·30 (4·26)†	1·72	− 0·35	0	1·37	2·93 (2·89)†	0·32 (0·32)†	0·68 (0·68)†	0·26 (0·26)†	0·54 (0·54)†

* See Table 1.
† Corrected for H_{COOH} in original material.

Table 4. Distribution of Carbon and Hydrogen in the Original Materials

Sample	Carbon			Hydrogen		
	Aromatic (%)	In COOH groups (%)	Aliphatic and/or alicyclic (%)	Aromatic (%)	In COOH groups (%)	Aliphatic and/or alicyclic (%)
A_0	49	3	48	36	3	61
B_h	48	22	30	21	28	51
Lignite	56	1	43	32	1	67

1,200 cm.$^{-1}$, indicated the probable formation of cyclic anhydrides[9]. Also, new bands appeared at 920 cm.$^{-1}$ (OH deformation vibrations of COOH) and at 740 cm.$^{-1}$ (polycyclic aromatic structures). All these bands increased in intensity with heating. From the infra-red spectra we estimated that not more than 10 per cent of the carboxyl groups of the B_h material had formed anhydrides. On this assumption the correction for H_{COOH} (Table 3) should be 10 per cent lower resulting in a correspondingly higher hydrogen aromaticity.

A carbon and hydrogen balance is shown in Table 4. Exclusive of the carboxylic carbon and hydrogen, the A_0 material contained more aliphatic and/or alicyclic carbon and hydrogen than the B_h preparation. This would indicate that the carbon skeleton of the B_h is more aromatic than that of the A_0 material. Similar conclusions were reached from oxidative degradation studies of these two materials[10].

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LETTERS TO THE EDITORS

RADIOASTRONOMY

Secular Variation of the Flux Density of the Radio Source Cassiopeia A

Högbom and Shakeshaft[1] have recently presented evidence for a secular variation of the intensity of radio emission from the source Cassiopeia A. Their observations, made at a frequency of 81·5 Mc./s. over a period of 12 years, suggest that the flux density of Cassiopeia A relative to that of Cygnus A is decreasing at a rate of about 1 per cent per annum. This type of measurement may be sensitive to unknown systematic instrumental effects, and the importance of the result makes independent confirmation desirable.

Observations of the ratio of flux densities of Cassiopeia A and Cygnus A were begun at the National Radio Astronomy Observatory in 1959, in order to search for a possible change in intensity of Cassiopeia A. While these observations cover a period of only one year, they do, in conjunction with earlier observations at about the same frequency obtained at other observatories, provide some confirmation of the result of Högbom and Shakeshaft.

The available 1,400-Mc./s. observations of the ratio of Cassiopeia A to Cygnus A are given in Table 1. The Leyden[2], Bonn[3] and National Radio Astronomy Observatory[4] observations were made with antennae of about the same diameter, 82–85 ft., and were corrected for atmospheric extinction and receiver non-linearity. The Naval Research Laboratory observations[5] were made with a 50-ft. diameter antenna. The Leyden, Bonn and National Radio Astronomy Observatory observations all have small internal uncertainties. Systematic effects should also be small, as in each case the observational procedures were specifically selected to minimize them so far as possible. Although the Naval Research Laboratory measurements are of lower accuracy than the others they are of particular value because they are the earliest measurements of these sources at this frequency. Details of the equipments, observing techniques and reduction procedures used in the various measurements are given in the references cited.

Fig. 1 shows the 1,400-Mc./s. flux density ratios as a function of time. The solid line in Fig. 1 represents the change in Cassiopeia A/Cygnus A at 81·5 Mc./s., found by Högbom and Shakeshaft. The 1,400 Mc./s. observations suggest a secular change in reasonable agreement with that found at 81·5 Mc./s. The vertical displacement of the 1,400 Mc./s. and 81·5 Mc./s. ratios is due to the difference in spectra of the two sources.

Table 1

Date	Cas. A/Cyg. A	Frequency (Mc./s.)	Source
1954	1·8 ± 0·1	1,394	NRL
1956	1·63 ± 0·01	1,395	Leyden
1958	1·63	1,419	Bonn
1959	1·608 ± 0·003	1,400	NRAO
1960	1·592 ± 0·003	1,400	NRAO

NRAO, National Radio Astronomy Observatory.

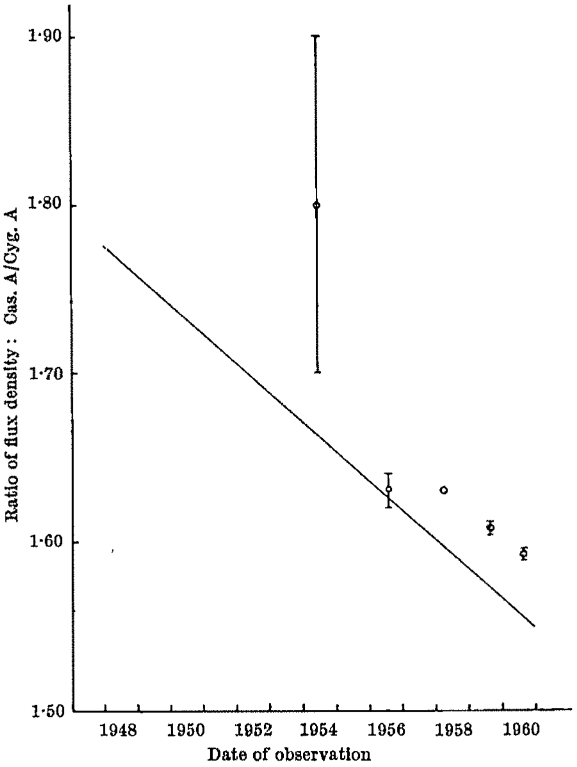

Fig. 1. Variation of the ratio of intensities of Cassiopeia A and Cygnus A as a function of time. Points with circles give measurements at a frequency of 1,400 Mc./s. Vertical lines show the published uncertainties in the measurements. No estimate of error was given for the 1958 measurement. The solid line is the variation of Cas.A/Cyg. A with time at 81·5 Mc./s., as found by Högbom and Shakeshaft (ref. 1)

The 1960 result, given in Table 1 and Fig. 1, is the mean of sixteen sets of observations made at one-week intervals in the period June 17–September 30, 1960. These observations were made with the same receiver and antenna, under as nearly identical conditions as possible. They have high relative accuracy and should be largely free of any systematic

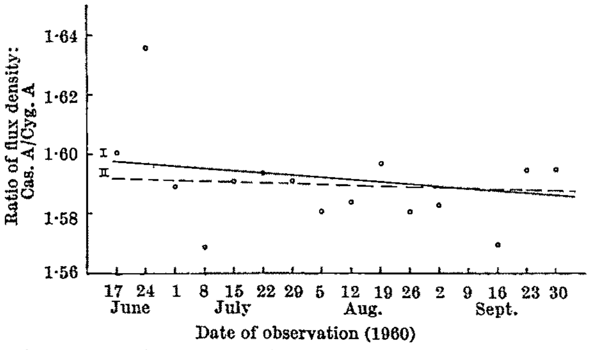

Fig. 2. Weekly measurements of Cas. A/Cyg. A at 1,400 Mc./s. The solid line (I) is the least squares straight line which best fits all the measurements. The dashed line (II) is the straight line which best fits the data if the four least accurate measurements are omitted

instrumental effects over the time-interval involved. The weekly measurements are shown in Fig. 2. Even over this short interval of time there is evidence of a systematic change in the ratio of Cassiopeia A to Cygnus A flux density. A least-squares solution for the best straight line representing all sixteen measurements gives a rate of decrease of 2·3 per cent per annum. This solution is shown by the solid line (I) in Fig. 2. A second solution was made omitting the four days on which the measurements deviated from the mean value by more than five standard errors. On two of these days (June 24 and July 8) the observer had noted on the records as they were obtained the probability of interference or marginal receiver performance. This solution, indicated by the dashed line (II) in Fig. 2, gives a rate of decrease of 0·9 per cent per annum. Both values are in good agreement with the results of Högbom and Shakeshaft.

The 1,400-Mc./s. measurements are not sufficiently accurate to determine the rate of decrease of intensity of Cassiopeia A with any reliability, so the question as to whether the rate of change is independent of frequency remains open.

We wish to thank Messrs. Högbom and Shakeshaft for a copy of their paper prior to its publication.

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ASTROPHYSICS

Solar Wind and Terrestrial Oxygen

STEFANSSON[1] pointed out that nothing gained such universal acceptance as an error. This should make us all wary of unanimity, and it has led me to question persistently the general assumption that Earth's original atmosphere contained little or no oxygen and that the origins of life are to be sought exclusively among anaerobic reactions. The atmosphere now contains 230 gm. of oxygen per sq. cm. and perhaps twice that amount must have passed through the atmosphere at an early stage of Earth's development to account for ferric iron, sulphates, etc., in sediments. These quantities are so small that slightly different assumptions lead to radically different conclusions about the amount of oxygen at various times during the past 3×10^9 years. It has, nevertheless, been difficult to account satisfactorily for the oxygen and there is opposition to all the mechanisms proposed for its formation.

Those interested in the origins of life are therefore eager for comment on the suggestion[2] that Earth is gaining 1·5 tons of hydrogen a second from the solar wind, for this rate of accretion would provide all the hydrogen in the ocean in 3×10^9 years. If this is so, the oxygen in the ocean comes into the category of 'fossil oxygen' along with ferric iron. There are 230 kgm. of it per sq. cm., that is, 500 times the amount that has been causing trouble hitherto. If we exist, and have always existed, in this hydrogen hurricane, the problem of the origin of oxygen is more intractable than we thought.

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RADIOPHYSICS

Variation in Height of Anisotropy and Random Drift Velocity of the Irregularities in the Ionosphere

HORIZONTAL ionospheric drift records taken by the spaced receiver method of Mitra[1] are used here to analyse the variation with height of anisotropy and random drift speed $(V_C)_v$ along the direction of steady drift of the irregularities.

Horizontal drift records were taken in rapid succession on several frequencies corresponding to different levels of reflexion on six days around the morning hours of 0900 I.S.T. The drift records were analysed by Phillips and Spencer's[2] correlation method to determine the steady and random drift velocities as well as the axial ratio r and orientation of the anisotropy ellipse. It is generally assumed that these values refer to the irregularities in the ionosphere. The true height of reflexion corresponding to each drift record was calculated, using Schmerling's[3] method, from an $h'(f)$ record taken at the same time.

Values of ν, the orientation of the major axis of the correlation ellipse in degrees east of north, showed no preferred direction, a feature which has also been reported earlier by Phillips and Spencer. However, on each occasion, the variation of ν with height in the ionosphere was found to be very systematic. A clockwise rotation of the major axis with increasing height is clearly evident on five of the six occasions. The gradient $\partial\nu/\partial h$ was found to vary between 54′ to 1° 42′ km.$^{-1}$ with an average of 1° 28′ km.$^{-1}$.

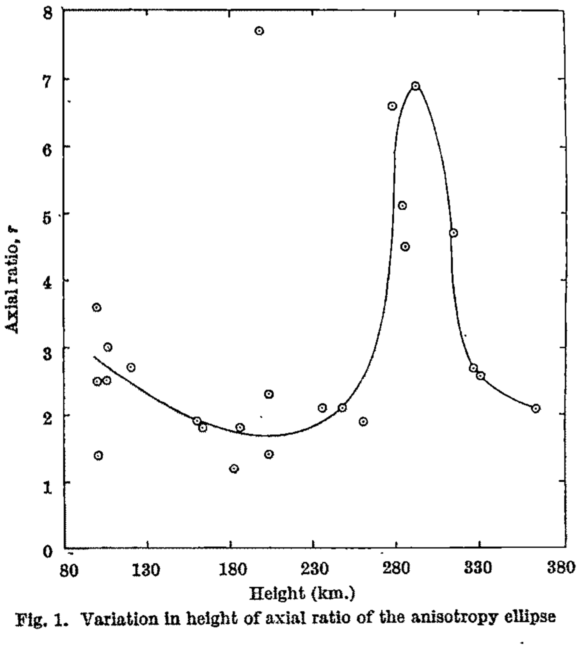

Fig. 1. Variation in height of axial ratio of the anisotropy ellipse

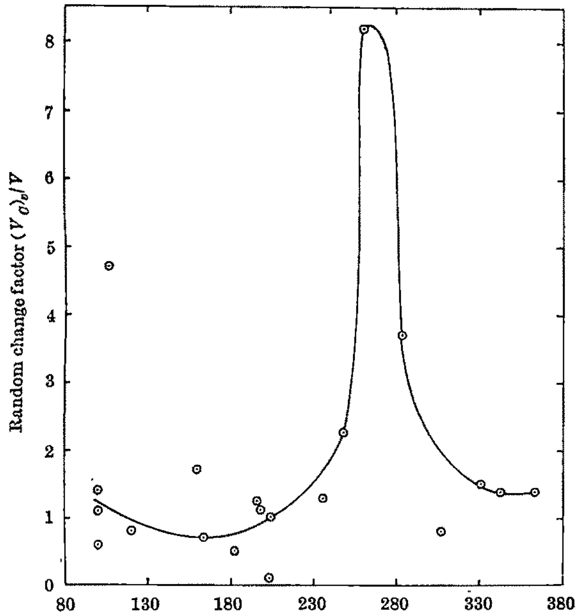

Fig. 2. Variation in height of the random change factor

The axial ratio r also showed a more or less systematic variation with height. In Fig. 1 is presented a plot of the axial ratio variation with height for all the six days. A smooth curve is drawn which passes through most of the observations. It can be seen that r decreases with height at first, slowly from about 100 km. to 190 km., then rises rapidly, reaching a maximum value of about seven at a height of about 290 km. Above this height, the value of r is found to decrease rapidly.

In Fig. 2 is presented a plot of the 'random change factor' $(V_C)_v/V$ versus height, where V is the steady drift velocity of the diffraction pattern on the ground. In this case, too, a smooth curve is drawn passing through most of the observations. This plot of $(V_C)_v/V$ is very similar to that in Fig. 1, except that the peak occurs at about 270 km. instead of at 290 km. It appears, from this work, that the ionospheric irregularities are highly elongated in regions where random motion predominates over the steady drift. It may be of interest here to point out that Yerg[4] also arrived at a similar conclusion when he stated that "large random changes are anisotropic and tend to cause the correlation pattern to be oriented along the magnetic field", although the latter trend is not clearly observed in our investigation. It is, thus, quite possible that, at this latitude, the irregularities responsible for the radio-star scintillations and spread F are located at about the level of 270–290 km. since $(V_C)_v/V$ and r are each a maximum in this region. However, it may be pointed out that the investigations were conducted by us only during daytime. Booker[5] suggested that radio-star scintillations cannot be explained in terms of turbulence if the height of irregularities causing the scintillation phenomena were taken to be located at 400 km. He concluded from certain theoretical considerations that a reasonable explanation can be offered for the scintillation phenomena if that height is about 200–300 km. The observation of large random drift velocities and also of intense spread F in the range of 200–300 km. at this station seems to lend support to this view.

A detailed paper on the investigations will be published elsewhere. We are indebted to the Council of Scientific and Industrial Research for supporting this investigation.

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[2] Phillips, G. J., and Spencer, M., *Proc. Phys. Soc.*, B, **68**, 481 (1955).
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Irregularities in the E_s Layer of the Ionosphere

It has been shown from oblique incidence backscatter experiments that the equatorial type of sporadic E ionization (E_s) contains irregularities which are elongated in a north–south direction, that is, along the direction of the Earth's magnetic field[1]. On the other hand, observations made at high latitudes have shown that the auroral type of E_s layer contains irregularities which are elongated in an east–west direction, that is, perpendicular to the field[2]. The results in the second case were based on observations of the diffraction pattern produced on the ground by a reflected wave. No corresponding observations relating to the E_s layer in medium latitudes have been reported.

In the present communication, some observations made at Cambridge (lat. 52° N., long. 0° E.) are described. The method was based on measurements of the diffraction pattern produced by a wave reflected at vertical incidence. The transmitted pulses were 100–200 μs. in length, and the frequencies used were in the range 4·0–4·5 Mc./s. during the day, and 2·0–2·4 Mc./s. at night. The reflected waves were received on three crossed-loop aerials, arranged to accept the 'ordinary' circularly polarized component of the down-coming wave, and placed at the corners of a right-angled triangle having two equal sides 130 m. long in the north–south and east–west directions. The amplitudes of the received pulses were recorded using a pen-recorder, or on film if the fading became too fast to be recorded faithfully by the pen-recorder. Correlation analysis was applied to typical portions of the records for eighteen occasions between March 1959 and August 1960. The auto- and cross-correlation functions of the fading curves were calculated using an electronic digital computer, and the graphical method of Phillips and Spencer[3] was used in subsequent calculations to determine the properties of the diffraction pattern.

The results of the analysis show great variability in the scale of the irregularities in the diffraction pattern on the ground. For only nine of the eighteen occasions was it possible to determine a characteristic ellipse representing an average single irregularity in the pattern ; on the other occasions the irregularities were either very large (1 km. approximately) or very small (80 m. approximately) and their scale could only be estimated very roughly.

While it is not possible in general to determine the size of irregularities in the ionosphere simply from measurements of amplitude in the diffraction pattern on the ground[4], the axial ratio and orientation of the characteristic ellipse of the diffraction pattern do give a measure of the elongation and orientation of the irregularities in the ionosphere. When the characteristic ellipse could be found, it was not usually very elongated; on only three of the nine occasions was the axial ratio greater than 2·0, and there was no evidence of any preferred direction of elongation. This is in contrast with the systematic elongation found at high and low latitudes.

On all the occasions when the fading of waves reflected from the E_s layer was observed at Cambridge, E_s was also found on ionograms obtained at Slough, 90 km. to the south-south-west. There was, however, no apparent association between the scale of the irregularities in the diffraction pattern at Cambridge and the type of E_s (c, f, h or l) as determined by its appearance on the Slough ionogram[5]. There was also no association between the scale of the pattern and the magnetic K-index (K was in the range of 0–4 for the occasions observed).

This work forms part of a programme of research on ionospheric irregularities supported by a grant from the Department of Scientific and Industrial Research. I was in receipt of a maintenance grant from the same Department. Thanks are due to the Director of the Mathematical Laboratory, University of Cambridge, for permission to use the computer *Edsac* 2.

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108–216 Mc./s. Radio Signals from Satellites below the Horizon

Smyth Research Associates have been conducting an experimental programme on low-angle refraction of radio waves penetrating the atmosphere.

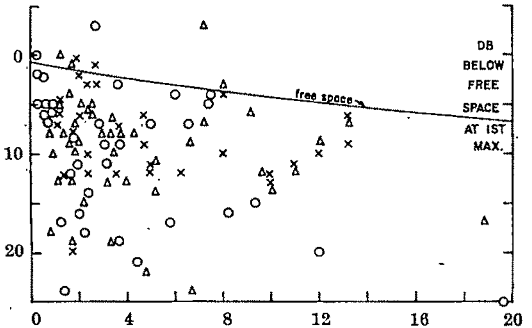

Fig. 1. Precursor signal-levels. ○, 108 Mc./s.; ×, 162 Mc./s.; △, 211 Mc./s.

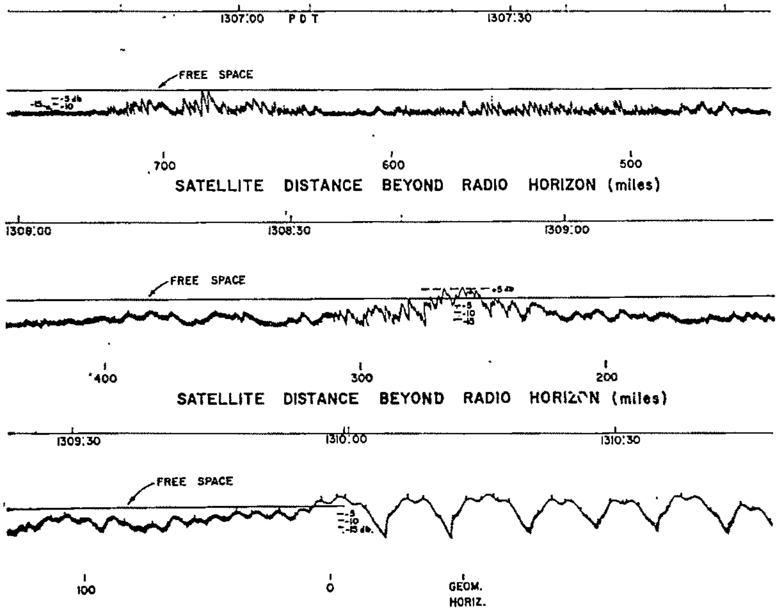

Fig. 2. Satellite precursor signals. Tiros I, 108 Mc./s. June 13, 1960

Satellite transmitters at 108, 162, and 216 Mc./s. have been used as signal sources in these studies. During the course of the measurements (June, July and August 1960) it was found that, on the majority of passes, signals were received while the satellite was well beyond the radio horizon. Out of a total of 49 cases when such 'precursor' signals were sought, only 14 failed to yield signals for at least one minute beyond the radio horizon.

In two cases, signals were first received when the satellite was 1,900–1,950 miles beyond the radio horizon point at orbit height. On these passes the satellite was roughly twice as far away as the distance to the horizon. The signal strength on these occasions was 11 db. below the free space-level on 216 Mc./s. and 18 db. below the free space-level on 108 Mc./s. As can be seen in Fig. 1, there appears to be no distinct dependence of received signal strength on frequency. Even simultaneous data on 162 and 216 Mc./s. show no consistent dependence on frequency.

The combination of low attenuation and no frequency-dependence suggests a tropospheric ducting mechanism. Correlation of local radiosonde data with signal strength and maximum path-length supports this view. The duct thickness and intensity in all cases when signals were observed exceeded the trapping requirements for frequencies greater than 100 Mc./s.

Fig. 2 shows the data record for one of these cases, together with a scale of the great circle distance of the satellite below the radio horizon. The record was taken at the Smyth Research Associates Point Buchon field site on the coast-line near San Luis Obispo, California, with a sea-interferometer system, giving rise to the pattern of maxima and minima above the horizon. Relatively minor tumbling nulls can also be seen superimposed on the interferometer trace.

This work was sponsored by Rome Air Development Center, Air Research and Development Command.

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A Transatlantic Communication Experiment via *Echo* I Satellite

THE article in *Nature* of January 28, p. 268, by Carru, Gendrin and Reyssat, described the reception by the Centre National d'Etude des Télécommunications of 960-Mc./s. signals reflected from the *Echo* I satellite during pass 70.

The signals originated at the Bell Telephone Laboratories' station in Holmdel, New Jersey, U.S.A. The transmitter frequency was measured just before pass 70 and found to be 960·045 Mc./s., which is in good agreement with the value of 960·045053 Mc./s. calculated by Carru *et al.* during reception some 20 min. later.

The 60-ft. diameter transmitting antenna used at Holmdel has a gain of 43·1 ± 0·1 db. instead of the value of 44 db. assumed by Carru *et al.* Transmitter power at the time of reception was 7·5 kW. and was further reduced by the 0·5 db. antenna-transmission line loss to 6·7 kW., which is 0·8 db. less than that assumed by Carru *et al.* Inspection of pointing records for the reception period shows that the Holmdel antenna was aimed to within 0·2° of the satellite, which would add an additional loss of no more than 0·1 db. These factors predict a smaller value for received signal by 1·8 db.; on the other hand, there is no justification for the assumption that the cross-section of the balloon varies with the geometry of the transmission path, as borne out by our own measurements on *Echo* I and the correct theoretical treatment[1]. Thus the 1·3 db. factor used by Carru *et al.* can be omitted, leaving a net loss of 0·5 db., or a predicted received level of −143·0 dbm. This is in good agreement with the value reported, −142 dbm. ± 1 db.

Accurate information regarding the Holmdel transmission is available on request to any independent experimenters who need such information to evaluate their own measurements.

The results of the radio transmission tests supported by the U.S. National Aeronautics and Space Administration and using the *Echo* I satellite over the period August 1960–March 1961 have been evaluated and will be published shortly.

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Bell Telephone Laboratories, Inc.,
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WE thank Mr. Jakes for the above information, which corroborates the order of magnitude of our measurements and assumptions. The reference which he points out had only just been published when we submitted our communication (we added it to our ref. 4) and we had not had time to investigate the case of circular polarization, which is not thoroughly examined in Morgan's paper.

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PHYSICS

Apparent Intermediate Supervelocity of Detonation

THE transition from the low to the high detonation velocity in a cylindrical dynamite charge is seen as a rather sharp bend in the streak camera curve. At the transition point the high velocity often starts with an apparent supervelocity which is much greater than the stationary high velocity. The reason is probably that the high velocity is initiated by the low velocity at a point on the axis. Good accordance between observed and calculated values is obtained if it is assumed that the high-velocity detonation sets off from a point a little behind the luminous zone of the low velocity. This accords with the fact that the latter, according to instantaneous photographs, is at the front of the preliminary reaction zone of Selberg and Sjölin[1].

An analogous phenomenon is the initiation of a cylindrical charge by an axial shock-wave. In that case the detonation sets out from a circular, coaxial area on the shock front, the radius of which is smaller than the radius of the charge[2], and the maximum apparent supervelocity goes to infinity.

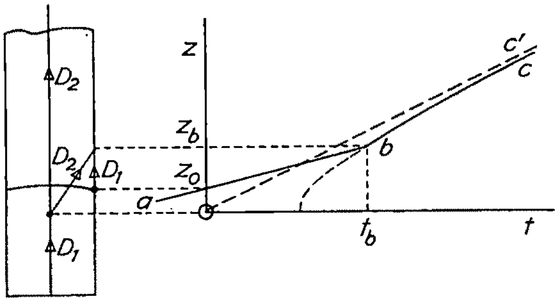

Fig. 1

Fig. 1 gives a schematic picture of the course of events. To the left is shown the cylindrical charge with the front of the low velocity and the starting point of the high velocity when the high velocity starts ($t = 0$). In the time diagram to the right, *abc* shows the course of the luminous front at the envelope surface of the charge. It is obtained from the streak camera photography. *b* is the point where the high-velocity front overtakes the low-velocity front. *Oc'* shows the front of the high-velocity detonation along the axis. The slope of *ab* gives the low velocity D_1, and the derivative of the curve

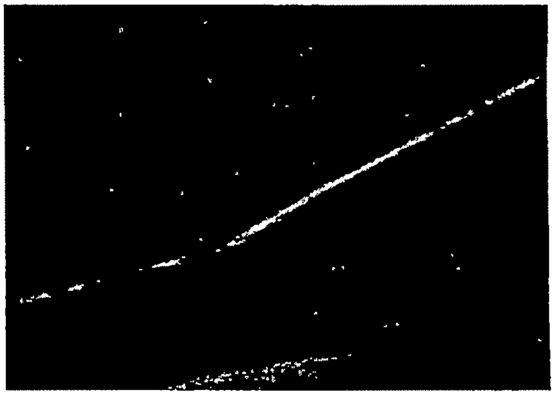

Fig. 2

bc in b gives the maximum apparent supervelocity D_2'. The slope of the asymptote Oc' to the curve bc gives the steady high velocity D_2. If R denotes the radius of the charge, z_b and z_o ordinate values according to Fig. 1, $n = D_2/D_1$ and $m = D_2'/D_2$, we get $z_b = R\sqrt{m^2 - 1}$, $z_o = z_b\ (1 - m/n)$ and $t_b = (z_b - z_0)/D_1$.

Fig. 2 shows a streak camera photo of a charge of gelatinized ammonium dynamite with 35 per cent nitroglycerine/nitroglycol (1 : 1). It was initiated by a donor charge of the same explosive detonating with high velocity. Both charges were in a common tube of 'Plexiglass' with 36 mm. interior and 40 mm. exterior diameter separated by an air gap of 40 mm. The transition point was 27 mm. from the end of the receiver. From Fig. 2 we get: D_1 = 3,030 m./s., D_2 = 5,760 m./s. and D_2' = 7,350 m./s. This gives $n = 1{\cdot}89$, $m = 1{\cdot}28$, $z_b = 22{\cdot}5$ mm., $z_o = 7{\cdot}3$ mm. and $t_b = 5{\cdot}0$ µs.

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Damped Free Oscillations of a Gyroscopic System

THE equations of motion of a gyroscopic system consisting of a symmetric rotor of axial moment of inertia C mounted in heavy gimbals are :

$$\left.\begin{aligned} A\ddot{\theta}_1 + c_1\dot{\theta}_1 + Cn\dot{\theta}_2 &= 0 \\ -Cn\dot{\theta}_1 + B\ddot{\theta}_2 + c_2\dot{\theta}_2 &= 0 \end{aligned}\right\} \quad (1)$$

where n is the constant angular velocity of the rotor about its axis ; θ_1 and θ_2 are small angles denoting rotations about the outer and inner gimbal axes respectively ; c_1 and c_2 are the corresponding coefficients of viscous friction ; A is the moment of inertia of the entire system about the outer gimbal axis and B that of the inner gimbal plus rotor about the inner gimbal axis. These equations are based on three principal assumptions : (1) the centres of mass of the rotor and of the gimbals lie at the intersection of the gimbal axes, (2) the axis of the rotor is nearly perpendicular to the axis of the outer gimbal, and (3) the axes of rotation are principal axes of inertia. If there is no friction at the gimbal axes, equations (1) define a free, undamped oscillation in which the rotor axis performs a conical whirl about its central position and the gimbals vibrate at frequency $p = Cn/\sqrt{AB}$.

Now it is well known that friction normally reduces the frequency of free vibrations of an oscillatory mechanical system. The gyroscopic motion defined by equations (1), however, provides an exception to the rule, as may be seen by eliminating θ_2 from (1). This gives

$$\dddot{\theta}_1 + \left(\frac{c_1}{A} + \frac{c_2}{B}\right)\ddot{\theta}_1 + \left(\frac{c_1c_2 + C^2n^2}{AB}\right)\dot{\theta}_1 = 0 \quad (2)$$

Equation (2) represents a damped free oscillation the frequency, p_d, of which is :

$$p_d = \sqrt{\left[\frac{C^2n^2}{AB} - \frac{1}{4}\left(\frac{c_1}{A} - \frac{c_2}{B}\right)^2\right]} \quad (3)$$

Thus the frequency of the damped oscillations will normally be less than that of the undamped oscillations. However, when:

$$\frac{c_1}{A} = \frac{c_2}{B} \quad (4)$$

an exceptional and interesting case occurs, for then p_d is independent of both c_1 and c_2. This condition might easily arise in practice. Even if it were only approximately true, the decrease in frequency due to damping would still be very small: it would, for example, be considerably less than that associated with a conventional system having a single degree of freedom.

It is also interesting to note that if (4) is satisfied, the motion can never be critically damped and must remain oscillatory, even although the damping coefficients and hence the rate of decay of oscillations may become very large.

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CHEMISTRY

Precision and Accuracy in Gas Chromatographic Analysis

THE extensive use of gas chromatography to provide quantitative information on the composition of mixtures of organic compounds has prompted an investigation designed to give some indication of the reliability expected. With the exception of the gas density balance, the commonly used detectors do not give a response which can be predicted accurately from the physical or chemical properties of the compounds concerned, so that for highest accuracy calibration is necessary. The development of high-sensitivity detectors has made possible the achievement of high column performance by virtue of the smaller sample loads used, but since reproducible loading becomes more difficult the use of internal standards becomes desirable.

The most widely used high-sensitivity detector is the argon ionization detector devised by Lovelock[1], which combines very high sensitivity with a high degree of stability to changes in rate of flow of gas, temperature and pressure. The linear range of the detector at any given applied voltage is limited but adequate, and a wide dynamic range is achieved by change of applied voltage.

The argon detector in its present form is of robust construction, and when used with suitable power supply and electronic circuitry is capable of giving very accurate and reproducible results. Experience shows that the major cause of detector malfunction is through contamination of the argon carrier gas with either organic or inorganic substances.

Quantitative results obviously rely on the proper functioning of the detector, and a knowledge of the way in which accuracy and reproducibility can be affected is essential.

Organic contamination of the argon entering the detector causes an increase in the background current. Superimposition of a chromatographic peak on this background could result in the observed response extending into the non-linear region. Variation in the level of organic contamination produces a variation in the detector background current, which appears as an unstable chromatogram baseline. Deposition of organic material derived from stationary phase decomposition on the collecting electrode can result in low detector response. Common sources of organic contamination are cylinder gauge heads, unclean tubing used to convey argon to the column and stationary-phase stripping from the column used.

The process of ionization in the detector is brought about by the transfer of the excitation energy of metastable argon on collision. The formation of sufficient metastable argon for normal detector operation requires the collision of molecular argon with electrons acquiring sufficient energy by acceleration in the applied potential field. The effect of some inorganic contaminants is to prevent the electrons acquiring sufficient energy to create metastable argon; the response of the detector is thereby destroyed. (This effect has been used by Willis[2] to produce a high 'negative' response for permanent gases in a detector fed with an argon stream containing an organic component.) Recent results published by Lovelock[3] show that the greatest effect is produced by water vapour, the sensitivity of the detector being reduced ten-fold by a change in water vapour concentration from about 30 p.p.m. to about 1,000 p.p.m. At less than 30 p.p.m. of water vapour the detector response is constant. The effect of other inorganic contaminants is similar but sets in at higher concentrations. With oxygen, reduction in sensitivity begins at 100 p.p.m. and with nitrogen at 1,000 p.p.m.

It is clear that in order to achieve constant sensitivity and hence reproducible chromatograms it is essential to eliminate or reduce to acceptable limits contamination of the argon supply or to maintain at a constant level deliberately introduced contaminants. Sources of contamination are listed in Table 1.

Table 1

Site of contamination	*Contaminant*	*Source*	*Effect*
Argon cylinder	Water vapour	Present in cylinder	Low and variable sensitivity
Rubber or plastic argon line	Water vapour, permanent gases	Diffusion through walls of tubing	Low and variable sensitivity
Column	Organic bleed	Unstable stationary phase or operation at too high temperature	High background current, non-linearity, variable sensitivity

Contamination can be avoided by conveying the argon from the cylinder to the column through clean metal tubing and a molecular sieve trap, and by pretreating the column at a temperature in excess of the proposed working temperature.

In order to determine the precision obtainable from a gas chromatograph employing an argon ionization detector, a two-component gas mixture was sampled automatically at regular intervals and chromatographed on a standard Pye argon chromatograph (Fig. 1). Contamination of the argon supply was prevented by passing the argon from the cylinder through a molecular sieve Linde 5*A* trap and thence to the column in copper tubing which had been cleaned previously by heating to a dull red in a stream of argon. The column used was 10 per cent squalane on 100/120 mesh alumina which had been rendered stable by heating at 100° C. for 24 hr. in a stream of argon. The column temperature was 75° C. and the carrier gas flow-rate 30 ml./min. The sample gas was passed through the automatic sampling valve at about 5 ml./min. to atmosphere, no attempt being made to control the pressure since small variations in the volume injected would not affect the results obtained. Under these conditions the gas mixture consisting of approximately equal amounts of propane and butane was eluted in 8 min. with complete resolution of the two components. A fresh injection of 0·30 ml. of argon containing 1,600 p.p.m. of organic material was made every 10 min. and the run was continued for 4 days. The ratio of the peak heights of the two components was then determined for each injection.

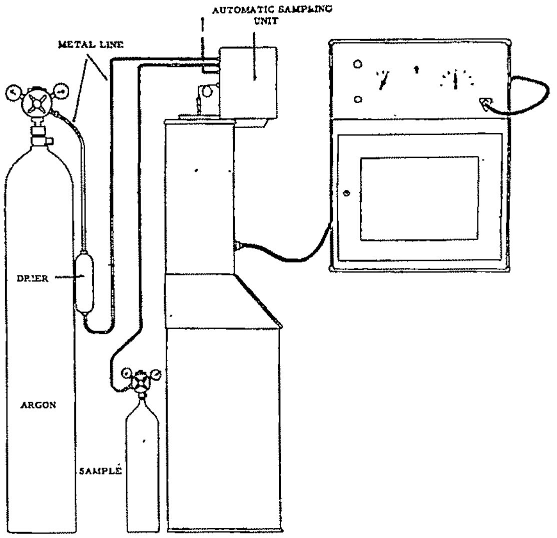

Fig. 1. Apparatus used in the determination of reproducibility

The results obtained (Fig. 2) show an overall spread of about 1 per cent for 110 consecutive determinations. The standard deviation is 0·23 per cent and the 95 per cent confidence limits are ± 0·35 per cent. The base-line deviation was 1·7 per cent full-scale deflexion over 4 days. The sample load added varied by about ± 4 per cent. A second series of injections was carried out, this time using an integrating amplifier to give an

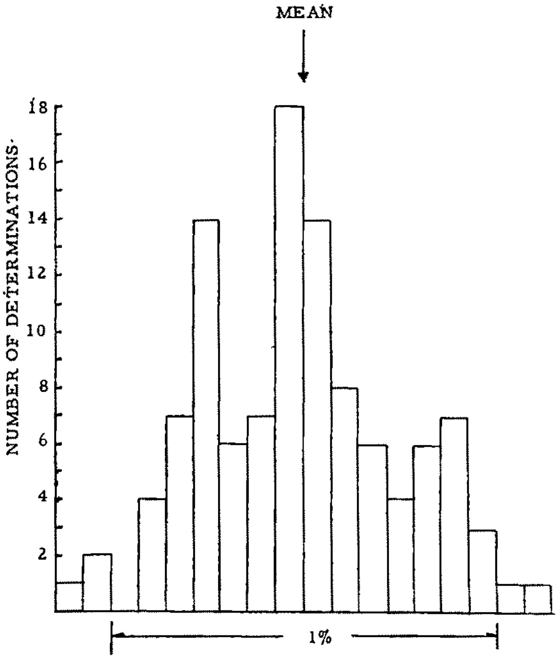

Fig. 2. Variation in peak height ratios from 110 consecutive chromatograms

accurate measurement of peak areas; an overall spread similar to that quoted was obtained.

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An Organo-clay Complex for the Separation of Isomeric Dichlorobenzenes using Gas Chromatography

White[1] has shown that dimethyldioctadecyl ammonium bentonite is particularly suitable as a stationary phase for the chromatographic separation of aromatic and aliphatic compounds, while Hughes, White and Roberts[2] have shown that this material is equally suitable for the separation of isomeric xylenes, toluidines and cresols. Further work by Cowan and White[3] has shown that similar organo-clay complexes are adsorbents for phenols, the adsorbents showing widely differing specificity for the various isomers, and from the data presented the authors propose that the electronic transitions occurring within the sorbate molecules are the main contributory forces governing the extent of adsorption. In consequence, the use of organo-clay complexes for separating isomeric compounds is particularly appropriate and a further example, the separation of dichlorobenzenes, has been investigated and the results obtained compared with those for a conventional liquid stationary phase.

The relative retention volumes for the dichlorobenzenes are given in Table 1 and these are expressed relative to the *para* isomer—in all cases the values are corrected for the dead space of the column. A typical chromatogram is shown in Fig. 1 and this was obtained with a column 200 cm. long × 0·4 cm. bore containing 10 gm. 60–80 mesh 'Celite' as a diluent for 1·0 gm. of dimethyldioctadecyl ammonium bentonite ('Bentone 34', Abbey Chemicals, Ltd.)—the detector was a β-ray ionization unit as supplied by Gas Chromatography, Ltd.

It is noted that using 'Bentone 34' as a stationary phase, a complete resolution of the dichlorobenzenes is obtained whereas with the silicone oil virtually no separation of the *para* and *meta* isomers is effected. Furthermore, even with a capillary column and dinonyl sebacate as a stationary phase complete resolution of these isomers is not possible. It is also noted that, with the solid 'Bentone' stationary phase, symmetrical elution peaks are obtained.

Table 1

	Relative retention volume			
	Silicone oil		'Bentone 34'	
	80° C.	110° C.	80° C.	110° C.
Benzene	0·08	—	0·11	—
Chlorobenzene	0·28	0·37	0·68	0·79
p-Dichlorobenzene	1·00	1·00	1·00	1·00
m-Dichlorobenzene	1·02	1·02	2·23	2·14
o-Dichlorobenzene	1·17	1·12	5·64	4·80
1,2,4-Trichlorobenzene	3·11	2·47	—	16·0
	Specific retention volume (ml.)			
p-Dichlorobenzene	440	161	840	246

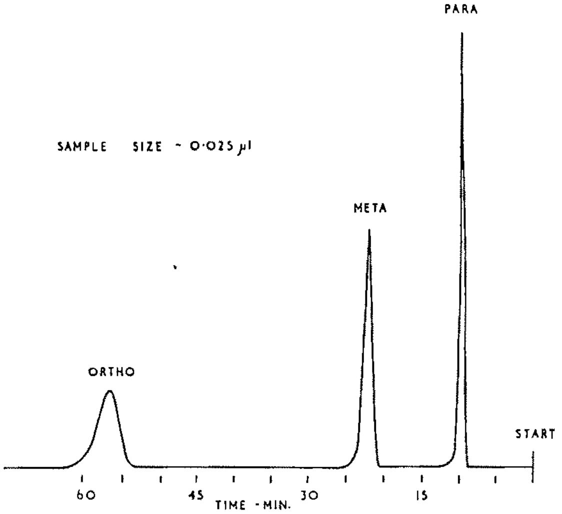

Fig. 1

From the results and those obtained by previous workers, it is suggested that the use of organo-clay complexes as stationary phases in chromatography warrants more detailed investigation. Since interlamellar distances within the clay complex play an important part in the adsorptive properties, and as these distances are governed by the size of the substituting amine ion[4], it is expected that further work will reveal that it is possible to prepare an organo-clay stationary phase of pre-determined resolving characteristics for a specific separation of organic compounds.

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The Possibility of obtaining E.M.F. directly from Acid-Base Reactions: Electrodes of the Third Kind reversible to Alkaline Earth and Other Ions

Some years ago Shedlovsky[1,2] suggested that proton transfer reactions could give rise directly to e.m.f., and apparently confirmed this idea by demonstrating that a glass electrode, coated with lauric acid and barium laurate, acted as a barium electrode. There is a simpler explanation of this result, however: namely, the glass electrode with lauric acid and barium laurate acts as an electrode of the third kind. In a barium chloride solution saturated with these substances, the activities of barium and hydrogen ions must be related by the equation:

$$a_{\mathrm{H}} = (k_{\mathrm{HL}}^{2}/k_{\mathrm{BaL}_2})^{1/2} \cdot a_{\mathrm{Ba}}^{1/2}$$

k representing the thermodynamic solubility product. In such solutions the glass electrode, responding to

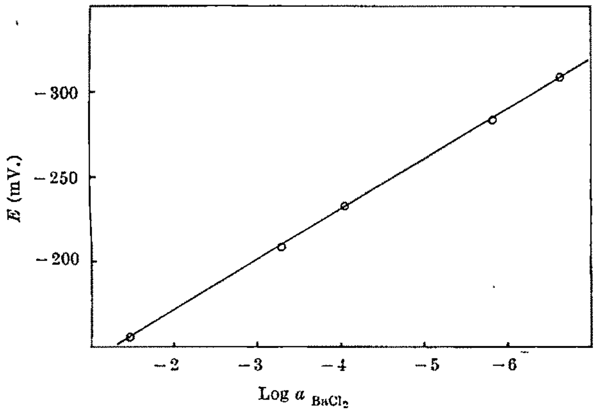

Fig. 1. E.m.f. of the cell: $\mathrm{Ag,AgCl}\Big/\begin{matrix}\mathrm{BaCl_2\ sat.\ with}\\ \mathrm{Ba}L_2\ \text{and}\ \mathrm{H}L\end{matrix}\Big/$glass electrode

hydrogen ion activity in the normal way, would therefore appear to act as a barium electrode.

To check this possibility, solutions of barium chloride ranging in concentration from 0·005 to 0·5 *M* have been shaken with mixtures of solid lauric acid and solid barium laurate at 25° C. and the potential difference between a glass and silver–silver chloride electrode then determined in each of these solutions. The results of these measurements are shown in Table 1 and Fig. 1, in which the straight line has been drawn with the theoretical slope of 29·6 mV. per logarithmic unit. The points referring to 0·5, 0·1 and 0·01 *M* solutions were obtained with one glass electrode and the others with a second glass electrode which was also measured in 0·5 *M* solution. For both electrodes the e.m.f. was set equal to -156 mV. in the latter solution in order to fit the data for the two electrodes together. The measurements are seen to give the expected result. A further check is possible, since the potential difference between the glass and silver chloride electrode in 0·01 *N* hydrochloric acid was also determined. The value was $-101·3$ mV. compared with the value of -156 mV. in the 0·5-*M* barium chloride solution. Since the e.m.f. of the chain:

$$\mathrm{Ag,AgCl}\Big/\begin{matrix}\mathrm{BaCl_2\ sat.\ with}\\ \mathrm{H}L\mathrm{(s)\ and\ Ba}L_2\mathrm{(s)}\end{matrix}\Big/\begin{matrix}\text{glass}\\ \text{electrode}\end{matrix}-\begin{matrix}\text{glass}\\ \text{electrode}\end{matrix}\Big/\mathrm{HCl}\Big/\mathrm{AgCl,Ag}$$

should obey the equation:

$$E=(RT/2F)\ln(k^2_{\mathrm{H}L}/k_{\mathrm{Ba}L_2})+(RT/2F)\ln(a_{\mathrm{BaCl_2}}/a^2_{\mathrm{HCl}})$$

the ratio $(k^2_{\mathrm{H}L}/k_{\mathrm{Ba}L_2})$ can be calculated. The value thus obtained was $3·9 \times 10^{-9}$. Combining this with the value $k_{\mathrm{Ba}L_2}=1·2 \times 10^{-11}$, calculated from solubility data[4], one then obtains for the solubility product of lauric acid in water at 25°, $k_{\mathrm{H}L}=2·15 \times 10^{-10}$, and hence for the molality of undissociated lauric acid in the saturated solution, $2·15 \times 10^{-5}$, if lauric acid is assumed to have a dissociation constant of 10^{-5}. Finally, the solubility of lauric acid at 25° C. is found to be $3·6 \times 10^{-5}$ mole kgm.$^{-1}$ water. This may be compared with the value $1·9 \times 10^{-5}$ mole l.$^{-1}$ reported by McBain and Eaton[5] from conductivity measurements.

This explanation of Shedlovsky's results implies that the laurate layers on his electrodes were porous or cracked, so that the underlying glass surface was in reality in contact with solution. It also implies that the solutions inside and outside his glass electrodes differed in *p*H, contrary to what he believed. This must have been so in fact, in view of the much smaller buffer capacity of his solutions compared with that of the solids barium laurate and lauric acid, which would therefore determine the equilibrium *p*H of the solution.

Table 1

E.m.f. of the cell: $\mathrm{Ag,AgCl}\Big/\begin{matrix}\mathrm{BaCl_2\ sat.\ with}\\ \mathrm{Ba}L_2\ \text{and}\ \mathrm{H}L\end{matrix}\Big/$glass electrode

M ($\mathrm{BaCl_2}$)	$\gamma_{\pm}$ (Ref. 3)	E_{25° (mV.)
0·005	0·774	$-308·8$
0·01	0·716	$-283·2$
0·05	0·564	$-233·1$
0·1	0.500	$-208·8$
0·5	0·397	$-156·0$

It should obviously be possible by this method to obtain electrodes reversible to the other alkaline earth ions, while by varying the acid the list might be extended still further. The method is especially convenient since it makes use of the glass electrode.

Although it is true that the e.m.f. of cells of the type tested does arise directly from acid-base reactions, the detailed mechanism is probably not that suggested by Shedlovsky. Even with the simpler mechanism suggested here, however, acid-base reactions might still be the origin of some bioelectric potentials.

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Spectrum of Vanadium in the Tri-positive Oxidation State (V^{3+}) in Tetrahedral Co-ordination[†]

Vanadium in the tri-positive oxidation state has two unpaired electrons in the 3*d* shell. The interaction of the crystalline electric field with the two *d*-shell electrons has been treated theoretically for fields of various symmetries[1–3]. Most of the information concerning the electronic structure of V^{3+} comes from work on compounds in which the metal ion is co-ordinated to six ligands.

The optical spectra of V^{3+} in alums[4] and in corundum[2,5], where the co-ordination is to six oxygens, agree reasonably well with the calculated energy-level diagrams. The *Dq*-value which fits these data best is 1,800 cm.$^{-1}$. Using the strong field theory, McClure[6] gives the octahedral site preference energy as 12·3 k.cal./mole for oxide lattices.

Since energy-levels are profoundly influenced by a change in co-ordination number, it is of interest to learn more about four co-ordinate V^{3+} (ref. 7). We have now obtained the spectrum of V^{3+} substituted for Al^{3+} in the $CsAlCl_4$ lattice (Fig. 1). Single crystals of this material were grown containing a few tenths per cent of V^{3+}. They are blue in colour. Tetrahedral $AlCl_4^-$ ions have been shown to exist as structural units in the $NaAlCl_4$ lattice[8] and, presumably, are present also in the $CsAlCl_4$ lattice.

The room temperature spectrum is characterized by four bands with maxima at 15,000; 10,250; 9,400; and 8,250 cm.$^{-1}$. The total oscillator strength

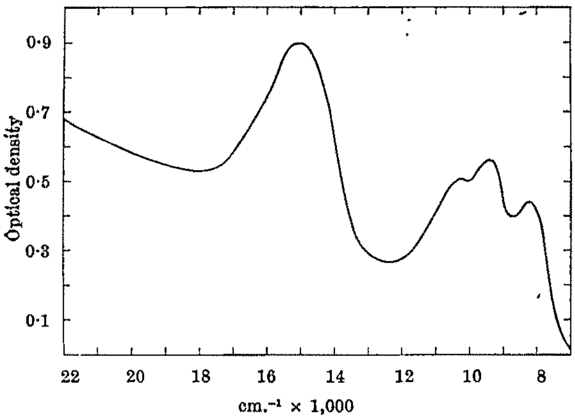

Fig. 1. Absorption spectrum of V^{3+} in $CsAlCl_4$ at room temperature

in the region 17,000–7,000 cm.$^{-1}$ is about 10^{-3}. The bands overlap and are not symmetrical. They can be expected to show additional structure at low temperatures. Liehr and Ballhausen's energy-level diagram, based on pure crystal field theory calculations for a d^2 system in a tetrahedral field[1], gives two $^3T_1$ levels at 16,000 and 9,000 cm.$^{-1}$ and a $^3T_2$ level at 5,000 cm.$^{-1}$ for $Dq = -500$ cm.$^{-1}$.

Tripositive vanadium can also be situated in an octahedral chloride ion environment. This would appear to be the case, for example, in molten lithium chloride-potassium chloride eutectic[9,10]. In this solvent, two bands are found at 18,000 and 11,000 cm.$^{-1}$ corresponding to transitions from the $^3T_1$ ground-state to $^3T_1$ and $^3T_2$ excited states, respectively. Good agreement with the theoretical energy-level diagram is obtained with $Dq = 1{,}200$ cm.$^{-1}$. Using Dq-values of -500 and 1,200 cm.$^{-1}$ for tetrahedral and octahedral environments respectively, one finds the octahedral site preference energy for V^{3+} in chloride lattices to be 10·5 k.cal. on the strong field model.

Spectral intensity calculations on $CuCl_4^{2-}$ and $CoCl_4^{2-}$ ions led Ballhausen and Liehr[11] to postulate that tetrahedral complexes display a certain degree of covalency. It would appear from the present work that covalent bonding in VCl_4^- affects not only the intensity of the spectrum but also the energy-level structure of the ion. An extension of the theory, which would include covalency effects, will undoubtedly be required in order to obtain better agreement with the experimentally observed spectrum.

The technique of isomorphous substitution of a transition metal ion in a colourless host lattice was applied in the present case to obtain a particular configuration difficult to produce by other means. Other examples of host lattice configuration stabilization have been reported for tetrahedral Co^{2+} and Ni^{2+} in oxide[8] and chloride[11] lattices. The phenomenon is being put to use in work on a number of other transition metal ions.

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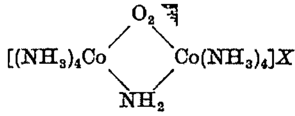
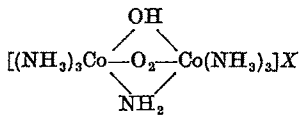
Structure of Polynuclear Cobalt Complexes

It is known that molecular oxygen will react with the hexaminecobalt(II)-ion, whereby different kinds of complexes are formed. Among these complexes, the following types are described in the literature:

$$[(NH_3)_5Co{-}O_2{-}Co(NH_3)_5]X_5$$





Lately the bonding in these compounds has been much discussed, and it therefore appears to be of considerable interest to establish their structures. Work on the crystal structures of compounds belonging to the first of the above-mentioned types have been going on at this institute for several years. The following compounds have been investigated by the usual X-ray methods:

$$[(NH_3)_5Co{-}O_2{-}Co(NH_3)_5](NO_3)_5$$

$$[(NH_3)_5Co{-}O_2{-}Co(NH_3)_5](NO_3)_4.HF_2.(H_2O)_2$$

The nitrate was found to have tetragonal symmetry, corresponding to the space group $P4_2nm$ (No. 102). The dimensions of the elementary cell are $a = b = 11 \cdot 94$ Å. and $c = 8 \cdot 06$ Å. The cell contains two formula units. Parameter values for all the atoms with the exception of the hydrogens have been determined by a three-dimensional Fourier summation (Table 1). The reliability index R is calculated to be 0·20.

Table 1. Parameter Values for $[(NH_3)_5Co{-}O_2{-}Co(NH_3)_5](NO_3)_5$. Space Group $P4_2nm$

Atom	Position	Number	x	y	z
Co	*c*	4	0·134	0·134	0·000
NH_3	*d*	8	0·213	0·054	0·181
NH_3	*d*	8	0·054	0·213	0·814
NH_3	*c*	4	0·250	0·250	0·012
O	*c*	4	0·457	0·457	0·512
N	*a*	2	0·500	0·500	0·988
N	*c*	4	0·265	0·265	0·550
N	*b*	4	0·500	0·000	0·000
O	*a*	2	0·500	0·500	0·138
O	*c*	4	0·438	0.438	0·913
O	*c*	4	0·192	0·192	0·500
O	*c*	4	0·299	0·299	0·407
O	*c*	4	0·295	0·295	0·680
O	*b*	4	rotating, $r = 1 \cdot 2$ Å.		
O	*b*	4			
O	*b*	4			

The constitution of the cation complex can be seen from Fig. 1. It will be noted that the axis of the peroxide group is perpendicular to a line connecting the two cobalt atoms, as predicted by Vlček[1].

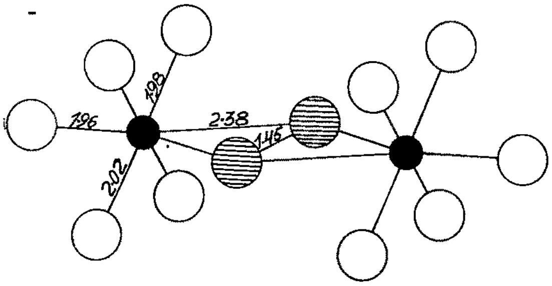


Fig. 1. Configuration of the ion $[(NH_3)_5Co-O_2-Co(NH_3)_5]^{5+}$. Shaded, O; white, NH_3; black, Co

The compound containing the fluoride as well as the nitrate anion has been prepared by treating the nitrate with a concentrated aqueous fluoric acid solution. The following crystallographic data have been obtained for this compound: space group *Pbca* (No. 61) and unit cell dimensions $a = 13{\cdot}14$ Å., $b = 12{\cdot}21$ Å. and $c = 14{\cdot}14$ Å. The unit cell contains four formula units.

As expected, the compound is composed of complex groups, as indicated in Fig. 1, in addition to water, nitrate groups and hydrogen difluoride groups. The atomic parameter values obtained so far give an *R*-factor of 0·34. A least-square refinement of the structure has therefore been undertaken.

A complete report of both these and related structure investigations will be published elsewhere.

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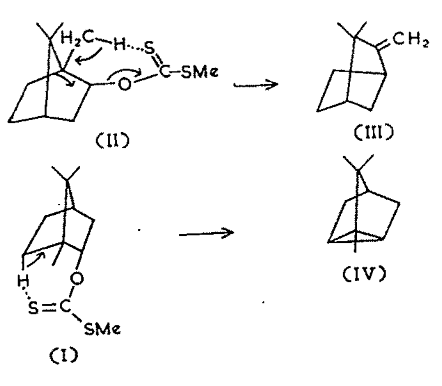
Pyrolysis of Esters of Borneol and *iso*Borneol

It is generally believed that esters pyrolyse to give the products of *cis* elimination in a six-membered cyclic transition state[1]. Recently, however, products corresponding to a *trans* elimination have been shown to be formed by the pyrolysis of methyl xanthates of some substituted *cyclo*hexanols[2]. The pyrolyses of xanthates and acetates of 1-alkyl*cyclo*hexanols give predominantly the more stable endocyclic olefins[3], and both *cis* and *trans* elimination have been observed in pyrolyses of chlorosulphites[2], and sulphoxides[4].



Tschugaeff isolated bornylene, containing some tricyclene, from the pyrolysis of bornyl methyl xanthate[5]. Hückel has isolated camphene from the pyrolysis of *iso*bornyl xanthate[6], and camphene has been shown to be a pyrolysis product of bornyl sulphites[7]. We have examined the pyrolysis products of methyl xanthates and benzoates of borneol and *iso*borneol; cf. (I) and (II), respectively. The methylxanthates were decomposed using a bath at 300°, at which temperature the decomposition was almost instantaneous; the benzoates decomposed comparatively slowly when the bath was at 350°. The products distilled off and were analysed and separated by gas-liquid chromatography (Table 1).

Table 1. Composition and Configuration of Pyrolysis Products. (Figures in parentheses are percentage retention of configuration)

	Bornylene (per cent)	Tricyclene (per cent)	Camphene (per cent)
Bornyl methyl xanthate	70 (100)	13·5	16·5 (~ 30)
*Iso*bornyl methyl xanthate	38·5 (92)	—	61·5 (~ 100)
Bornyl benzoate	24·5 (97)	21·5	54 (~ 30)
*Iso*bornyl benzoate	—	13	87 (~ 30)

The bornylene was probably formed through the classic *cis* elimination mechanism[1], but this mechanism cannot explain the formation of camphene (III) and tricyclene (IV). Maccoll[8] and co-workers have emphasized the similarity between the gas-phase pyrolyses of alkyl halides and their unimolecular solvolyses, and emphasize the importance of charge separation in the transition state[2]. Following these ideas, we can explain the composition of the product by assuming that the part of the reaction which gives camphene and tricyclene is 'quasi-heterolytic'[8], because the initial elimination product of the solvolyses of bornyl and *iso*bornyl chlorides is always camphene. The amount of camphene is greater in the pyrolyses of the *iso*bornyl esters, and *iso*bornyl compounds form carbonium ions much more readily than do bornyl compounds. Similarly, carbonium ions should be generated much more readily by benzoates than by xanthates, and the former always give more camphene and less bornylene; although the temperature difference may make this comparison misleading.

The formation of camphene of high optical purity from *iso*bornyl methyl xanthate suggests that there is a seven-membered cyclic transition state, in which breaking of a C—H bond is synchronous with the migration of C—C bond electrons, and separation of the leaving group, but the formation of partially racemic camphene from the other esters shows that for them the decomposition must be step-wise, with loss of asymmetry at some stage. A similar synchronous mechanism can be written for the formation of tricyclene (IV).

As might be expected, the bornylene is more than 90 per cent optically pure, and bornylene and camphene and reactants have the same configuration. Because in these pyrolyses we observe products typical of a Wagner–Meerwein rearrangement, we have stressed the analogy with carbonium-ion chemistry, but our cyclic mechanisms could be considered to have some homolytic character, and it is also possible that some 1 : 1-elimination might lead to a carbene intermediate.

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BIOCHEMISTRY

Hydrolysis of Paraoxon in Mammalian Blood

Several publications have indicated that in the detoxication of paraoxon (diethyl *p*-nitrophenyl phosphate, '*E*600,' 'Mintacol') the mammalian blood serum might be of importance[1-3]. Aldridge[4] was first to describe the existence of an *A* esterase (aryl-, aromatic esterase) which hydrolyses paraoxon. This enzyme occurs in the sera of various species[1], but the extent of its involvement in the metabolism of paraoxon is not yet clear[5,6]. Among other properties, the arylesterase cleaves phenyl acetate[7], and it has been shown with this substrate that the enzyme requires the presence of calcium for activity[8,9]. The arylesterase is activated by calcium and inhibited by sodium ethylenediaminetetraacetate and numerous other compounds[8-11]. In human blood plasma it occurs in fraction IV–1[12,13]. Since preliminary experiments indicated that in addition to this arylesterase, at least one more factor might be responsible for the hydrolysis of paraoxon in blood serum or plasma, we undertook the present investigations.

The cleavage of paraoxon was followed in a Cary recording spectrophotometer, by determining the amount of *p*-nitrophenol liberated at a wave-length of 4000 Å., or in some of the experiments at 3470 Å. The temperature was kept constant at 37° C., the *p*H at 7·9. The 0·1 *M* *tris*(hydroxymethyl)aminomethane buffer was 4·5 × 10^{-3} *M* in substrate and 2·4 per cent in propylene glycol. The results were calculated from the initial rates of hydrolysis.

Fig. 1 summarizes some of the results. As shown in Fig. 1*A*, fresh pooled human serum hydrolyses paraoxon at an appreciable rate. This activity was inhibited only 86 per cent by a 0·01 *M* concentration of ethylenediaminetetraacetate. Since the cleavage of paraoxon by purified human arylesterase (fraction IV–1) was completely abolished by a concentration of ethylenediaminetetraacetate 20 times lower (*D*), clearly the residual activity in the serum was not due to arylesterase, but to something else. The splitting of paraoxon, however, was completely blocked when the serum, in addition to ethylenediaminetetraacetate, contained 10^{-3} *M* aurin tricarboxylic acid ammonium salt (aluminon) (*A*). While looking for the source of the activity resistant to ethylenediaminetetraacetate we found that human albumin (fraction V or crystallized) also hydrolyses paraoxon, and indeed this hydrolysis is inhibited by aluminon (*C*). Even after 18 hr. of incubation ethylenediaminetetraacetate had no effect on the albumin. On the other hand, the enzymic hydrolysis of paraoxon by fraction IV–1, as indicated above, was inhibited by ethylenediaminetetraacetate, and unaffected by aluminon (*D*). When plasma arylesterase (fraction IV–1) and albumin (fraction V) were mixed (*B*) the results with selective inhibitors were similar to those with normal serum. However, in the presence of albumin the activity of fraction IV–1 is somewhat higher than expected.

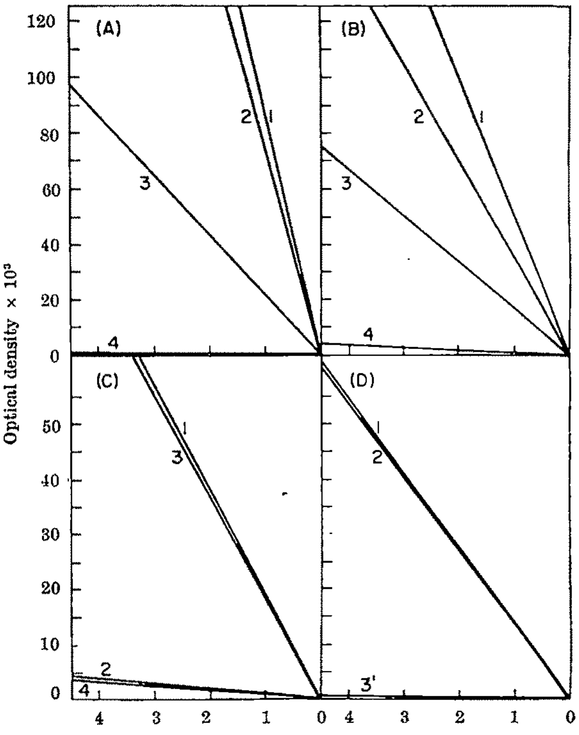

Fig. 1. Inhibition of the hydrolysis of paraoxon in human blood serum (*A*) and plasma fractions (*B*, *C*, *D*). Dilutions : *A*, pooled serum, 1 : 20 v/v; *B* fractions IV–1, 1 mgm./ml. + V, 4 mgm./ml. w/v; *C*, fraction V, 4 mgm./ml.; *D*, fraction IV–1, 0·5 mgm./ml. (1) control; (2) aluminon 1 × 10^{-3} *M*; (3) ethylenediaminetetraacetate 1 × 10^{-2} *M*; (3′) same 5 × 10^{-4} *M*; (4) aluminon 1 × 10^{-3} *M* + ethylenediaminetetraacetate 1 × 10^{-2} *M*

The behaviour of albumin with paraoxon was further explored with the help of various agents. Albumin (or a factor in albumin) was inhibited to about 50 per cent (I_{50}) by 2 × 10^{-4} *M* aluminon. This inhibition was not reversed by overnight dialysis. Lineweaver–Burk-type plots[14] showed a non-competitive inhibition. Some other enzyme inhibitors or activators such as oxalic acid (0·01 *M*) or mercuric chloride (5 × 10^{-4} *M*) nevertheless gave negative results. Since the preparations used were not activated by Sr^{2+} and Ba^{2+}, the presence of a phosphoryl phosphatase[15] is unlikely. But albumin was definitely inhibited by sodium, potassium or lithium chloride. The I_{50} values were roughly 0·5 *M*. Albumin was more sensitive to monovalent anions than to cations; the I_{50} of sodium bromide is about 0·1 M; that of sodium iodide, 0·01 M.

Fluoride was not tried as an inhibitor because we had seen that 10^{-3} M potassium or sodium fluoride catalyses the hydrolysis of paraoxon at a relatively high rate. The rate of this reaction was similar to the one catalysed by albumin in the concentration shown in Fig. 1,*C*. Other metals and organic compounds, among them the cholinesterase reactivator pyridine-2-aldoxime methiodide (2–PAM), used frequently in paraoxon poisoning[16,17] had an analogous effect. (The subject was recently reviewed extensively by O'Brien[18].) This catalytic activity at a 1 × 10^{-3} M concentration decreased in the following order: 2–PAM, sodium fluoride, *p*-chloromercuriphenylsulphonic acid, zinc sulphate, cadmium sulphate, manganese chloride, cobalt chloride, mercuric chloride. The zinc-ethylenediaminetetraacetate was not active.

Reagents and procedures known to denature proteins also destroyed the activity of albumin. For example, albumin became inactive after heating it at 70° C. for 10 min. Furthermore, 4 M urea abolished 80 per cent of the activity in 1 hr. Calcium chloride protected the protein; in the presence of 0·01 M calcium chloride only 40 per cent was inactivated. In contrast to the results with arylesterase[19], lanthanum chloride was less effective than calcium chloride. Other alkaline earth salts (magnesium chloride, strontium chloride) also protected the albumin, but less than calcium chloride.

When the investigations were expanded to serum proteins of other mammals, swine serum was found to behave like human serum (see Fig. 1,*A*). Serum albumin of other animals also destroyed paraoxon. The relative activity of the various albumin fractions decreased in the following order: rabbit, human, porcine, bovine, equine. Under our conditions, 100 mgm. of human albumin hydrolysed 2·4 μM paraoxon per hour. These albumins were also inhibited to a varying extent by aluminon but unaffected by ethylenediaminetetraacetate, etc. On the other hand, ethylenediaminetetraacetate blocked partially or completely the hydrolysis of paraoxon in the sera of pig, horse, rabbit, guinea pig, and sheep.

Lactalbumin, lysozyme, and human plasma cholinesterase (fraction IV–6–3) hydrolysed paraoxon to a negligible extent. Like arylesterase, albumin also splits phenyl acetate. While the relative ratio of hydrolysis-rates of phenyl acetate to paraoxon by fraction IV–1 was about 600 : 1 in the two systems employed, it became 1 : 1 with albumin as source of 'enzyme'.

In preliminary studies with the Spinco model *E* analytical ultracentrifuge fraction V gave a single peak, but in starch-gel electrophoresis human albumin from various sources showed contamination by globulin. However, in experiments where the sample had been pre-treated with the inhibitor aluminon, the albumin band migrated at a higher speed in front of the field.

The similarities and dissimilarities between the hydrolysis of paraoxon and other 'substrates' of albumin, for example, β-naphthylacetate, β-carbonaphthoxycholine[10], carbamates[12,20], di*iso*propoxy phosphoryl fluoride[21], indophenyl acetate[22] and other details are discussed elsewhere.

Our present experiments indicate that mammalian serum albumin (or a factor in it) hydrolyses paraoxon at an appreciable rate. Selective inhibitors can distinguish its activity from that of serum arylesterase.

The kind co-operation of Drs. W. Jensen, R. Rice and M. Westerman in some of the experiments is gratefully acknowledged.

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Precautions in the Use of Ferri- or Ferrocyanide for the Study of Hæmoproteins

POTASSIUM ferri- and ferro-cyanide are extensively used as oxidizing and reducing reagents respectively, or as buffer systems in the study of the oxidation-reduction potentials of hæmoproteins and other biological compounds. It was shown, however, by Schönbein and some other workers, especially Baudisch and Bass[1], that these reagents easily undergo photodecomposition accompanied by the liberation of a certain amount of cyanide. Solutions of these reagents must therefore be not only freshly prepared, but also well protected from light, especially when used for the study of hæmoproteins which may have high affinities for cyanide, forming with it well-defined complexes.

In this respect it may be interesting to recall a red compound described by Bock[2] as 'photomethæmoglobin', which he obtained from methæmoglobin exposed to strong light. He also recorded the absorption spectrum of this derivative, which differed markedly from those of methæmoglobin and hæmoglobin. Bock's 'photomethæmoglobin' was re-examined by Haldane[3], who identified it as the cyanmethæmoglobin compound discovered and well described by Kobert[4]. Cyanide, according to Haldane, originated in Bock's experiments from the photodecomposition of ferricyanide present in his solution of methæmoglobin when it was exposed to light. Haldane[3], being unaware of the fact that the light-sensitivity of ferricyanide had already been recorded before, carried out some interesting experiments on this subject. Thus, on treating methæmoglobin in the dark with a solution of ferricyanide previously exposed to light, Bock's 'photomethæmoglobin' was immediately obtained, and was indistinguishable from cyanmethæmoglobin formed by treating methæmoglobin with cyanide. Haldane's conclusion as to the nature of 'photomethæmoglobin' was corroborated by Zeynek[5].

On several occasions in the course of recent experiments, I have observed the formation of the cyanferriperoxidase complex during spectroscopic examinations of samples of pure peroxidase treated with ferri- or ferro-cyanide solutions freshly prepared, but not protected from light. These experiments suggest that if a hæmoprotein compound, treated with ferri- or ferro-cyanide solutions, shows certain changes in its absorption spectrum, or in some of its other properties, which cannot be accounted for by simple oxidation or reduction reactions, it is advisable to compare the derivative thus obtained with the same hæmoprotein solution treated with different concentrations of cyanide.

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A New Technique of Chromatography and Ionophoresis on Ion-exchange Paper. Application to Separation of Barbiturate, Salicylate, Acetophenetidin and *p*-Acetylaminophenol

IN a previous communication, we[1] showed that a mixture of salicylate, barbiturate, and acetophenetidin could be separated by a combination of chromatography and ionophoresis on a sheet of cellulosic anion-exchanger.

According to Stewart and Stolman[2], a major metabolite of acetophenetidin in the human body is *p*-acetylaminophenol. When *p*-acetylaminophenol is subjected to chromatography on Whatman *DE*20 ion-exchange paper, it moves at approximately the same speed as phenobarbitone, so that a mixture of these two compounds is not resolved. However, if the paper is now subjected to ionophoresis, the *p*-acetylaminophenol moves more slowly than the barbiturate. This means that, by using our new technique[1] of chromatography and ionophoresis on ion-exchange paper, it is possible to obtain complete resolution of a mixture of salicylate, phenobarbitone, acetophenetidin and *p*-acetylaminophenol. The results are illustrated in Fig. 1.

It also has been observed that when the wet sheet (following ionophoresis) is viewed in the light from a mercury-vapour lamp emitting radiation at 2,537 Å., the *p*-acetylaminophenol shows up as a dark purple absorbing spot. However, after the paper has dried, the spot shows a very light blue fluorescence when viewed in the ultra-violet light. This phenomenon is observed when only *p*-acetylaminophenol is present. It occurs following chromatography in dilute ammonia solution, but not when applied to the paper without ammonia treatment. Hence, the final dried sheet when examined in ultra-violet light shows the pale-blue fluorescent spot of *p*-acetylaminophenol differentiated quite clearly from the faster moving and dark purple absorbing spot of phenobarbitone.

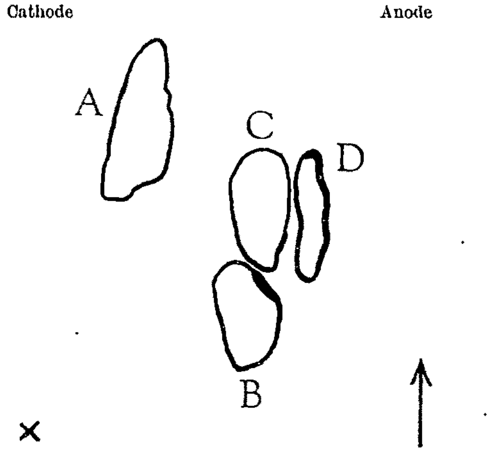

Fig. 1. Chromatography in 0·2 *N* ammonia (15 min.) followed by ionophoresis in 0·2 *N* ammonia (60 min.; constant current, 5 m.amp.; initial voltage, 250 V.) of a mixture of acetophenetidin (*A*), salicylate (*B*), *p*-acetylaminophenol (*C*) and phenobarbitone (*D*). Arrow indicates direction of ascending solvent-flow during chromatography; mixture applied at *X*

The chromatography separation takes about 15 min., ionophoresis about 1 hr. 0·2 *N* ammonia solution is used as 'solvent'.

A full account of these procedures will be published elsewhere.

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Isolation of Beta-Alanine from *Iris tingitana* (var. Wedgewood)

BETA-ALANINE has often been reported (for example, Hulme and Arthington[1]) in plants on the basis of chromatographic evidence and is now considered to be a common plant constituent[2].

Virtanen and Laine[3] isolated beta-alanine from the root nodule bacteria of peas, but there is no published report of the isolation of beta-alanine from higher plants. In the process of isolating beta-amino*iso*butyric acid from iris bulbs[4], beta-alanine has been obtained in crystalline form and characterized by classical physical and chemical methods.

Beta-alanine and beta-amino*iso*butyric acid were separated from the other neutral amino-acids in the 80 per cent ethanol extract of iris bulbs (45 kgm.) on 'Dowex I' in the salt form[5]. The two beta amino-acids were separated from each other by chromatography with *n*-butanol/methanol/water (2 : 2 : 1) on a paper roll. Final purification was accomplished by crystallization from aqueous ethanol, resulting in 300 mgm. of material. Quantitative analysis of an extract of a different sample of Wedgewood iris resulted in a value of 0·045 µmole of beta-alanine per gm. of tissue fresh weight. The elemental analysis of the isolated material was 40·5 C, 7·93 H and 15·7 per cent N (theoretical values for beta-alanine are 40·3 C, 8·15 H and 15·6 per cent N). The infra-red spectrum of the isolated material in a potassium bromide disk was identical with that of authentic beta-alanine. The iris amino-acid co-chromatographed with beta-alanine in phenol/water (8 : 3), butanol/acetic acid/water (9 : 1 : 2·5) and *n*-butanol/methanol/water (2 : 2 : 1).

The 2,4-dinitrophenyl derivatives of the isolate and authentic beta-alanine were prepared by Levy's method[6]. After several recrystallizations from 95 per cent ethanol, the derivatives of the isolated and synthetic amino-acids melted at 146–147° C. (uncorrected). A mixed melting point of 146–147° C. was observed. Co-chromatography of the two derivatives in Levy's solvent system produced no separation.

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Possible Role of Nucleopeptides in Protein Biosynthesis

WE have reported on the isolation of nucleopeptides from guinea pig liver[1]. Further work[2,3] has revealed that these compounds were oligonucleotides combined with various peptides containing more than ten amino-acids. Steinberg *et al.*[4] have isolated some compounds of similar kind from rat kidney and liver, as well as from rabbit liver.

This communication presents some results concerning nucleopeptide biosynthesis within a cell-free system, as well as their incorporation into protein.

Guinea pigs were used for experiments. The nucleopeptide synthesis was carried out by a method identical with that applied for protein biosynthesis *in vitro*. The mixture composition was that described under Fig. 2, the only difference being that in this experiment 1 ml. of the mixture contained, instead of nucleopeptides, 0·66 μc. of L-glutamate, 0·66 μc. of L-aspartate, 0·66 μc. of L-arginine and 0·3 mgm. of each of the twenty various non-radioactive L-amino-acids ; the radioactive ones just mentioned were omitted. One-half of this mixture was sampled at the beginning of the experiment and saturated with solid ammonium sulphate. The other half was incubated at 37° for 20 min., then treated with ammonium sulphate in the same manner. Nucleopeptides were isolated from these solutions by means of ethanol according to the procedure described in ref. 1. The residue obtained, after removal of ethanol, was eluted with acidified acetone (0·4 ml. of 1 *N* hydrochloric acid per 10 ml. of acetone), transferred on strips of Whatman No. 1 paper (5 × 57 cm.) and electrophoresis was carried out in the Markham and Smith apparatus[5]. The applied conditions were : ammonium acetate buffer 0·08 *M*, *p*H 5·0, potential gradient 50 V./cm., temperature 10–15°, and time 2 hr. The material examined was separated into three fractions visible in ultra-violet light and moving towards the cathode. The control test showed that these fractions did not contain free glutamate at all and neither aspartate nor arginine. The fractions were excised, eluted with 0·01 *N* hydrochloric acid, concentrated by evaporation and chromatographed within the solvent system containing butanol–acetic acid–water (4 : 1 : 5). The chromatography of radioactive glutamate, aspartate and arginine was performed under identical conditions. The photograph of the chromatogram was then taken in ultra-violet light and autoradiographed for 6 weeks (Fig. 1). The darkened areas of the autoradiograph corresponded to spots in the picture taken in ultra-violet light, although some spots showing absorption at 260 mμ did not contain any radioactivity. The paper was then sprayed with 0·5 *N* salt-free hydroxylamine and dried in air. After treatment with acidified ferric chloride some pink-brown spots arose which indicated the presence of hydroxamic acids. They corresponded in their localization to the spots found in the picture taken in ultra-violet light, as well as to the darkened areas of the autoradiogram. The spots showing high radioactivity were excised, eluted with 0·01 *N* hydrochloric acid and the eluate hydrolysed with 6 *N* hydrochloric acid for 16 hr. at 105°. Then hydrochloric acid was evaporated, iron removed with ammonium hydroxide, the residue chromatographed, as described, and the chromatogram developed with ninhydrin. Three to seven spots were found. The spots corresponding to glutamate, aspartate and to arginine were excised and their radioactivity was recorded.

Experiments were undertaken afterwards to establish whether the peptide part of nucleopeptides might

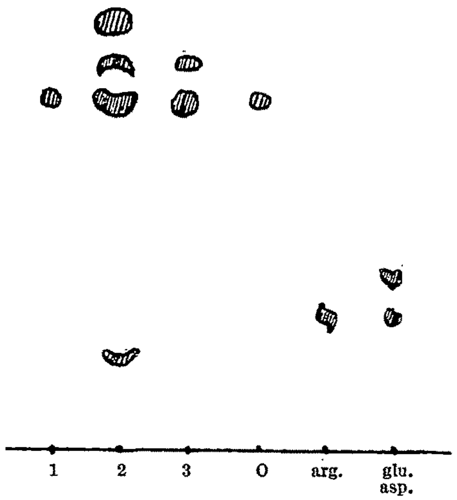

Fig. 1. The autoradiographed chromatogram of the nucleopeptides obtained *in vitro* in the presence of carbon-14 labelled glutamate, aspartate and arginine, as well as that of the standard amino-acids. 1, 2 and 3 are fractions of nucleopeptides after incubation and electrophoresis; O, before incubation

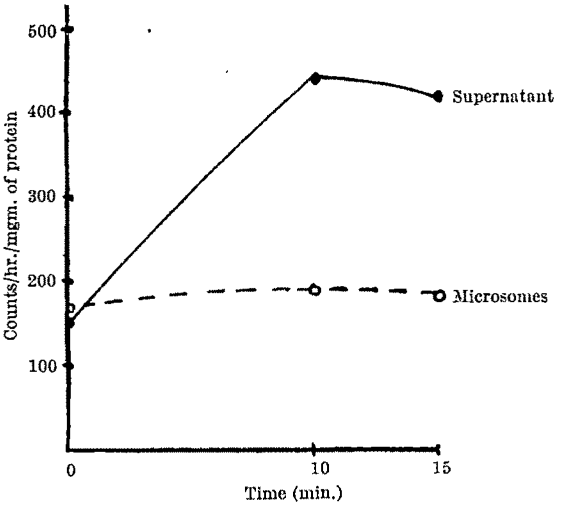

Fig. 2. Incorporation of nucleopeptides into proteins within a cell-free system. Incubation mixture contained in 1 ml. : 12 mgm. of the guinea pig liver microsomal protein (obtained in 0·25 *M* sucrose between 10,000*g* and 40,000*g*), 5·6 mgm. of the *p*H 5 enzymes from the supernatant at 40,000*g*, 0·58 mgm. of ATP (potassium), 4·4 mgm. of potassium chloride, 0·12 mgm. of $MgCl_2$, 4·6 mgm. of phosphocreatine (sodium), 0·3 mgm. of the phosphocreatine kinase, 0·07 mgm. of GTP ($2Na.2H_2O$) ^{14}C-nucleopeptides, 3,200 c.p.m. Components were dissolved in 0·1 *M tris* buffer, *p*H 7·5. Samples for the radioactivity measurement were diluted with a threefold volume of 0·25 *M* sucrose and centrifuged at 40,000*g*

be incorporated into protein when preparations for synthesizing protein *in vitro* were applied. Radioactive nucleopeptides were derived from the liver of a guinea pig which had been injected into the heart with carbon-14 labelled glutamate, aspartate and arginine at doses of 45 μc./kgm. body-weight. The animal was killed by decapitation 3 min. after injection and nucleopeptides were isolated[1,2], the free radioactive amino-acids being removed by means of paper electrophoresis as described. The nucleopeptides formed *in vivo* eluted from electropherograms were added to the system for synthesizing protein *in vitro*. Then the radioactivity of protein was measured in microsomes as well as in the supernatant after centrifugation at 40,000*g*. The purification of proteins for this estimation was carried out according to the commonly applied procedure[6]. The radioactivity was measured with an end window Geiger–Müller counter. Fig. 2 shows that radioactivity appears mainly in the supernatant proteins, whereas its incorporation into microsomes is considerably less.

Our experiments prove that nucleopeptides are formed in the system for synthesizing protein *in vitro* and it seems that they participate in protein biosynthesis.

Details of these investigations will be published in *Acta Biochimica Polonica*.

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Configuration of the 6-Hydroxyl Group in Phenolic Steroids

DURING recent years reports from various laboratories have indicated that phenolic steroids substituted at C_6 may have biological significance. Thus, Mueller and Rumney[1] provided evidence for the conversion of radioactive œstradiol-17β to 6-hydroxyœstradiol-17β by mouse liver microsomes *in vitro*. Furthermore, 6-hydroxyœstradiol-17β has been isolated as a metabolite after incubation of œstradiol-17β with slices of rat liver[2] and human fœtal liver[3]. Bush *et al.*[4] detected 6-hydroxyœstradiol-17β in the follicular fluid of the mare, and Marrian[5] reported on the isolation of a new Kober chromogen (*KC–6B*) which appears to be identical with a 6-hydroxyœstrone.

The 6-hydroxyœstradiol-17β which served as a reference compound in this work was obtained by borohydride reduction of 6-oxo-œstradiol-17β [6]. Wintersteiner and Moore[6] have given reasons for believing that the 6-hydroxyl group thus formed may have a quasi-equatorial configuration; accordingly the compound was provisionally designated as 6'α'-hydroxyœstradiol-17β.

There seemed to exist various possibilities of obtaining more decisive configurational evidence, one of which involved the biological aromatization of 6α- and 6β-hydroxylated C_{19}-steroids to the corresponding phenolic steroids. After preliminary experiments it was decided to employ the aromatizing enzyme preparation from human placenta which has been shown by Ryan[7] to be most efficient in converting C_{19}-steroids to C_{18}-steroids. The investigation of the problem necessitated a paper chromatographic system for the separation of the two epimeric 6-hydroxyœstradiols. Both compounds are easily separated from each other in the system formamide/chloroform (5): ethyl acetate (1), the 'α'-epimer (formed by reduction of 6-oxo-œstradiol-17β with sodium borohydride[6]) travelling faster than the 'β'-epimer (formed by catalytic reduction of 6-oxo-œstradiol-17β with platinum oxide in ethanol[6]).

The incubation of 6α-hydroxy-Δ^4-androstene-3.17-dione with the 10,000*g* supernatant fraction of human placenta homogenate in the presence of adenosine triphosphate and diphosphopyridine nucleotide resulted in the formation of a phenolic metabolite which had the same R_F value as 6'α'-hydroxyœstrone[8] in the system formamide/chloroform. After reduction of the 17-oxo-group with sodium borohydride and subsequent chromatography in the formamide/chloroform-ethyl acetate system the substance showed the same mobility (14·7 cm./14 hr.) as the 6-hydroxyœstradiol-17β provisionally designated by Wintersteiner and Moore[6] as the 6 'α'-compound. When 6β-hydroxy-Δ^4-androstene-3.17-dione was incubated with the placental enzyme a phenolic metabolite was formed having a similar R_F value to 6'β'-hydroxyœstrone in the system formamide/chloroform. Reduction with sodium borohydride yielded a substance which on chromatography in the system formamide/chloroform-ethyl acetate showed the same mobility (12·8 cm./14 hr.) as the 6-hydroxyœstradiol-17β provisionally designated as the 6'β'-compound. After elution from the paper the material was acetylated and subjected to sublimation under atmospheric pressure. The melting point of the crystals (175–177°) was identical with that of authentic 6'β'-hydroxyœstradiol-17β-triacetate. An additional experiment was carried out with 6β-hydroxy-19-nor-testosterone. The phenolic substance obtained after incubation of this steroid with the placental enzyme showed the same mobility in the formamide/chloroform-ethyl acetate system as 6‘β’-hydroxyœstradiol-17β. By control experiments, the possibility was excluded that epimerization or oxido-reduction of the 6-hydroxyl groups in the neutral and phenolic steroids had occurred during incubation with the aromatizing enzyme.

Since the configuration of the 6-hydroxyl groups in neutral steroids[9] is well established, the experiments reported here now permit the definite assignment of configurations to the two epimeric 6-hydroxyœstradiols. The 6-hydroxyœstradiol-17β which results from reduction of the 6-oxo group with sodium borohydride is assigned the 6α-configuration, whereas the other epimer which is formed by catalytic reduction with platinum oxide has the 6β-configuration.

Full experimental details will be published elsewhere. We wish to express our thanks to Prof. M. Ehrenstein, University of Pennsylvania, Philadelphia, for a generous gift of 6α- and 6β-hydroxy-Δ^4-androstene-3.17-dione, and to Dr. A. Szpilfogel, N.V. Organon, Oss, for a sample of 6β-hydroxy-19-nortestosterone. We are also indebted to Dr. G. F. Marrian, Imperial Cancer Research Fund, London, and to Prof. M. Ehrenstein for reading the manuscript and for their helpful suggestions.

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Determination of Enzyme Activity by a Linear Measurement

The determination of the activity of proteolytic enzymes usually requires chemical analysis. A substrate, which can be either natural or synthetic, is being hydrolysed by the enzyme under specified conditions, and after a certain time the concentration of either the products or the remaining substrate is determined analytically. These methods are generally rather cumbersome. Furthermore, it is essential to perform the reaction under conditions such that only a small percentage of the substrate is transformed, so that the effect of the products on the reaction-rate should be negligible. This again leads to some analytical difficulties.

Let us suppose that a natural polymeric substance can be prepared in a homogeneous hydrated form, such as a gel, and then it can be cross-linked by some chemical means. A small number of cross-links, while leaving the chemical nature of the substrate virtually unchanged, will render the system insoluble, and relatively rigid. The matrix of the cross-linked substance may permit the penetration of small molecules or ions, but will be impenetrable to macromolecules, such as enzymes. Consequently, the action of the enzyme will be restricted entirely to the surface. Since the enzymatic breakdown products are usually soluble in aqueous media, these products will constantly be removed, and a fresh surface exposed to the enzyme.

If the enzyme-substrate interaction is restricted to the surface of the substrate, it is reasonable to assume that the reaction-rate will be proportional to the exposed surface area. Provided the substrate is homogeneous the reaction-rate will appear as a rate of change of volume of the substrate. These considerations can be expressed in the following relationship: $\frac{dV}{dt} = -k\,A$, where V is the volume of the substrate, t is the time, and A is the exposed surface area.

Denoting by dx the thickness of the layer of cross-linked substrate solubilized during time dt, then $dV = A dx$ or $\frac{dx}{dt} = -k$. In other words, the rate of change of any linear dimension perpendicular to the surface is constant. To determine k one measures a linear dimension perpendicular to the surface, for example, an edge of a cube, and plots the change of this length against time. A straight line should result, the slope of which is k. Since the concentration and the degree of cross-linking of the substrate are kept constant, different slopes will correspond to different enzyme activities. Measurement of the slope thus furnishes a means to determine enzyme activities.

The above principle has been applied successfully to the case of a number of proteolytic enzymes. A slightly modified gelatin was used as substrate, which is being manufactured by Schwarz BioResearch, Inc., under the trade name of ‘Thiogel’. ‘Thiogel’ differs from ordinary gelatin in that it has a few homocysteine residues attached to the chain through peptide bonds with the lysine ε-amino groups. By virtue of this, ‘Thiogel’ has approximately 10 sulphydryl groups per 100,000 molecular weight. Oxidation of these sulphydryls to disulphide groups provides a convenient way to cross-link the polymer. ‘Thiogel’ gels can be cross-linked, for example, by atmospheric oxygen at slightly alkaline *p*H values.

The properties of cross-linked ‘Thiogel’ gels make them well suited for enzyme studies. These gels are rigid, unmeltable and can be cut into convenient geometric shapes. The chemical characteristics of gelatin are fully preserved. The equilibrium volume of the gel changes only very slightly with temperature, *p*H or ionic strength of the medium. For example, the linear expansion of the gel is only about 4 per cent when the temperature is raised from 4° C. to 40° C. The gel represents a protein in a highly hydrated form, and due to its high water content (94 per cent) it is freely permeable to small molecules and ions, such as the hydronium ion, yet completely impermeable to macromolecules, such as enzymes.

Cross-linked ‘Thiogel’ gel of about 6·5 per cent concentration has been prepared and cut into cubes of about 2 ml. volume. These cubes can be stored under water at room temperature, if some preservative, for example a few drops of chloroform, is added to the water. Before use a cube is immersed in about 200 ml. buffered solution and equilibrated for 20–30 min. at the temperature and *p*H of the determination. After this the distance between two parallel planes of the cube is measured with a micrometer. This initial

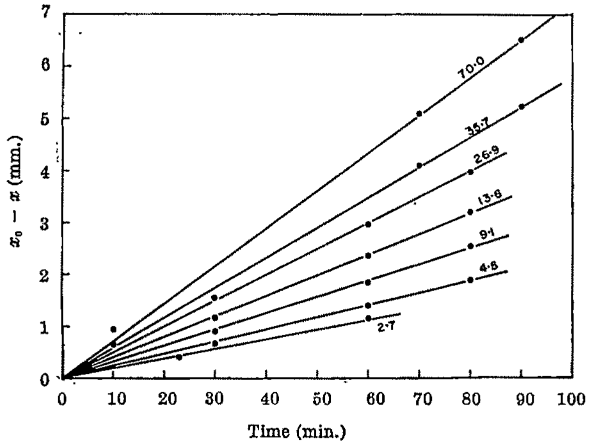

Fig. 1. Crystalline trypsin (pH 7·6, 36° C.)

distance was designated as x_0. At zero time a known amount of enzyme solution is added, and constant stirring applied. At regular intervals the cube is removed from the reaction mixture, rinsed with water to stop the reaction, and the distance x between the two parallel planes is again measured with the micrometer. After each measurement the cube is put back into the solution and the reaction continues. When plotting x_0–x against time, good linearity is generally obtained, so the slope can easily be determined and expressed in mm./hr. Sometimes at low enzyme concentrations a short induction period could be observed. In such cases it is advisable to determine x_0 about 10–20 min. after the addition of the enzyme.

Results obtained with crystalline trypsin are illustrated in Fig. 1. The trypsin concentrations (in mgm./l.) employed are given on the lines. All determinations were made at $36 \pm 0{\cdot}5°$ C. and at pH 7·6. The crystalline trypsin was prepared by Worthington Biochemical Corp., and its activity assayed about 8,500 units per mgm. determined with N-benzoyl arginine ethyl ester as substrate. Good linearity of $x_0 - x$ versus time was observed for at least 90 min. The slopes were reproducible to about 5 per cent. The magnitude of the slope is not proportional to the enzyme concentration, but follows the relationship: $\frac{x_0 - x}{t} = 0{\cdot}74\ c^{0{\cdot}427}$, where x is the distance in mm., t is the time in hours, and c is the trypsin concentration in mgm. per litre.

Other proteolytic enzymes are also being investigated using cross-linked 'Thiogel' as a substrate. Activated papain solubilizes the substrate readily. The change in linear dimension is proportional to time for about an hour. The slope is again proportional to a fractional power of the enzyme concentration. The action of crystalline pepsin and α-chymotrypsin was also studied, and it has been found that the rate of solubilization of cross-linked 'Thiogel' by these enzymes is lower by orders of magnitude as compared to the rate with trypsin. Thus the determination of the activity of trypsin can be performed in the presence of chymotrypsin.

The principal advantages of our method are that: (1) simple distance measurements are used instead of chemical analyses; (2) the enzyme–substrate interaction can be arrested merely by physical removal of the cross-linked gel from the solution; (3) the reaction can proceed over a long period of time without the reaction products having any effect on the rate since the region near the surface of the substrate may be continuously renewed. The sensitivity of our method depends on the precision of the distance measurement. The measurement need be made only over a short period of time if the substrate is a very thin sheet and one is observing the time of complete dissolution of the film. In Nature, proteases act on the surface of insoluble substrates and hence our system perhaps more closely resembles the natural condition than that with soluble protein substrates. When the cross-linked gel is removed from the enzyme solution, rinsed with water, and placed in a fresh buffer solution the dissolution of the substrate continues but at a progressively slower rate. In this manner the rate constant for the enzyme–substrate complex can be studied without the presence of free enzyme.

Our method should be applicable to other enzyme and substrate combinations. All that is required is that the enzyme be capable of cleaving either the polymer chain or the cross-linking bond of the hydrated cross-linked polymer substrate.

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Inhibition of Proteolytic Enzymes by Decarboxylated Amino-Acid Derivatives. Effect of Toluenesulphonyl(Tosyl)agmatine (4-Toluenesulphonamidobutylguanidine) on Thrombin and Trypsin

PROTEOLYTIC enzymes are known to catalyse the hydrolysis of specific amino-acid esters and amides[1]. Specificity is usually determined by the side-chain of the amino-acid contributing the carbonyl function to the bond being split, and, for several reasons, it may be inferred that this ester or amide bond lends very little to the initial binding of the substrate to the enzyme. Thus we suggest that derivatives of specific decarboxylated amino-acids would act as good competitive inhibitors of these enzymes. Since these derivatives would not be subjected to simultaneous hydrolysis, the effect would be longer lasting than that obtained with competing substrates.

One can readily construct a list of decarboxylated amino-acids which, when N-(amino) acylated, would be potential inhibitors for a number of enzymes. For example:

(1) Agmatine for thrombin, trypsin, plasmin and papain.

(2) 1,5-diaminopentane for trypsin and plasmin.

(3) 2-phenylethylamine or tyramine for chymotrypsin and pepsin.

(4) β-alanine or γ-aminobutyric acid for pepsin.

This list could, of course, be extended to include other enzymes with known specificities.

Thrombin (prepared by Rasmussen's method[2]) and trypsin (Worthington) were the enzymes chosen to test these ideas, and the kinetics of the hydrolysis of N$^{\alpha}$-tosyl-L-arginine methyl ester (L-TAMe)[3] were studied with the use of a pH-stat (pH 7·7; μ, 0·069; 37°). Tosylagmatine (m.p. 178°) was synthesized and tested for inhibitory activity. The latter compound inhibited the ester hydrolysis by both enzymes in a competitive manner, with the results summarized in Table 1.

Table 1. TOSYLAGMATINE INHIBITION OF L-TAME HYDROLYSIS

Enzyme	Inverse Michaelis constant l./mole	K_I (inhibition constant) l./mole
Thrombin	$1 \cdot 79 \times 10^3$	$7 \cdot 6 \times 10$
Trypsin	$2 \cdot 78 \times 10^3$	$2 \cdot 9 \times 10^2$

The inhibitory effect of tosylagmatine on thrombin was also examined by the independent technique of measuring *p*-nitrophenol production from the substrate carbobenzoxyglycine *p*-nitrophenyl ester[4]. Tosylagmatine again proved to be a competitive inhibitor, $K_I = 8 \cdot 12 \times 10$ l./mole (at μ 0·133; *p*H 7·5; 38·4°). Furthermore, this compound effectively inhibited clotting in the thrombin-fibrinogen system, and it also greatly delayed the clotting time of whole plasma after recalcification.

One may conclude that, in spite of the fact that optical activity is lost by decarboxylation, derivatives of decarboxylated amino-acids retain most of the required characteristics of fit to the enzyme surface to classify them as competitive inhibitors. We have reasons for believing that modifications to the acylating group added to the decarboxylated amino-acids will yield even better inhibitors.

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PHYSIOLOGY

Effect of Iproniazid on the Antileukæmic Action of Reserpine

AN earlier communication reported the ability of reserpine to exert an antitumour effect[1]. This effect has been, in general, associated with the depressant action of the drug on the central nervous system[1-3]. Treatment with reserpine produced a significant increase in the survival-time of mice carrying advanced lymphoid leukæmia (*L*1210) and caused regression of the local tumour which develops at the inoculation site[1]. Robison *et al.* have made similar observations[2]. Belkin observed that reserpine and chlorpromazine inhibited the growth of the solid tumour, sarcoma 37 [3]. Cranston[4], on the other hand, failed to demonstrate inhibitory action on recent transplants of spontaneous mammary adenocarcinoma in mice with reserpine, chlorpromazine, promazine or promethazine. Chorazy[5] found no inhibitory effect of chlorpromazine on Crocker sarcoma or Ehrlich carcinoma in mice.

Shore and Brodie[6] showed that, following pretreatment of rabbits with iproniazid (a monoamine oxidase inhibitor), reserpine administration resulted in excitatory and sympathomimetic responses. However, when iproniazid was administered after reserpine, it failed to alter the depressant effects on the central nervous system[6,7]. In an effort to dissociate the central depression induced by reserpine from its antitumour activity, experiments were conducted to determine the effect of prior administration of iproniazid on the antileukæmic effectiveness of reserpine in mice.

The reversal by iproniazid of the action of reserpine against advanced leukæmia is illustrated in Fig. 1. In this experiment daily injection of iproniazid (200 mgm./kgm.) was begun 6 days after the inoculation of mice with leukæmic cells. Reserpine was administered on day 8. The median survival-time of control leukæmic mice was eleven days. The median survival-time was increased progressively by increasing the dose of reserpine, reaching a maximum of 17 days at a dose of 12·5 mgm./kgm. of the drug. Daily injection of iproniazid reduced the median survival-time to that of the controls over the entire dose range of reserpine. Mice receiving iproniazid alone had the same median survival-time as the controls (11 days).

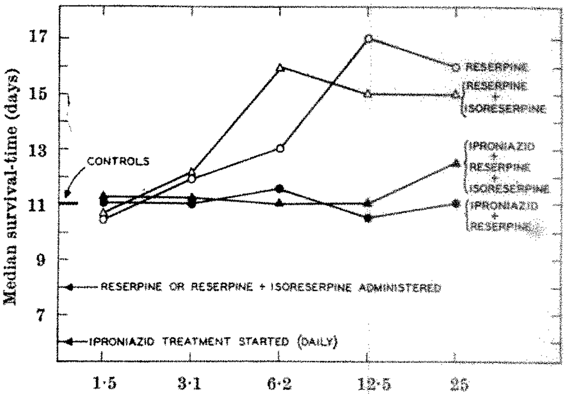

Fig. 1. The general methods have been previously described by Goldin *et al.* (refs. 9 and 10). Experiments were conducted in hybrid male mice ($(BALB/c\ An \times DBA/2J)F_1$) 8–10 weeks old and 20–25 gm. in body-weight. They were inoculated in the right hind leg with 0·1 ml. of a suspension of *L*1210 leukæmic cells. Treatment of advanced leukæmia was initiated when the local tumour reached a diameter of 9–12 mm., at which time the disease is systemic and can be transmitted by the blood. Iproniazid (200 mgm./kgm.) was administered intraperitoneally daily from day 6 until death. *Iso*reserpine (50 mgm./kgm.) was given subcutaneously on day 8. Reserpine at the doses noted was administered subcutaneously on day 8, 30 min. after the *iso*reserpine. Ten mice per group

The activity of 3-*iso*reserpine, a stereoisomer of reserpine, was of particular interest, since this compound is known to be devoid of depressant effects on the central nervous system. (One preparation of 3-*iso*reserpine produced marked central nervous system depression and antileukæmic activity similar to that seen with an effective dose of reserpine[1]. This preparation was later shown to be contaminated with 5 per cent reserpine.) The combination of 3-*iso*reserpine (50 mgm./kgm.) and reserpine (6·2 mgm./kgm.) prolonged survival-time three days over that observed with reserpine alone at this dose. This is suggestive of a potentiating effect by *iso*reserpine. This dose of *iso*reserpine alone did not produce depression of the central nervous system or increase the survival-time of mice with acute leukæmia. (This potentiating effect by 3-*iso*reserpine has been seen in several experiments. In addition, potentiation of reserpine action by 3-*iso*reserpine was also demonstrated in the prolongation of pentobarbital-induced anæsthesia. In one experiment, reserpine alone (2·5 mgm./kgm.) prolonged pentobarbital (70 mgm./kgm.) anæsthesia approximately four times, while in combination with 3-*iso*-reserpine (50 mgm./kgm.), anæsthesia was extended six times[8]. Reserpine and 3-*iso*reserpine were injected subcutaneously four hours prior to intraperitoneal pentobarbital.) However, when the same dose of 3-*iso*reserpine (50 mgm./kgm.) was used in combination with larger doses of reserpine, no further enhancement of survival-time was obtained. Injection of iproniazid decreased the median survival-time to that of the controls up to a dose of 12·5 mgm./kgm. of reserpine with *iso*reserpine (50 mgm./kgm.). At the highest dose (25 mgm./kgm. reserpine with 50 mgm./kgm. *iso*reserpine), the median survival-time of 15 days was reduced by iproniazid to 12·5 days.

Iproniazid appeared to reverse the depressant action both of reserpine and of reserpine in combination with 3-*iso*reserpine. Iproniazid also prevented the loss of body-weight and inhibition of local tumour growth. Only in the group receiving 25 mgm./kgm. of reserpine and 50 mgm./kgm. of 3-*iso*reserpine with iproniazid was depression of the central nervous system evident. The reversal of reserpine action by iproniazid was also demonstrated in other experiments, including one in which the drugs were administered early (3 days after inoculation with leukæmic cells) in the course of the disease.

The experiments reported in this communication failed to demonstrate a dissociation of the depressant action of reserpine on the central nervous system from the ability of reserpine to inhibit leukæmic tumour growth. Therefore, it remains to be determined whether this correlation is indicative of a non-specific host-mediated antileukæmic effect or whether there is a specific antitumour action of reserpine.

We wish to thank Ciba Pharmaceutical Products, Inc., for the reserpine ('Serpasil') and 3-*iso*reserpine used in this study and Hoffmann-LaRoche, Inc., for the iproniazid ('Marsilid phosphate').

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Influence of Gamma-Aminobutyric Acid on the Acetylcholine Responses of the denervated Rat-Diaphragm Preparation

THE literature on the actions of γ-aminobutyric acid was reviewed comprehensively by Elliott and Jasper in 1959 [1]. Several investigators have demonstrated that it and related substances are normal products of brain metabolism, and that it has an important action on brain function as well as on certain peripheral nerve structures[2]. It is known to reverse the surface-negative post-synaptic potentials of the apical dendrites of the cerebral cortex[3] and also blocks transcallosal conduction when injected into the internal carotid artery[4]. The exact mechanisms by which these effects are produced are still unknown. Hence work on the influence of γ-aminobutyric acid on the acetylcholine responses of the denervated rat's diaphragm was undertaken.

Denervation was carried out on the right side by section of the roots of the phrenic nerve and the preparations were used at variable times after the denervation.

A triangular strip of the right half of the diaphragm was dissected carefully and fixed by means of threads to a special electrode holder made of 'Perspex'. The whole preparation was immersed in a bath containing Krebs–Henseleit solution[5] and oxygenated by passing very fine bubbles of 95 per cent oxygen and 5 per cent carbon dioxide. The temperature of the bath was maintained at 37° C. The muscle was connected by its tendinous portion to a lever, and the activity of the muscle was recorded on a slowly moving smoked drum.

The muscle could be stimulated either electrically using a physiological stimulator (Ritchie–Sneath) or chemically using acetylcholine. The complete details of the technique are published in my thesis on the denervated rat-diaphragm preparation published in 1951 (ref. 5).

Several experiments were conducted and the results were accepted only after each new finding could be repeated several times. The following results were obtained:

(1) 400–1,000 µgm. γ-aminobutyric acid had no action on the responses of the innervated rat's diaphragm, when stimulated by maximal stimuli through its nerve (rate 4–5/min.). This work confirms that of Hobbiger[2].

(2) 1,000 µgm. of the drug has no action on the responses of the denervated rat-diaphragm prepara-

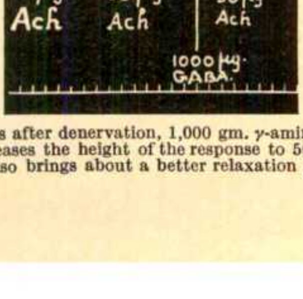

Fig. 1. 7 days after denervation, 1,000 gm. γ-aminobutyric acid not only increases the height of the response to 50 µgm. acetylcholine but also brings about a better relaxation of the muscle

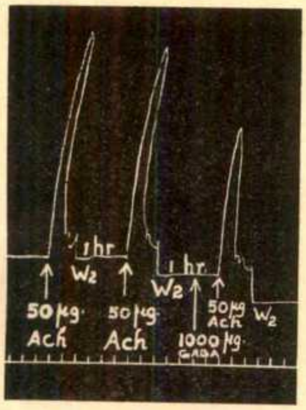

Fig. 2. 8 days after denervation, 1,000 gm. γ-aminobutyric acid not only reduces the height of the response to 50 μgm. acetylcholine but also brings about better relaxation of the muscle

tion (8–10 days after denervation) when stimulated by maximal stimuli at a rate of 4–5 per min.

(3) In my thesis on the denervated rat diaphragm preparation[5], I observed that the first noticeable response to a test dose of acetylcholine (50 μgm.) was obtained on the third or fourth day after nerve section. The sensitivity thereafter increased progressively and was fairly well established by the second week. This state was maintained up to the fifth to sixth week, and then there was a steady decline to almost nothing by the third month after denervation.

As the sensitivity of the denervated preparation alters with time, the influence of γ-aminobutyric acid on the acetylcholine responses was studied with preparations 7, 8, 9 and 10 days after denervation. It was noticed that 1,000 μgm. produced two main changes to the responses of the denervated preparation to 50 μgm. of acetylcholine: (*a*) An alteration in the height of the contracture. The height was always increased in 7-day preparations and diminished in 8-day preparations (Figs. 1 and 2). (*b*) A more complete relaxation of the muscle was noticed in both preparations. It was also found that 1,000 μgm. γ-aminobutyric acid had variable actions on the acetylcholine responses of 9-day-old preparations and no influence whatsoever on 10-day-old preparations.

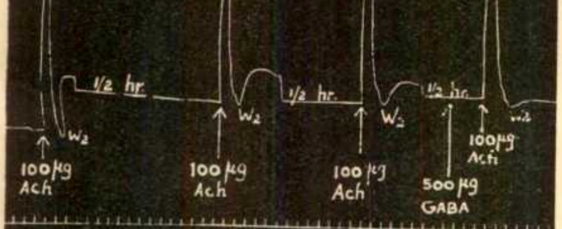

Fig. 3. Responses of a denervated preparation (8 days) when immersed in Krebs–Henseleit solution containing one quarter the normal concentration of potassium chloride. 500 μgm. γ-aminobutyric acid is found to bring about a better relaxation of the muscle after the same dose of acetylcholine (100 μgm.)

(4) It was noticed that 500 μgm. γ-aminobutyric acid abolished the second contracture that occurs when 100 μgm. of acetylcholine was introduced into a bath containing a denervated preparation with a potassium concentration one quarter that found in normal Krebs solution (normal Krebs has 5·52 gm. potassium chloride/15 litres, Fig. 3).

(5) 1,000 μgm. γ-aminobutyric acid had no influence on the contracture produced by 50 μgm. adrenaline on the denervated rat-diaphragm preparation.

This work has been done in the Department of Physiology, University of Ceylon, since 1958.

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Effect of Duodenal Administration of Highly Unsaturated Fatty Acids on Composition of Ruminant Depot Fat

Although linolenic acid is the main fatty acid of pasture lipids, the fats of pasture-fed ruminants contain little if any of this constituent[1]. Also, feeding large quantities of highly unsaturated oils to ruminants has had no perceptible effect on the unsaturation of their depot fats[2]. Various workers[3–5] have shown that micro-organisms in the rumen hydrogenate dietary unsaturated fatty acids, and this has been presumed to explain the low content of highly unsaturated fatty acids in the depot fats of ruminants.

To test whether this explanation was correct the rumen micro-organisms have been by-passed and dietary lipids rich in linoleic and linolenic acids presented to the tissues by administering linseed oil through a duodenal cannula to a sheep, and the effect on the composition of the depot fat observed.

A mature Merino ewe was cannulated about 6 in. from the pylorus and fed on chaffed lucerne hay. About 40 ml. of an emulsion of 8 vol. of linseed oil and 1 vol. of bile were given each day, half in the morning and half in the evening, for six weeks. The perinephric fat was then obtained after killing and the fatty acids subjected to gas-liquid chromatography. Results are given in Table 1.

Palmitic, stearic and C_{18} monoene (oleic) contents of the fat were within the normal range, but the C_{18} diene (linoleic acid) and triene (linolenic acid) contents were greatly above the normal range of 1–2 per cent. This shows that the main fatty acid constituents—linoleic and linolenic acids—of linseed oil

Table 1. FATTY ACID COMPOSITION OF PERINEPHRIC FAT (MOLAR PER CENT)

	n-Saturated	Branched saturated	Unsaturated
C_{10}	0·20	—	—
C_{12}	trace	—	—
C_{14}	1·20	0·10	—
C_{15}	1·00	0·89	—
C_{16}	26·83	—	1·09
C_{17}	2·99	2·19	—
C_{18}	27·12	—	Monoene 18·55 Diene 7·87 Triene 9·97

are capable of being deposited in the depot fats of ruminants as in the case of non-ruminants provided they are not first subjected to the action of the rumen micro-organisms.

Thus the hypothesis mentioned here, that the differences between the depot fats of ruminants and non-ruminants arise from the hydrogenation of the unsaturated dietary fats in the rumen, receives further support.

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Photomotor Reflex as an Indicator of Consciousness during Curarization

WHEN a relaxant is used in conjunction with a general anæsthetic, a new problem arises in assessment of the depth of anæsthesia: the reflexes which normally indicate returning consciousness and awareness of pain cannot appear if the patient is under the influence of a relaxant. Thus, if only reflexes involving voluntary muscle are used, it is possible, in the presence of a relaxant, for anæsthesia to lighten so that surgery is performed on a conscious paralysed patient without surgeon or anæsthetist being aware of the situation. This has been recorded more than once in human medicine, the patient having described the situation after the operation[1]. The same situation can occur in operations on animals when an anæsthetic is used in conjunction with a relaxant; but in this case the patient cannot afterwards direct attention to the mistake. Such circumstances produce problems with humanitarian and legal aspects for the veterinarian, as Finn[2] has pointed out; they also complicate the interpretation of the pain clause for research workers holding a vivisection licence.

The electroencephalograph can be used to assess depth of anæsthesia in a curarized man or animal, but it is not always available, and needs some skill in interpretation. Experiments have therefore been carried out to discover a simple method of assessing depth of consciousness in curarized dogs, the electroencephalograph being used as a criterion of the activity of the brain. Pentobarbitone sodium, thiopentone sodium and ether were used as anæsthetics, and succinylcholine chloride as the relaxant.

It was found that the photomotor reflex (change in size of pupil of eye with sudden change in light intensity), unlike the other reflexes commonly used for testing anæsthesia, gave a reliable indication of returning consciousness in the presence of a relaxant. It became positive as the dog passed from stage III (surgical anæsthesia) to stage II (excitement). Pre-medication with atropine did not invalidate the reflex, provided that the dose of atropine did not exceed 0·032 mgm./kgm. body-weight. It seems likely that this reflex would also be useful in human patients in similar circumstances. The work will be described in more detail elsewhere.

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Spiral Nerve-endings (Proprioceptors) in the Human Vocal Muscle

THE problem of the proprioceptive innervation of the human vocal muscle is still controversial[1]. The presence of a proprioceptive mechanism controlling the fine adjustments of the vocal cords has been postulated by some authors; it has been rejected on theoretical grounds by others. Paulsen[2] assumes that the tension of the vocal muscle would have central control alone. A peripheral proprioceptive mechanism would be rather a hindrance for normal phonation. The same was said to be true of the extrinsic eye muscles, in which nevertheless proprioceptors have been demonstrated within the past ten years histologically as well as by electrical records[3-5].

To clear up the question, we have cut the vocal muscle of men who had shown no evidence of neurological disease during life, in longitudinal frozen sections of 40–60μ thickness. With a modification of the silver staining Bielschowsky–Gros method, we succeeded in showing spiral nerve-endings in this muscle, which may be interpreted as mechanoreceptors responding to stretch or shortening of the fibres (Fig. 1).

These spiral structures have been found to form a group of 3–5 nerve-endings occupying a small place within the motor innervation zone[6] near the free inner board of the muscle at the level of the vocal

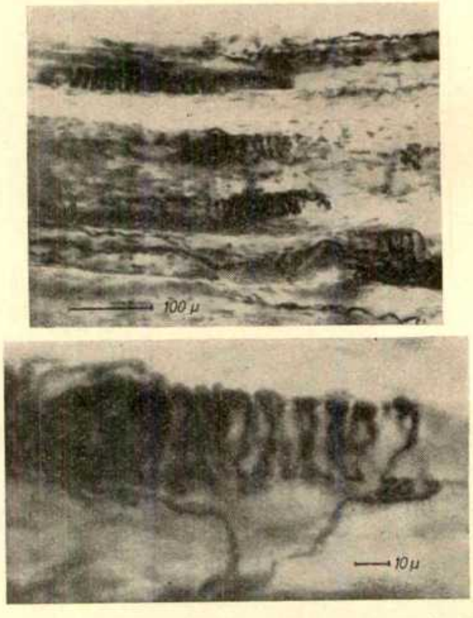

Fig. 1. Spiral nerve-endings in the human vocal muscle; silver staining. Top: Group of receptors in the small diameter muscle fibre area (inner board) of the muscle. Bottom: One receptor with higher magnitude; double spiral turns in the left part around two small muscle fibres

string. They look like the annulo-spiral endings in the equatorial region of muscle spindles. Their length varies from 100 to 200μ. The nerve fibre is coiled up around one or two small muscle fibres in steep turns. Between the spiral endings are closely packed nuclei, suggesting a region of intense metabolic activity. The size of muscle fibre varies in the vocal muscle from 10 to 60μ. The spiral nerve-endings and their associated muscle fibres lie within a region where the diameter of most of the muscle fibres does not exceed 10μ. One or two end-plates of the associated fibres which stained in some cases were found within 100μ from the spiral district. There is no histological evidence that an efferent gamma-system exists in the vocal proprioceptive mechanism. A connective tissue wrap around the spindle organ does not seem to be present.

These structures differ from the classical form of spindles described by Kühne and Ruffini. There is perhaps some relation to the spiral nerve-endings in extrinsic eye muscles of which the receptor properties have been shown[4]. The spiral ending in the vocal muscle may be a rather primitive form of proprioceptor, which is no surprise in a muscle of such particular origin (Goerttler).

The fact that these spiral nerve-endings are not encapsulated and the inner muscle fibre diameter does not differ markedly from the diameter of the surrounding muscle fibres may be the reason why they have been overlooked in transverse sections. They could relate to the recently described myotatic reflex elicited in the human vocal cord[7].

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Active Transport of Foreign Pyrimidines across the Intestinal Epithelium

THE small intestine of mammals possesses at least three independent active transport processes for the absorption of organic compounds: one for monosaccharides like glucose and galactose[1]; one for L-amino-acids[2]; and one for pyrimidines like uracil and thymine[3]. These specialized absorption processes have a number of characteristics in common which distinguish them from the process of simple diffusion: the substrate is transferred against a concentration gradient; the transport systems become saturated at high concentrations of substrate; and one substrate depresses the passage of another by competing with it for the transport process.

In contrast to the natural cell substrates, most drugs and other foreign organic compounds are absorbed mainly by simple diffusion[4,5]. The intestinal epithelium behaves towards these substances as an inert, lipid-like layer, readily penetrated by lipid-soluble, undissociated molecules, and penetrated with difficulty by lipid-insoluble molecules and ions.

The present work indicates that foreign organic compounds similar in structure to a normal substrate can be absorbed by a specialized transport process. Evidence is presented that two foreign pyrimidines with anti-tumour activity, 5-fluorouracil and 5-bromouracil, are actively transported by the pyrimidine transport system of the intestine.

For the experiments, male Sprague–Dawley rats (120–130 gm.), fasted for 20 hr. but allowed free access to water, were killed by decapitation, and the upper part of the small intestine was removed. Sacs of everted intestine[6], 20 cm. long, were filled with 2 ml. of bicarbonate–saline solution[7] which contained 1 gm. of glucose per litre and various concentrations of uracil-2-^{14}C, 5-fluorouracil-2-^{14}C, or 5-bromouracil-2-^{14}C. The sacs were suspended in 15 ml. of the same fluid contained in 50-ml. beakers, and the beakers shaken in a Dubnoff metabolic shaker (90 oscillations per min.) at 37° C. in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. After 1 hr., the mucosal and serosal solutions were collected, and the concentration of pyrimidine estimated from the radioactivity of the solutions.

Evidence that the labelled compounds are not altered metabolically by the intestine was provided by paper chromatography of the pyrimidine solutions incubated with the intestine. Chromatograms developed in either *n*-butanol/acetic acid/water (20 : 6 : 17 v/v/v) or ethyl acetate saturated with 0·1 *M* acetate buffer (*p*H 5·3) revealed single radioactive spots with R_F values the same as those of the non-incubated pyrimidines chromatographed on the same sheets.

The extent of transfer of the pyrimidines across the intestinal epithelium, against a concentration gradient, is shown in Table 1. When the initial concentration of pyrimidine was 0·02 m.mol./litre, fluorouracil was transported to about the same extent as uracil, attaining a serosal/mucosal concentration ratio of 4·5; bromouracil was transported to a somewhat smaller degree, attaining a serosal/mucosal ratio of 3.

Table 1. INTESTINAL TRANSFER OF PYRIMIDINES AGAINST A CONCENTRATION GRADIENT *in vitro*

Pyrimidine	Concentration of pyrimidine (m.mol./l.)			Final, serosal to mucosal concentration ratio
	Initial, mucosal and serosal solutions	Final, mucosal solution	Final, serosal solution	
Uracil	0·020	0·012	0·056	4·7 ± 0·3*
5-Fluorouracil	0·020	0·011	0·049	4·5 ± 0·3†
5-Bromouracil	0·020	0·013	0·039	3·0 ± 0·2†

* Mean value for 27 animals ± *S.E.*
† Mean value for 8 animals ± *S.E.*

The transport process became saturated at high concentrations of the pyrimidines. For example, on raising the concentration of fluorouracil or bromouracil to 0·5 m.mol./litre, the serosal/mucosal concentration ratios declined to values approaching unity.

An indication that fluorouracil, bromouracil and uracil cross the intestinal epithelium by the same transport system was obtained from experiments in which the foreign pyrimidines depressed the transfer of uracil. For example, at a concentration of 5 m.mol./litre, fluorouracil and bromouracil inhibited completely the active transport of 0·02 millimolar uracil.

The nature of the substituent at the 5-position of the uracil molecule does not appear to be of great importance in determining the extent to which the molecule is actively transported, since uracil, 5-methyluracil (thymine), 5-fluorouracil and 5-bromouracil are all transported across the intestinal epithelium to a similar extent. On the other hand, since the hydrogen, fluorine and bromine atoms and the methyl group have Van der Waals radii of a similar magnitude (1·2–2·0 Å.)[8], it is possible that a considerably larger substituent would interfere with transport of the molecule. Investigations are now being made to determine the structural characteristics of pyrimidines which are required for active transport.

The results of this work suggest that anti-metabolites may not only compete with normal substrates for metabolic processes within the cell, but may also compete with these substrates for transport into the cell.

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Changes of Quantity of Flavin in the Brain after Peripheral Administration of Flavins

IT has been reported from our laboratory[1] that flavins produce a change in the electroencephalogram, and that the effect produced by chlorpromazine is partly reversed by flavin adenine dinucleotide. These results suggest that the flavins act on the brain *in situ*. In an attempt to discover the mechanism of action of flavins, their penetration of the blood–brain barrier was examined by determining the amount of flavin in the rat brain after peripheral injection of flavins.

Flavin adenine dinucleotide was prepared by the method of Yagi *et al.*[2]. Flavin mononucleotide and riboflavin were purified chromatographically from commercial samples. Albino rats weighing about 100 gm. were used for the experiment. Fifty animals, 10 days after feeding under the same conditions, were divided into five groups, each group consisting of 10 animals. The animals of the first group were used as control, and injected with water intra-abdominally. Each animal of three groups was injected intra-abdominally with 20 mgm./kgm. (calculated as riboflavin) of flavin adenine dinucleotide, flavin mononucleotide, or riboflavin aqueous solution respectively. The animals of the fifth group were injected with 50 mgm./kgm. of an aqueous solution of riboflavin. One hour after the injection, a blood sample was collected from the carotid artery, and then the animal was killed by decapitation. The quantity of flavin in the brain was measured by the lumiflavin fluorescence method described by Yagi[3], and that in the blood sample was measured by the micro-method for riboflavin in blood described by Yagi *et al.*[4].

Table 1. QUANTITY OF FLAVIN (CALCULATED AS RIBOFLAVIN) IN RAT BRAIN AFTER THE INJECTION OF FLAVINS (μgm./gm. OF WET TISSUE)

	Means of population (n = 10), (α = 0·01)
Controls	3·32 ± 0·35
After the injection of:	
Flavin adenine dinucleotide (20 mgm./kgm.)	3·75 ± 0·69
Flavin mononucleotide (20 mgm./kgm.)	3·73 ± 0·85
Riboflavin (20 mgm./kgm.)	5·18 ± 0·74
Riboflavin (50 mgm./kgm.)	7·37 ± 0·87

The quantities of flavin in the brain after the injection of flavins are shown in Table 1. The variances in the normal cases were rather small, and the values were in good agreement with the earlier results[5]. After the injection of flavin adenine dinucleotide, the increase of flavin in the brain was significant by the t-test with $\alpha = 0{\cdot}1$. In the case of flavin mononucleotide, the increase was significant with $\alpha = 0{\cdot}2$. However, after the administration of 20 mgm./kgm. of riboflavin, flavin in the brain increased, the difference being significant even with $\alpha = 0{\cdot}01$. After the injection of 50 mgm./kgm. of riboflavin, further increase of flavin was observed.

The increases of flavins in blood at 1 hr. after the administration to each animal of 20 mgm./kgm. of flavin were almost equal for the three types of flavins, being less than 1 μgm./ml. of blood. Since the brain contains 0·024 ml. of blood per gm. on the average[6], the increase of flavins due to the blood content of the tissue was about 0·02 μgm./gm. The corrected amounts found by subtracting the values due to the blood from that of the tissue gave a similar result.

It seems, therefore, that the increase of flavin in the brain was due to the penetration of injected flavins from the blood into the brain tissue. It is considered that the flavins penetrate the blood–brain barrier, and that riboflavin can cross this barrier more easily than flavin mononucleotide or flavin adenine dinucleotide.

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Reversible Block of Axonal Conduction by Curare after Treatment with Cobra Venom and a Detergent

THE recent experiments of Abbott, Hill and Howarth[1] have revealed that the remarkably high initial heat produced in nerve fibres coincides roughly with electrical activity. This finding re-emphasizes the necessity of explaining electrical activity on a chemical basis. Nachmansohn's theory[2] attributes the change of conductance which initiates the ion movements to the reaction of acetylcholine with a receptor protein. One of the main objections to this theory is the lack of effect of acetylcholine and curare on axons in contrast to their powerful action at junctions. This failure was attributed to the existence of structural barriers for lipid-insoluble quaternary ammonium compounds. Evidence supporting this contention was obtained in experiments on squid giant axons: acetylcholine, labelled with nitrogen-15, and prostigmine failed to penetrate into the axoplasm whereas lipid-soluble tertiary compounds did so readily[3,4]. However, at the nodes of Ranvier, where these barriers are relatively small, curare can rapidly and reversibly block conduction, as shown in recent work by Dettbarn[5]. Walsh and Deal[6] succeeded in partially breaking down these barriers in frog sciatic nerve by treatment with the cationic detergent cetyltrimethylammonium bromide, after which curare and other quaternary ammonium compounds, ordinarily inert on the intact axon, caused reversible block of conduction.

In the present work the squid giant axon was pretreated with a variety of enzymes and detergents in an attempt to permit the action of curare and other compounds. Of the many pre-treatments used, the combination of *Naja naja* cobra venom (10 μgm./ml.) and cetyltrimethylammonium chloride (20 μgm./ml.) applied for 30 min. was most effective in permitting the action of curare applied afterwards on electrical activity. In many experiments, complete and reversible block of conduction was obtained (Fig. 1), sometimes at a curare concentration as low as 5×10^{-4} *M*; $1 \cdot 4 \times 10^{-2}$ *M* curare (10 mgm./ml.) on the intact nerve produced little if any effect.

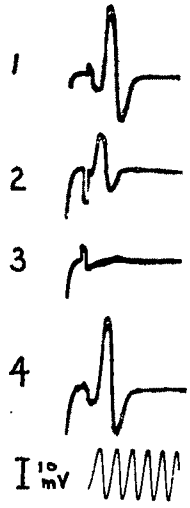

Fig. 1. Effect of curare on squid axon following pre-treatment with 10 μgm./ml. cobra venom plus 20 μgm./ml. cetyltrimethylammonium chloride. (1) Control after 30 min. pre-treatment with cobra venum plus cetyltrimethylammonium chloride. Pretreatment had no effect on spike height. (2) and (3), 5 and 10 min. after application of $1 \cdot 4 \times 10^{-3}$ *M* curare. Conduction was completely blocked, as shown in 3, despite greatly increased stimulus strength. (4) 10 min. after return to sea water. Time signal: 850 c./sec.

The action of a series of tertiary compounds including local anæsthetics (procaine and dibucaine) on the untreated axon was also studied. In each case, these compounds affected conduction at concentrations similar to those required to block synaptic junctions of a single isolated electroplax preparation[7]. Local anæsthetics such as tetracaine and dibucaine, known to block all axonal activity, have recently been shown to react in a highly specific way with the acetylcholine receptor protein[8]. Curare has a high affinity to this protein[9] and has now been shown to block axonal conduction. These results suggest that the receptor protein is present in the axon and is essential for electrical activity. Failure of lipid-insoluble cationic substances such as curare to act on the intact nerve is evidently due to the protection of such receptors by permeability barriers and not to a mechanism which differs basically from that at the junction. Cobra venom and cetyltrimethylammonium chloride probably break down these barriers to an extent which permits curare to react with the receptors and block conduction. The evidence for the action of curare on axonal conduction, to be published in greater detail elsewhere, together with the other results mentioned above, provide new support for Nachmansohn's theory.

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HÆMATOLOGY

Hæmoglobin Variants in Sarawak and North Borneo

ABNORMAL hæmoglobins are frequent in south-east Asia[1]. However, only one report of a search for abnormal hæmoglobin variants in Sarawak has so far appeared: among 101 blood samples from Land Dyaks and 85 from Sea Dyaks, only one (from a Land Dyak) contained an abnormal variant and represented the heterozygous state for hæmoglobin *E* [2].

Recently, 1,355 blood samples from Sarawak were studied by the technique of filter-paper electrophoresis at *p*H 8·6. The distribution of samples was Sea Dyaks 68, Land Dyaks 341, Chinese 742, Malay 166, Indian 13, European 23, Eurasian 1 and Filipino 1. All the samples were taken from persons in the First Division of Sarawak and largely from areas within reach of Kuching, and came from hospital in-patients, ante-natal cases and a few out-patients and candidates for government posts. The age distribution was 3–72 years and the sexes were approximately equally represented. All the samples from Sea Dyaks, Land Dyaks, Indians and the Eurasian and Filipino were found to contain only normal adult hæmoglobin (hæmoglobin *A*). Among the samples from Chinese, Malay and European subjects, respectively, 2, 5 and 1 contained a mixture of hæmoglobins *A* and *E*, while 2 samples from Chinese contained a mixture of hæmoglobins *A* and a 'slow' hæmoglobin with properties very similar to those of hæmoglobin *Q*. It has not proved possible to characterize this slow hæmoglobin in great detail. The sample containing hæmoglobins *A* and *E* from a European 'white' came from an English woman whose lineage it was not possible to trace.

Through the courtesy of Prof. T. A. Lloyd Davies, of the Department of Social Medicine and Public Health, University of Malaya in Singapore, it was possible to study 53 blood samples obtained from Muruts inhabiting the Crown Colony of North Borneo. The Muruts are "small, stocky, mongoloid people, with light brown skin and straight black hair"[3]. They are a group of related pagan tribes which have been relatively isolated because they were practising head-hunters until recently. All but 3 of these samples contained only hæmoglobin *A* ; in the rest the pattern was that of a mixture of hæmoglobins *A* and *E*. The slow component in these three samples was probably not hæmoglobin A_2, since it comprised more than 20 per cent of the total hæmoglobin[4].

The absence of any abnormal variants among the Dyaks examined and the solitary finding of one example of hæmoglobin *E* trait by Colbourne *et al.*[2] indicates the infrequency of abnormal variants in these people. The finding of hæmoglobin *E* in 2 Chinese and in 5 Malays parallels similar findings among these ethnic groups in Singapore and Malaya[5]. The existence of a focus of a hæmoglobin, which is probably *Q*, among the Chinese in Kuching is indicated.

We are grateful to Dr. W. Glyn Evans, director of Medical Services, Sarawak, for permission to carry out this investigation.

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Production of *B* Character on Human Erythrocytes by Hæmosensitization with Purified *Escherichia coli O* 86 Substance

The serological specificity of the human blood group substances *A* and *B* is due to characteristic polysaccharide components[1]. Recently, the same has been found to apply to the *Salmonella O* antigen[2]. The serologically active component, a lipopolysaccharide, was isolated from the bacteria and submitted to chemical analysis, which showed that in various strains within the same *O* group or *O* sub-group, the *O* antigen is of an identical qualitative-chemical composition, that is, it contains the same 'Zuckerbausteine'.

A lipopolysaccharide from *E. coli O* 86, obtained by this procedure, was placed at my disposal. An investigation was conducted to ascertain whether this purified preparation, of known qualitative-chemical composition, might possess blood-group activity of *A* and/or *B* specificity. By previous work[3,4] and my earlier work it had been shown that *E. coli O* 86 contained *A*-like and *B*-like substances. The investigation was carried out by hæmosensitizing human *O* cells with the purified lipopolysaccharide by the following procedure :

Table 1. Sensitized *O* Cells and Normal *B* Cells in different Anti-*B* Reagents
(All reactions performed on a glass plate at 20° C. ; readings after 10 min.)

	Human *AB* serum or anti-*A*	Human anti-*B*	Human anti-*B* absorbed with human *B*	Human anti-*B* absorbed with rabbit *B* (β_1)	Human anti-*B* absorbed with sensitized *O*
Normal *O* cells	–	–	–	–	–
Sensitized *O* cells	+	+++	+	+	–
Normal *B* cells	–	+++	–	+	++

	Rabbit immune anti-*B* (β_1)	Chicken immune anti-*B* (β_2)	Chicken immune anti-*B* absorbed with human *B*	Chicken immune anti-*B* absorbed with rabbit *B*	Chicken immune anti-*B* absorbed with sensitized *O*
Normal *O* cells	–	–	–	–	–
Sensitized *O* cells	–	++	–	–	–
Normal *B* cells	+++	+++	–	–	++

	Eluate from human *B* cells incubated with human anti-*B*	Eluate from sensitized *O* cells incubated with human anti-*B*	Eluate from sensitized *O* cells incubated with human anti-*B* and absorbed with human *B*	Eluate from sensitized *O* cells incubated with human anti-*B* and absorbed with rabbit *B*
Normal *O* cells	–	–	–	–
Sensitized *O* cells	–	++	–	–
Normal *B* cells	+++	+++	–	–

10 mgm. of lipopolysaccharide from *E. coli O* 86 was dissolved during heating in 10 ml. re-distilled, sterile 0·9 per cent saline solution (stock solution). For purposes of the hæmosensitization the stock solution was diluted 10 times with sterile 0·9 per cent saline solution. To 20 volumes of this solution was added 1 volume of packed, thoroughly washed *O* cells, and the mixture was incubated in a water-bath at 37° C. for 1 hr. After incubation, the blood cells were again washed and then tested in various blood-group reagents of anti-*A* and anti-*B* specificities. The results are given in Table 1.

The sensitized *O* cells agglutinated in human anti-*B* sera, showing a strength of agglutination apparently dependent on the strength of the anti-*B* present. In undiluted serum the strength of agglutination was equal to or slightly weaker than that with normal *B* cells, but the titre against sensitized *O* cells was lower than against normal *B* cells. The agglutination was weakened or abolished by addition of *B* saliva, but not of *A* saliva or *O* saliva, by absorption with *B* cells, but not with *A* cells or *O* cells. In human anti-*A* sera or *AB* sera, there was generally a weak reaction of the same order of magnitude as the reaction which remained in the neutralized or absorbed anti-*B* sera.

Incubation of the sensitized *O* cells in human anti-*B* sera with elution afterwards of the antibody (by the Landsteiner method) resulted in an eluate reacting with *B* cells and sensitized *O* cells, but not with *A* cells or with normal *O* cells. On the other hand, an eluate made by using the same serum and *B* cells reacted exclusively with *B* cells, not with sensitized *O* cells.

Absorption of human anti-*B* sera with sensitized *O* cells induced an unmistakable reduction of the anti-*B* content. In the same way, the lipopolysaccharide solution was able to abolish the reaction of *B* cells in anti-*B* sera. Conversely, there was no neutralizing effect of the solution on the agglutination of *A* cells in anti-*A* sera.

Sensitized *O* cells were tested in rabbit immune anti-*B* (specificity β_1) and chicken immune anti-*B* (specificity β_2)[5]. The cells gave a negative reaction with rabbit immune anti-*B*, but a positive reaction with chicken immune anti-*B*. Absorption of chicken immune anti-*B* with rabbit cells abolished the reaction with sensitized *O* cells.

As mentioned previously, the sensitized *O* cells reacted with practically all the human anti-*A* sera and *AB* sera tested. There was no distinct difference between anti-*A* sera and *AB* sera. The sensitized blood cells gave negative reaction with anti-*T*. Thus, the purified lipopolysaccharide from *E. coli O* 86 must contain a reactive group against which most human beings possess an antibody.

Analysis of the preliminary results, as shown in the table, supports the presumption that the purified lipopolysaccharide from *E. coli O* 86 contains a reactive group similar to blood-group factor *B* in man and the *B*-like factor in rabbit cells. However, it does not seem to be identical with human *B* or rabbit *B*.

The so-called A^b cells[6-9] also had an atypical *B* character which was weaker than the normal *B* factor on human *B* cells. It might be imagined, therefore, that they bear a certain relation to the artificial *B* cells produced in this work. It has, in fact, been suggested that the A^b character might be considered to be acquired[7-9]. There are points of resemblance between the *B* character on the A^b cells and the *B* character on the sensitized blood cells, but there are differences as well. While the A^b character has been found only in *A* persons, there was no demonstrable difference, qualitative or quantitative, between sensitized *A* cells and sensitized *O* cells, when tested in a series of anti-*B* sera and *AB* sera. Further work is needed, however, to elucidate the relation between the *B* characters on the different types of blood cells.

I am indebted to Dr. O. Westphal, A. Wander Forschungsinstitut, Freiburg-Zähringen, for the generous supply of purified lipopolysaccharide from *E. coli O* 86, and I wish to thank Dr. F. Kauffmann and Dr. F. Ørskov, of the International Salmonella and Escherichia Centre, Statens Seruminstitut, Copenhagen, for valuable assistance.

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PATHOLOGY

Effect of Essential Oils on Atherosclerosis of Cholesterol-fed Rabbits

On glancing through the international literature we do not find any information on the therapeutical application of well-known pharmacological products, like the essential oils, in the treatment of atherosclerosis, and no experiments have been carried out on this subject. The essential oils are ingredients of plants and are readily soluble in most of the organic solvents. They contain mainly various terpenes like menthol, camphor, pinene, borneol, cineol and other compounds. The chemical structure of the majority of terpenes has been determined.

The terpenes preferably penetrate into cells of high lipoid content. Menthol presents an affinity to nerve receptors, camphor—according to several authors—to the vasomotor centre. Alpha-pinene, like the bicyclic terpenes, exerts a chemotactic effect on leucocytes[1-4].

The physico-chemical properties of essential oils and their remarkable affinity to cells with high lipoid content induced us to test their efficacy in atherosclerosis. In our investigations we used a terpene mixture consisting of borneol, camphenes, cineol, menthol, menthone and pinene. (We owe thanks to Messrs. Rowa-Wagner G.m.b.H., Bensberg/Cologne, Western Germany, for providing us with the necessary quantities of this product.)

Our investigations were carried out on male rabbits weighing 3,000 gm. We induced atherosclerosis by cholesterol feeding as described by Anichkov and Chalatov[5]. 1 gm. of cholesterol added to bran-balls was given daily to 20 rabbits of the first test group and to 12 rabbits of the second. Basically, we applied 1 ml. of the terpene mixture by subcutaneous injection every other day to ten rabbits of the first test group. Due to the occurrence of sterile abscesses in some rabbits 6 wk. later, the administration of the terpene mixture was continued orally for another 6 wk. Six animals of the second test group received the remedy orally throughout the whole test period of 3 months. Rabbit food consisted of lucerne and beet besides the bran-balls.

In both test groups four animals died. Thus 12 animals of each group remained for evaluation. Blood cholesterol-level was determined at the beginning, and at the end of our investigations showed an increase up to 1,048 ± 82 mgm. per cent, in comparison with an initial average level of 130 ± 24 mgm. per cent. We encountered a similar rise of cholesterol esters, the rate of which, however, was somewhat less than that of total cholesterol. Paper electrophoresis revealed a very significant increase of the beta-lipoprotein fraction in both groups. In autopsy we established hepatomegaly; a considerable storage of cholesterol in the liver was found in the animals of both groups by histochemical methods. Examination of the aortal intima revealed decisive differences between the terpene-treated animals and the controls. In the controls of the first series, we observed widespread atherosclerotic plaques in the aortal intima amounting to more than 45 per cent of total aortal surface as measured by planimeter. The number as well as the extension of these plaques in the aortæ of the treated animals of the same group were much less than in the controls; they affected merely 13 per cent of the total aortal intima (Fig. 1). In the other test group, however, essentially no atherosclerotic

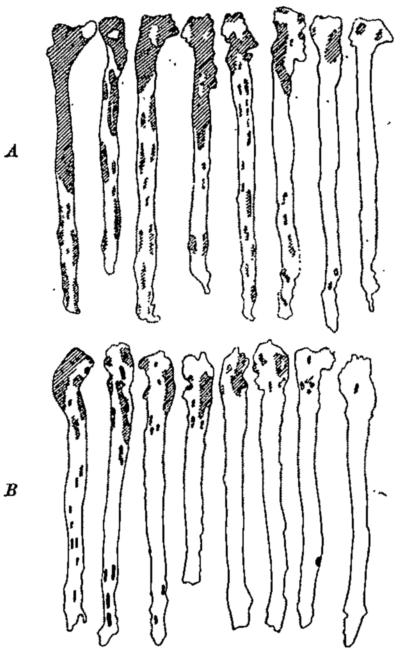

Fig. 1. *A*, Aortæ of terpene-treated animals; *B*, aortæ of controls

plaques appeared in the aortæ of the treated animals compared with those of the untreated ones (Fig. 2).

These findings are rather noteworthy in view of the fact that terpene treatment did not impair the absorption or storage of cholesterol, and that it did not inhibit the increase of serum cholesterol-level. Nevertheless, the terpene compound prevented remarkably the development of atherosclerotic plaques.

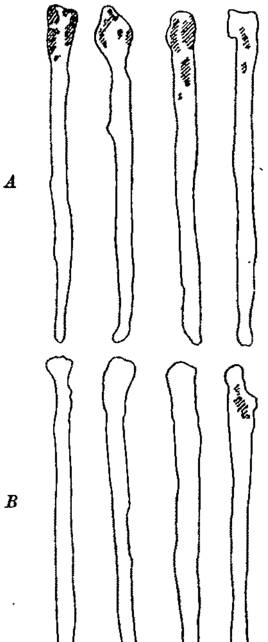

Fig. 2. *A*, Aortæ of terpene-treated animals; *B*, aortæ of controls

Impaired lipoid metabolism is nowadays generally considered to be one of the most important pathogenic factors causing atherosclerosis[6–9]. All the therapeutical measures have in common the tendency to remove these disorders. In dietetics, the important role of unsaturated fatty acids has been pointed out recently[10]. All the procedures aim at normalizing the blood lipoid-level. It is well known, however, that a high level of serum cholesterol is not always associated with atherosclerosis, as demonstrated by cortisone-induced hypercholesterolæmia, by nephrosis, and by certain forms of cirrhosis. Gerő and co-workers[11] reported a significant decrease of atherosclerosis originating from lipoprotein immunization in spite of high cholesterol-levels. We also suggest that the formation of atherosclerotic plaques is prevented by a terpene treatment, notwithstanding the high levels of serum cholesterol. We suppose that this effect of the terpenes partly results either from some competitive inhibition, or perhaps they act by blocking mediatory factors promoting the penetration of lipoids into the arterial wall.

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Influence of Uræmia on the Survival of Skin Homografts

Couch *et al.*[1] have shown that homografts of skin may survive for prolonged periods in a uræmic patient. They considered that this extended survival might be due to an impairment of immune responses in the patient. However, there is evidence to show that in patients with both acute and chronic uræmia the production of certain classical antibodies is within normal limits[1–4]. Stoloff *et al.*[3] have suggested that while the synthesis of classical antibodies may be impaired by uræmia, the production of intracellular antibodies may be inhibited. However, in chronically uræmic patients the Mantoux test may be positive, implying that the formation of intracellular antibodies may also be maintained[2].

Since the principal response to orthotopic skin homografts occurs in the draining lymph nodes[5,6], the effect of acute uræmia on the regional lymph node reactivity to skin homografts has been investigated in 33 uræmic and 23 control rabbits. The animals used were adult females of albino and Flemish Giant strains. Animals from each strain were paired,

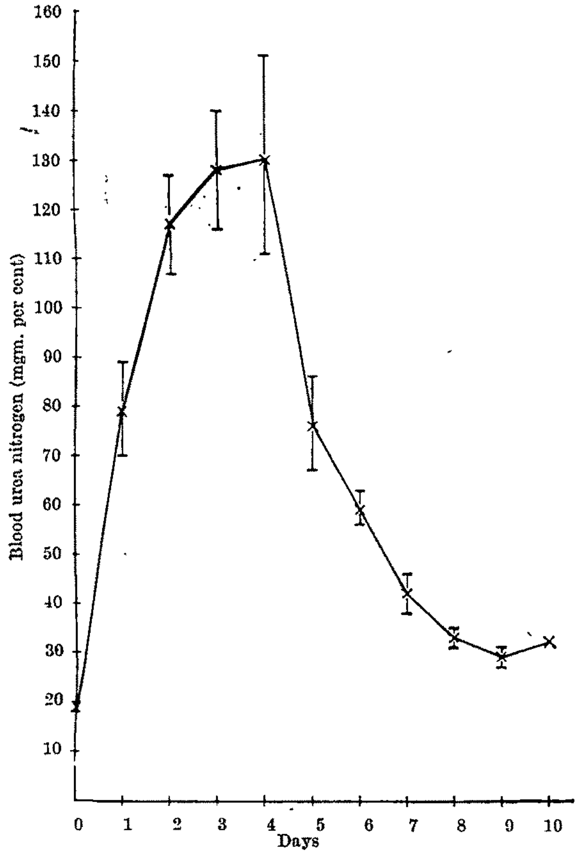

Fig. 1. Graph showing daily level of the blood urea nitrogen after occlusion of the right renal pedicle for 2 hr. Nephrectomy simultaneously performed on the opposite side

and under sodium pentobarbitone anæsthesia circular skin grafts 2 cm. in diameter were exchanged between the right ears. Acute uræmia was produced by means of unilateral nephrectomy, after which the pedicle of the other kidney was clamped for 2 hr.

Each day the grafts were inspected, the animals weighed and blood samples were taken from the left ears of the uræmic animals for blood urea nitrogen estimations. The animals were killed at intervals of time up to 14 days after operation. The parotid lymph nodes on the side containing the graft and on the contralateral side were removed, weighed and fixed in Carnoy's fluid.

The mean initial rejection-time of skin homografts exchanged between twelve healthy rabbits was 5·1 ± 0·9 days, whereas in eight uræmic animals, the mean initial rejection time was 10·8 ± 1·5 days. These observations have not been subjected to a *t*-test because the data are censored, implying that the sample of rejection times is not representative of the values which would have been obtained if all the rabbits had been allowed to live until the homografts were rejected. The criteria used for assessing the earliest signs of graft rejection were cyanosis, papillary congestion and intra-epidermal œdema appearing in skin which had shown evidence of survival. The blood urea nitrogen-levels in the uræmic group (Fig. 1) rose to a maximum between the fourth and the sixth day after grafting, and then fell rapidly towards normal levels. Two rabbits in which a secondary ante-mortem rise in blood urea nitrogen occurred have been excluded in the preparation of this graph. The mean absolute weight of the first regional lymph nodes draining the skin homografts at each time-interval after grafting in the control animals increased rapidly to a maximum by the sixth day after operation (Fig. 2), confirming the findings of Scothorne and McGregor[6]; the secondary rise in weight of lymph node after the tenth post-operative day was probably due to separation of the graft and infection of the graft bed. In the uræmic animals (Fig. 2) increase in the mean absolute weight of the first regional lymph nodes on the side receiving the graft was delayed until after the sixth day, by which time the azotæmia was returning towards normal levels (Fig. 1). A mean maximal weight was not reached until the tenth day, at about the time of graft rejection, which occurred at 10·8 ± 1·5 days. A secondary rise in weight of lymph node similar to that previously observed in the control group occurred after the twelfth post-operative day. Statistical analysis of the mean absolute lymph node weights between the uræmic and the control groups at each time interval has revealed a highly significant difference at the sixth ($P < 0.001$) and eighth ($P < 0.001$) days after operation.

Histological examination of first regional nodes draining tissue homografts has revealed the appearance of great accumulations of 'large lymphoid cells'[6-10] which reach a maximum between the fourth and sixth days after grafting[10]. The histological aspects of the present work will be reported in a further communication.

It is possible that acute uræmia exerts its effect of prolongation on skin homograft survival by a direct depression of the immunological reactivity of the lymph nodes draining the graft. However, the non-specific stress of the operation together with the acute uræmia may result in adreno-cortical stimula-

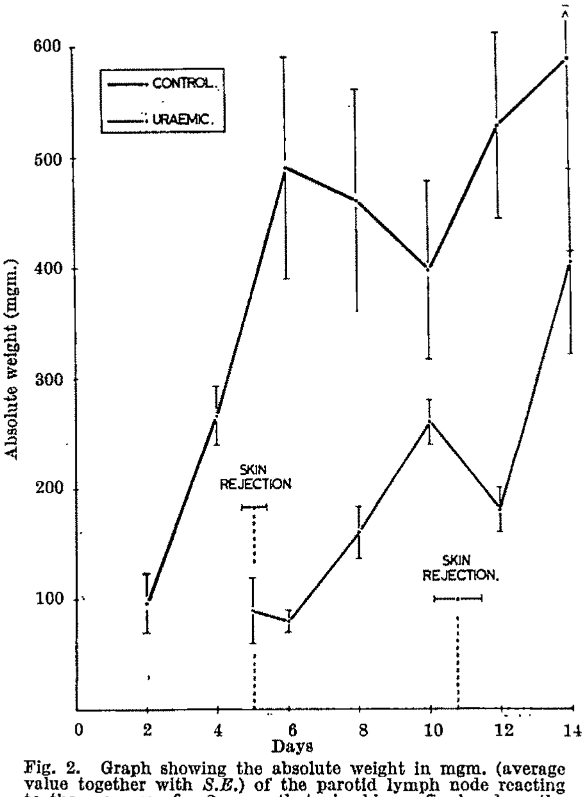

Fig. 2. Graph showing the absolute weight in mgm. (average value together with *S.E.*) of the parotid lymph node reacting to the presence of a 2-cm. orthotopic skin graft placed on the ventral aspect of the right ear. Control group above; uræmic animals below

tion. There is evidence to show that systemic or topical administration of cortisone acetate will prolong survival of skin graft in rabbits[11–13]. The possibility that the observed prolongation of skin homograft rejection time in rabbits with acute uræmia is due to adreno-cortical hyperfunction is being investigated at the present time. Further, the uræmic state of the recipient may inhibit the antigenicity of the homografted skin, a view which has been suggested as one of the ways in which cortisone may extend skin homograft survival[14]. An attempt was made to put the possibility of reduced antigenicity by uræmia to the experimental test, by grafting skin from severely uræmic rabbits on to six healthy rabbits. The mean initial rejection time of the skin homografts in this group of animals was 5·4 ± 0·5 days, a rejection time which is little different from the control group. Finally, Stoloff *et al.*[3] suggested that uræmia may act in prolonging homograft survival by diminishing the receptivity of the graft to the immune responses of the host. In the light of the impaired reactivity of regional lymph nodes draining skin homografts in the presence of acute uræmia, diminished receptivity of the grafts would seem unlikely to play anything more than a minor part in the extension of skin graft survival time produced by acute uræmia. A further and more detailed account of our findings will be given later.

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A New, Highly Effective but Non-Toxic Antibacterial Substance

WE have discovered an entirely novel drug which is a polycondensed urea-formaldehyde having quite unexpected properties.

This polycondensate was found during a routine search for new classes of compounds with bacteriostatic properties. It is produced by a simple process of 'curing' a water-soluble pre-condensate of formaldehyde and urea units by high-temperature evaporation in a spray-tower at ordinary pressure and an alkaline *p*H. The resultant material is known as 'Anaflex', which is an amorphous white powder soluble to the extent of 0·28–0·31 per cent in water. It shows broad-spectrum bacteriostatic properties which are discussed here.

This product is a distinct entity, and differs from the well-known products resulting from 'curing' the water-soluble pre-condensates of urea formaldehyde with acid reagents at either high or low temperature as these are always devoid of any bacteriostatic activity.

Because of the low solubility of the new material it has proved difficult adequately to assess its physical constants. It is not crystalline and when heated it decomposes without melting.

Carbon–hydrogen–nitrogen analysis gives constant values, namely: C, 35·96; H, 5·88; N, 27·72; C, 35·68 ; H, 6·05 ; N, 27·76 per cent. This indicates $C_6H_{12}O_4N_4$ as a unit of the polycondensate molecule. $C_6H_{12}O_4N_4$ calculated gives: C, 35·29 ; H, 5·92 ; N, 27·44 per cent.

Both the oxygen and hydrogen content of the molecule is higher than would be expected from simple cross-linking of urea and methylene groups. We, therefore, feel that many free oxymethylene-substituents occur within the molecule and are responsible for the strong bacteriostatic activity.

This new material has been investigated to assess its effect as a bacteriocide against the following organisms :

Staphylococcus albus
Staph. albus (penicillin insensitive)
Staph. aureus, coagulase positive
Staph. aureus, coagulase positive, type 43
Staph. aureus, coagulase positive and penicillin and tetracycline insensitive
Strept. viridans (α-hæmolytic)
Strept. pyogenes (β-hæmolytic)
Micrococcus tetragenus
Strept. lactis (from fermented milk)
Pneumococcus
B. coli strain 1
B. coli strain 2
B. coli strain 3
Ps. pyocyanea, type 1
Ps. pyocyanea, type 2
Proteus morgani
Salmonella paratyphosum A
S. paratyphosum B
S. paratyphosum C
S. mixed organisms
S., local type
Sh. dys. sonne
Sh. dys. schmidt
Lacto bacillus
N. catarrhalis sicca
N. catarrhalis, smooth type
Alkaligenes alkaligenes
Candida albicans, type 1
Candida albicans, type 2
Unidentified yeast
Trichophyton, type 1
Trichophyton, type 2
C. diphtheriae
C. hoffmani
C. xerosis
Aspergillus niger
A. rubrum
B. subtilis

Because of the low solubility in water the tests were carried out by Fleming's disk and ditch methods and the resulting zones of inhibition have been assessed.

In addition, in suitable liquid media which include 1 per cent glucose nutrient broth and modified brewer's broth, a suspension of 1 per cent of the new material w/v has shown similar inhibiting effects. Detailed tests have been made to eliminate bacteriostatic results by serial sub-dilutions in the appropriate media.

On serial sub-cultures of known micro-organisms from the periphery of the zones of inhibition repeated up to 100 times, no resistant strain of any of the test organisms has emerged. After these striking laboratory results the investigations have been extended to more than 200 human patients and these comprise at least :

40 cases furunculosis
20 ulcus cruris
22 infected pressure sores
14 infected acne
44 gynæcological discharges
14 napkin rash
20 infected industrial wounds
7 fungal paronychia
9 athlete's foot
15 proctological cases
20 cutaneous hypersensitivity
11 axillary infections
2 seborrhœa capitis

Method of use. Clinically it only appears necessary to apply the medicament twice daily—preferably directly to the affected part—with a 'ring' of dressing to the circumambient areas to minimize secondary spread. In most cases occlusive dressings are contra-indicated.

The antibacterial spectrum is very wide, both *in vitro* and *in vivo*. It is not claimed that all the patients treated were cured ; but it should suffice to indicate that more than 80 per cent of the furunculosis cases were cured in less than 4 days. In cases where treatment was instituted within 48 hr. of the appearance of the initial lesions, these failed to develop into classical boils and the lesions disappeared in a further 48 hr.

A small comparative trial of nasal barrier creams was instituted. A well-known commercial cream containing chlorhexidine was used as the control. 'Anaflex' removed the infection from nasal carriers of penicillin-resistant *Staphylococcus* within 4–5 days; chlorhexidine produced a similar effect in 2–3 days but relapses occurred within 3 days. With 'Anaflex' relapses to the carrier state took at least 7 days.

It has proved in our hands extremely difficult to account for the mode of action of 'Anaflex'. It might be argued that it is due to a slow release of formaldehyde and indeed on treating this material with 4*N* sulphuric acid, 53 ± 1 per cent, formaldehyde is quantitatively liberated. But it has been found to be effective in urinary dermatitis (which has an ammoniacal basis). It is not possible to detect formaldehyde in the solution derived from 'Anaflex'. The distillate from such a solution is completely devoid of bacteriostatic effect and also of formaldehyde.

We have reason for believing that the bacteriocidal effects of the new material are enhanced by the presence of electrolytes and/or body proteins.

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HISTOCHEMISTRY

Cholinesterase in the Chicken Nervous System

WHILE making a topographical survey of the distribution of cholinesterases in the spinal cord and brain stem of the chicken in relation to the question of the selective neurotoxicity of certain organophosphorus compounds, we were led to make a comparison in four laboratory mammals (to be published). Koelle[1] had shown histochemically in the rat that considerable variation exists in the cholinesterase content of neurones from one centre to another. Hebb[2] also recognized in mammals neurones where the cholinesterase was for the most part intracellular (cholinergic) and neurones where it was largely distributed on the neuronal membrane (cholinoceptive). In contrast to this variability in mammals, the distribution of cholinesterase in chicken neurones is strikingly uniform.

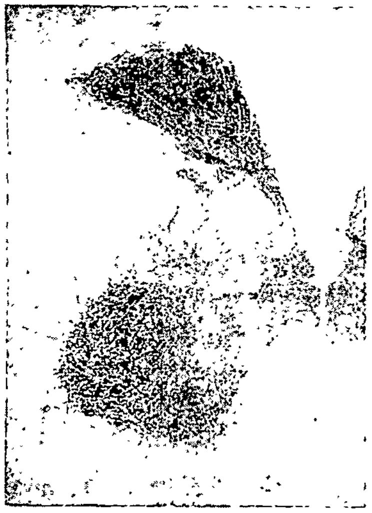

Fig. 1. Cervical spinal cord of guinea pig. Acetylthiocholine; Gömöri technique; *p*H 6·5; 2-hr. incubation

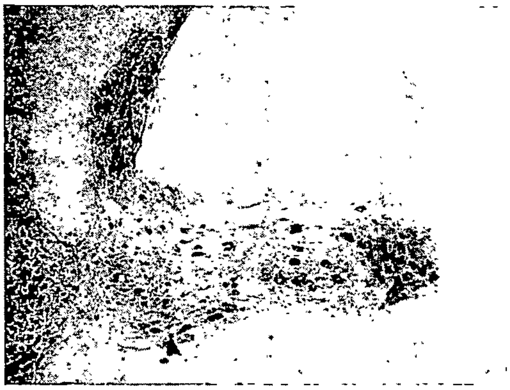

Fig. 2. Cervical spinal cord of chicken. Acetylthiocholine; Gömöri technique; *p*H 6·5; 2-hr. incubation

All nerve cells of sensory ganglia, spinal cord and brain stem contain an abundance of this enzyme, and variation in content seemed to be related to neurone size. It is particularly interesting that the dorsal root ganglia cells are so active, for in mammals these cells are very poor in this enzyme while many contain none at all[3]. Hebb[2] likewise found that the second-order sensory cells in the cat were rich in enzyme, but not the primary cells. In the mammalian spinal cord (Fig. 1) cholinesterase is seen histochemically to be concentrated in the lateral and ventral regions and appears to be related both to neuronal surfaces and perikarya, the relative abundance at each area varying with the species. In the chicken relatively little is seen at cell surfaces, while all neurones show cholinesterase activity in their perikarya (Fig. 2). A similar constancy of distribution is found in the brain stem neurones.

On the other hand, certain richly synaptic zones such as the posterior horns of the spinal cord (Figs. 1 and 2), the descending root of the Vth nerve and certain cochleo-vestibular centres in the chicken contain an abundance of cholinesterase just as they do in the mammals examined. But the molecular layer of the cerebellum, which is also a richly synaptic area and also contains much cholinesterase in the chicken, is negative in the rat, rabbit and cat. In the guinea pig, however, it is almost as active as in the chicken.

If the presence of cholinesterase, shown in this work by its substrate and inhibitor characteristics probably to be true cholinesterase, is an index of the presence of a cholinergic transmission mechanism, then it would appear that the chemical transmission system of the chicken is probably of a simpler overall type than in higher species. On the basis of evidence, largely drawn from work on mammals, Hebb[4] has concluded that cholinergic transmission is probably not universal and is limited to only a proportion of cells, and that the rest must use some alternative chemical system for this purpose. It is to be questioned whether these conclusions are in fact true for submammalian species or even whether absolute uniformity is to be expected among mammals as to which chemical system is employed in each region. Apart from the bearing that these findings have on the evolution of possible transmission systems, they emphasize the necessity for not drawing too close a comparison between the chicken and higher species when this animal is used for the purposes of experimental neurology.

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BIOLOGY

Sucker Display of Octopus

As a cephalopod, *Octopus vulgaris* is capable of the fastest and some of the most varied colour changes in the animal kingdom; yet there is a remarkable lack of evidence for these being used in courtship or mating displays of the kind associated with brightly coloured vertebrate animals. Courtship colour display by a less studied octopus (probably *O. horridus*) has been observed by Young[1], and the dramatic 'zebra' pattern of the decapod, *Sepia officinalis*, which is used both in courtship and as a threat between males, is well known.

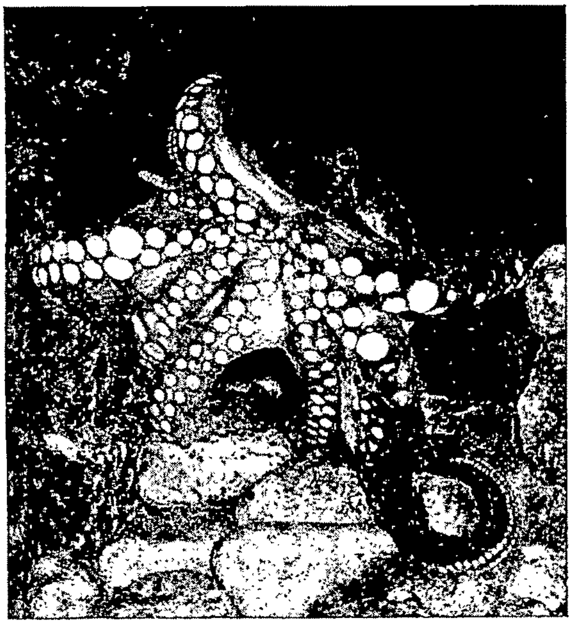

Fig. 1. Photograph of a male (about 4 kgm.) displaying its suckers in response to a female approaching on its left side. The uppermost arm on the right of the photograph is arm 2 of the animal's left side; arm 1 left being held over towards the right side; arm 1 right is regenerating. Note the characteristic large suckers of the male on arms 2 and 3

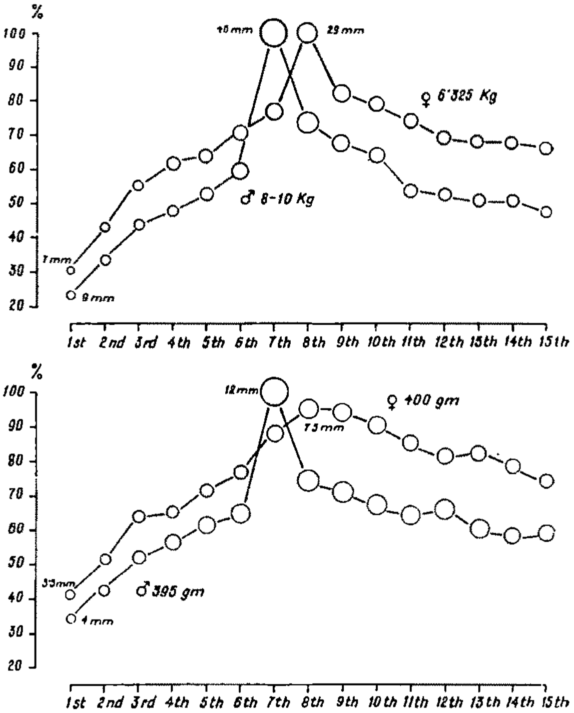

Fig. 2. Relative sizes of suckers at different levels on two males and two females of comparable weights. At each level outwards from the mouth the size of the sucker is given as a percentage of the diameter of the largest in the row. Plots are the average of measurements for single rows of suckers on arms 2 and 3 of either side. The diameters of the largest and of the most proximal suckers are indicated in millimetres. Measurements are from dead specimens

Reviewing the colour patterns of *O. vulgaris*, it soon becomes obvious that the well-known ones, for example, the dymantic 'startling' pattern described by Young, the deep reddening during an attack on a prey, and the various mottled patterns that clearly serve as protective coloration in different backgrounds, have an interspecific value rather than being intraspecific. The lack of information about colour patterns used intraspecifically may be due to the fact that the observer invariably takes part in an interspecific relationship with the animal and only rarely finds himself in a position to watch an intraspecific one. But a recent observation suggests that chromatophore colour patterns used in communication between one octopus and another may be of less importance than another signalling device, namely, the suckers.

During an encounter between one male *O. vulgaris* and a female in the Naples Aquarium, the male was seen to display its upturned arms while holding a position among the rocks from which it had just ousted the female. A photograph, taken at the time (Fig. 1), shows that certain suckers on the second and third arms of either side are larger than the others. It has not apparently been realized before, unless by Aristotle[2], that these are a regular external sexual character of the male *O. vulgaris* in addition to the hectocotylized tip of the third right arm. The presence of extra-large suckers at the level of the seventh or eighth pair, counting outwards from the mouth on arms 2 and 3 on either side, has now been found to be a reliable guide for the determination of mature males from females. At this level in both sexes the suckers, which increase in size outwards from the mouth, begin to decrease in size towards the arm tips, but in the male the largest sucker is proportionately larger than that in the female (Fig. 2).

A second observation made when a small but mature male was introduced to a female (four times his size) confirmed the impression that these large suckers are used as display between male and female. The body of the male was raised above the floor of the tank in such a way that the suckers near the mouth were showing with their undersides towards the female, and he approached with the third right arm outstretched in her direction. The proximal suckers were closed, by being folded on themselves, but the extra-large ones were, by contrast, expanded. Fig. 1 was taken at a moment when a female, attracted by a crab held close to the male, was approaching from the right of the photograph. It will be noticed that the closed suckers (here considered to be part of the complete display providing maximum contrast of expanded white on a dark ground) occurs on the side of the approaching female only. This is another example of unilateral response often observed in *Octopus* (also *Sepia*) when monocular vision is being used.

Another form of the sucker display used during copulation, and mentioned by Racovitza[3], has also been seen in the Naples Aquarium. While the male, sitting at right angles to the female, has his third right arm stretched into her mantle opening, the second right arm is curved back over the head so that the female is presented with a vertical double row of open suckers, the extra-large male suckers being the most conspicuous and directed with their expanded surfaces towards her.

Possible use of the sucker display by the male is as a recognition signal: male-to-female, especially important when the male is smaller than the female and might otherwise be attacked. Males mature at weights as little as 200 gm., females not until 2·5 kgm. or more[4], and it may be a normal requirement for small males to copulate with much larger females. To do so is to reverse the tendency of a small animal to withdraw from a larger one, as from a source of potential danger. A common feature of the withdrawal response of *O. vulgaris* is a turning up of the edges of the withdrawn arms so that the suckers, the surfaces of which are usually hidden by being in contact with the substratum, become visible especially on the stimulated side where the withdrawal proceeds further. This is often seen in training situations when animals are presented with a stimulus associated with punishment. In extreme withdrawal the arms are brought back over the head and mantle so that the suckers of all arms are visible. A film taken underwater of an octopus disturbed by a fish, shows very clearly how the moment in which the edges of the first arms are upturned, exposing the suckers, coincides with the closest approach of the fish, which then immediately halts and backs away. Such an interspecific display—as the movement clearly has become—develops directly from the protective withdrawal of the arms over the head. The sudden appearance of white circles, as upturning begins, startles the intruder.

The appearance to an octopus of white shapes on a dark background have for the past years been the subject of many experiments at Naples, and a white object such as a handkerchief or piece of plain cloth or white stone lowered into the water and dangled in front of an octopus has long been used by Neapolitan fishermen to lure an octopus out of its hole when no bait is handy or when other methods have failed. Boycott and Young[5] have considered the relative value as stimuli of rectangular and circular figures of the same area and Young[6] concludes that circles are attacked more readily than rectangles, while N. S. Sutherland (unpublished) has evidence that, of two different sized white circles presented together, the larger (within a diameter range of 2·7–5·6 cm.) is preferred even when attacks on both are rewarded.

The infundibular surfaces of the suckers are circular; they are white because the skin here lacks chromatophores: the outer keratinous layer of the skin of these surfaces is repeatedly renewed to maintain the efficiency of the suckers as adhesive organs. Therefore, given the existence of white circles as part of the anatomy of the animal and their visible appearance in only certain behavioural situations, it is not difficult to imagine that they should also have evolved a significance in communication, though we know almost nothing of how specific or capable of refinement this communication is.

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Movement of Coarse Fish in Rivers

IT is generally accepted[1] that coarse fish move to shallow water in the spring in order to spawn, and retire to deep water in the winter, but how extensive these movements are is not known. Work on American species has given rise to the concept of a 'home range'[2] which may be restricted to as little as a few 100 ft., and it has also been suggested that some species, including carp, are best regarded as having populations composed of mobile and sedentary fractions the relative proportions of which determine the degree of mobility shown by the species[3].

Investigations of coarse fish movements were made in the River Thames, near Reading, in 1959 and in the River Mole, in Surrey, between July and September 1960. The fish population in the Mole was repeatedly sampled by electric fishing at 14 stations, each about 200 ft. long, over a mile of the River. The catch, mostly roach and gudgeon, was marked either by fin clipping or by the attachment of a light metal tag, and released at the centre of the station in which it was caught. Altogether, 3,252 fish were marked over a period of five weeks and a total of 374 was recaptured during this period and a subsequent sampling period of two weeks.

The results showed no correlation between the time that fish had been at liberty and the distance travelled. Instead the frequency distribution of recaptures showed marked grouping about the point of release. In order to ascribe proper weighting to the distribution, the sampling effort exerted and the numbers of marked fish present have to be taken into account. The efficiency with which a station was sampled has been calculated as the fraction obtained by dividing the number of recaptured fish which were marked in the first week of the investigation by the

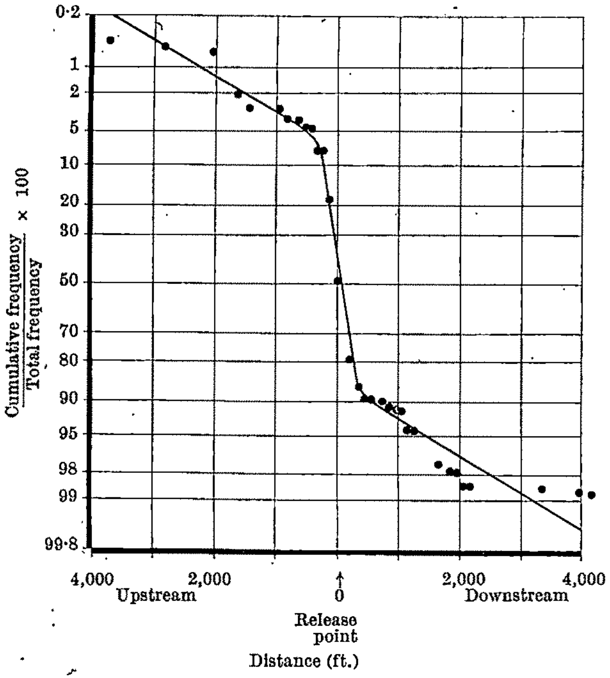

Fig. 1. Distribution of roach recaptures about the release point

total number of fish marked in the station during the first week. An analysis of variance showed that all samples could be regarded as having been taken with similar efforts. Therefore the weighting due to an individual recapture is given by :

$$\frac{1}{M_t.S_t}$$

where M_t is the number of marked fish present in the station of marking up to time t, and S_t is the number of samples taken up to and including time t at the station of recapture.

The corrected distribution is illustrated for roach in Fig. 1, where the cumulative frequencies are expressed as percentages of the total frequency and plotted on a probit scale against the distance from the station of marking. Using this method, a normal distribution gives a straight line with the slope of the line being a measure of the dispersal. However, in this case, a sigmoid curve is obtained which could be interpreted as the result of a combination of two normal distributions of differing dispersions. This seems to suggest that the roach population in the River Mole is made up of two components : a larger group comprising about 80 per cent of the individuals which remains within a range of 650 ft., and a smaller group of 20 per cent which moves more freely.

The weighting formula used introduces bias against recaptures showing no movement since M_t should be decreased by the unknown number which have left the station of marking. This makes the estimate of the proportion formed by the less-mobile group too low and its range too high ; but, since relatively few fish were taken outside the station in which they were marked, the differences will not be great.

The division of fish populations into two groups of differing mobility could provide an explanation of the apparently speedy recolonization of waterways by fish which, by the nature of their habitat, would be expected to exhibit station-holding behaviour.

Similar work is planned to investigate the movements of coarse fish throughout the year.

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Unilateral Implantation in the Uganda Kob

The Uganda kob, *Adenota kob thomasi* (P. L. Sclater), an antelope almost entirely endemic to Uganda, East Africa, inhabits primarily grassy plains along major rivers and lakes in the Western Rift Valley at elevations of 625–900 m. Its range extends approximately from 1° S. latitude to 4° N. latitude. Annual rainfall averages about 100 cm., and daily temperature varies approximately between 20° and 30° C. Breeding occurs throughout the year on special territorial grounds[1]. Gestation probably lasts about 7½ months, and normally the females are monotocous.

In the Semliki Game Reserve, 30 km. north of Fort Portal, Uganda, at 1° N. latitude, 150 mature female Uganda kob were collected. 12–15 individuals were shot each month during July 1957–June 1958. In addition, 10 females were taken immediately north of Murchison Falls National Park in the vicinity of Lolim Ranger Post. The position of the conceptus and distribution of cotyledons with respect to right and left horns of the bicornuate uterus were noted. Location of the most recent corpus luteum was recorded as to right and left ovary. Additional linear, weight and volume measurements were made of the uterus, extra-embryonic membranes and fluids, fœtus and ovaries. Analyses of these results are being prepared for publication. Samples of extra-embryonic tissues, ovaries, embryos, and fœtuses of less than 100 mm. crown-rump measurement were preserved.

Ovulation occurred at random between right and left ovaries (Table 1). In the tabulation, size of ovary is based on linear measurement, to the nearest millimetre, of the greatest three dimensions of the ovary prior to fixation. In pregnant females the larger size indicates the presence of a corpus luteum of pregnancy ; in non-pregnant females the larger size represents either a degenerating corpus luteum or an enlarged follicle. The present results support the principle of random distribution of ova released between right and left ovaries in monotocous species. Brambell[2] cites evidence for random distribution in the cow, sheep and monkey. Annual alternation between ovaries may occur, or there may be several successive ovulations from the same ovary. In the fur seal, *Callorhinus ursinus cynocephalus* (Walbaum), Enders *et al.*[3] found that a single ovum is ovulated annually from alternate ovaries, as shown by the persistent remains of the corpus albicans. The pattern of ovulation between right and left ovaries remains to be determined for the Uganda kob.

Implantation occurred uniformly in the right horn of the uterus, as indicated by the presence of the fœtus and the number of cotyledons in each horn (Table 1). Chorionic attachments to uterine caruncles occurred in the left uterine horn when growth of the conceptus was sufficiently advanced. Unilateral

Table 1. FREQUENCY OF OVULATION BETWEEN RIGHT AND LEFT OVARIES; NUMBER OF COTYLEDONS IN GRAVID AND NON-GRAVID UTERINE HORNS IN UGANDA KOB, *Adenota kob thomasi* (P. L. SCLATER)

Phase of reproduction	No. of animals	Site of implantation		Larger ovary		Mean No. of cotyledons (range in parentheses)	
		Right	Left	Right	Left	Right horn (Gravid)	Left horn (Non-gravid)
Pregnant	116	116	0	57	59	14·0 (8–20)	7·6 (3–12)
Non-pregnant (lactating)	39	39	0	18	21	15·0 (10–21)	7·2 (5–10)
Non-pregnant (non-lactating)	5	5	0	2	3	15·0 (13–16)	7·0 (6–8)
Total	160	160	0	77	83	14·9 (8–21)	7·4 (3–12)

implantation appears to be a phenomenon that is unique in the Uganda kob. Since about 50 per cent of the ova are released from the left ovary, trans-uterine migration of the ovum or embryo must occur. This does not preclude the possibility of trans-peritoneal migration of the ova, although this route seems less likely. Trans-uterine migration has been recorded in the mink, *Mustela vison* Schreber[4]; the raccoon, *Procyon lotor* (L.)[5]; and the skunk, *Mephitis mephitis nigra* (Peale and Palisot de Beauvois)[6]. In sows, Hafez[7] found 41 per cent migration of ova on the basis of excess of ovulation sites over the number of implantations in the corresponding uterine horn. The physiological significance of unilateral implantation is not understood. E. M. Amoroso (personal communication) has shown that the distal portion of the uterus in the cat is more favourable for implantation than the proximal end. Possibly the endometrium of the right uterine horn provides the most favourable site for implantation in the Uganda kob.

One case (No. 47) of twins was discovered by W. O. Pridham, game ranger, Uganda Department of Game and Fisheries, immediately after parturition. Although the fawns (1 male and 1 female) were fully haired and approached mean parturition weight, they were dead. The female was shot while the extra-embryonic membranes were still retained in the uterus. At autopsy it was found that both fœtuses had been in the right horn of the uterus, and two corpora lutea were present in the right ovary.

The results presented here were obtained during a Fulbright appointment to Uganda. I am indebted to Prof. E. M. Amoroso of the Royal Veterinary College (University of London) for encouragement in this work. A. C. Brooks, biologist of the Uganda Department of Game and Fisheries, transmitted Prof. Amoroso's ideas for field collection of the data and assisted with most of the field work. Eleven of the Uganda kob were collected by I. O. Buss, professor of wildlife management, Washington State University.

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Experimental Elimination of *Trypanosoma melophagium* Flu from its Hosts the Sheep and the Ked, *Melophagus ovinus* (L.)

Trypanosoma melophagium Flu is a non-pathogenic parasite of sheep, and its intermediate host is the sheep ked[2]. The parasite is world-wide in its distribution and is found wherever there are keds[1]. Infection of sheep usually takes place via the oral mucosa following ingestion of infected keds[2,4]. If sheep are treated to destroy keds, it is several months before the flagellates disappear from the host's blood. I[3] reported the death of large numbers of keds due to blockage of the midgut by masses of crithidia. Control over the trypanosome infection would be a useful tool for investigating this phenomenon further. The organism is not trans-ovarially transmitted in the ked[2] and thus flagellate-free insects should be easy to obtain.

Infected populations of keds on sheep were destroyed by spraying with 0·5 per cent toxaphene or malathion 6–8 weeks in advance of experiments to avoid residual toxicity to keds.

A week before re-infestation, sheep were given quinapyramine sulphate ('Antrycide', Imperial Chemical Industries, Ltd.), 8 mgm./kgm. subcutaneously. Sheep were infested with puparia which had been disinfected in 1 per cent mercuric chloride. Effectiveness of the drug in suppressing trypanosomes in the blood was checked weekly by dissecting 4–6 keds from each animal, wet-smearing the posterior midgut beneath a coverslip, and examining for crithidia under high dry magnification.

Lower doses of 'Antrycide' were not completely effective, and infection appeared in some keds within a few weeks. Higher doses caused toxic symptoms and occasionally death in sheep. At 8 mgm/kgm., trypanosome-free units can be maintained for several months provided there is no opportunity for re-infection. Sheep should be housed separately in clean quarters which have not recently contained infected animals. Even when trypanosome-free sheep were kept 50 ft. from infected sheep in the same barn, infection appeared in keds within a few weeks. This suggests that the infective forms present in ked fæces may be air-borne and may enter the sheep via the respiratory mucosa as well as by the oral route.

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Culicoides adersi Ingram and Macfie, 1923, a Presumed Vector of *Hepatocystis* (= *Plasmodium*) *kochi* (Laveran, 1899)

MONKEYS of tropical Africa are well known to harbour a malaria parasite *Hepatocystis* (= *Plasmodium*) *kochi*, characterized by the presence of gametocytes only in the blood, and by the occurrence of schizogony in the form of macroscopic merocysts in the liver. As a malaria parasite, its vector was suspected for a long time as being a mosquito and several attempts were made experimentally and in Nature to incriminate these insects[1,2]. All failed, not altogether unexpectedly, when it was shown[3] that the parasite

Table 1. ATTEMPTS TO INFECT MONKEYS WITH SPOROZOITES FROM *Culicoides adersi*

Species of monkey	Serial No.	Inoculum	Route	Date	Result	Remarks
aethiops	35	15 positive glands	Subcutaneous	4.9.60	Negative for 7 months	Suspensions ? viable
aethiops	28	27 positive glands	Subcutaneous	28.9.60	Monkey died after 1 month; negative	Suspensions ? viable
aethiops	50	16 positive glands	Subcutaneous	30.9.60	Negative for 6 months	Suspensions ? viable
aethiops	30	4 positive midges	Bite	28.9.60	Gametocytes appeared on 1.2.61	
aethiops	31	Suspension of 69 midges (heavily positive batch)	Intravenous	6.1.61	3	Garnham technique
rhesus	23	Suspension of 21 midges (heavily positive batch)	Intravenous	7.1.61	3	Garnham technique

belonged not to the family Plasmodiidae but to the Haemoproteidae in which the sporogonic cycle was known to take place in *Simulium*, *Culicoides* and hippoboscids, but never in mosquitoes. The search was therefore widened to include other arthropods and a range of species was examined[3], but again with negative results. There was a slight indication that midges might be concerned, and in July 1959 an attempt was made to test this hypothesis in East Africa in a place where the monkeys were heavily infected with the parasite. We selected Port Reitz on the mainland near Mombasa, and worked at a small field laboratory conveniently situated in the middle of the area.

Heavily infected *Cercopithecus aethiops* monkeys were obtained, anæsthetized with 'Sernyl' (Parke Davis) and exposed to the bites of *Culicoides adersi* at dawn and dusk in the mangrove swamps, where these insects abound during the spring tides. Simultaneously, *C. adersi* were allowed to feed on human bait, and all the specimens were kept singly in tubes 2 in. × $\frac{1}{2}$ in. at a relatively uniform temperature (about 80° F.) in the laboratory. They were supplied with dilute glucose solution from the second day onwards on filter paper 'wick'.

Oökinetes were found in smears of the gut contents 20 hr. after the infective feed, but at first no later stages were detected. Then in August 1959 one of us (M. I.) found sporozoites in the salivary glands of a midge which had fed on an infected monkey 13 days before. Later dissections revealed a high rate of infection in the experimental insects, but a much lower incidence in the controls which had fed on man. Thus during August 1959–January 1961, the salivary glands and guts of 1,928 *C. adersi*, which had fed on an infected monkey 5–15 days previously, were examined and 568 or 29·5 per cent were found to show sporozoites. At the same time, 247 control midges which had fed on man 5–15 days previously were examined and 4 or 1·6 per cent were found to contain sporozoites. All these insects were caught in the wild, and an infection-rate of 0·2 per cent (4 in 2,400) was found in unfed specimens, dissected on the day of capture.

The sporozoites were curved bodies, about 13μ in length, and usually had a single, solid bead-like nucleus, often placed nearer the pointed end of the sporozoite; the other end was blunt. In serum Locke fluid, the sporozoites showed a peculiar type of movement, one-half contracting with a snake-like motion, yet rather jerkily.

The sporozoites were first detectable in small numbers around the mid-gut and Malpighian tubules 5 days after an infective feed; they tended to increase during the sixth and seventh days when the salivary glands became invaded. Infection persisted in the glands for at least 17 days, but declined in intensity after the thirteenth day. It was difficult to be sure about the latter point, because the majority of infections at any time were light; it was quite exceptional to find, for example, several hundred sporozoites.

Attempts were made to transmit the infection to clean monkeys and the results are shown in Table 1. A vervet monkey (No. 30) was bitten by four midges on September 28, 1960; the salivary glands were then extracted and all four were found to contain sporozoites. The blood of the monkey was examined twice a week until February 1, 1961, when parasites were first detected. There is thus a pre-patent period of four months.

Many of the batches of midges which had fed on *kochi*-infected monkeys were found to be infected in a range of 60–90 per cent, while controls fed on man were almost entirely negative (4/247). We have sought in vain for any explanation other than the obvious one that the midges were acting as vectors of *H. kochi*.

The experiments were tainted because we were compelled to use wild-caught flies, and they are therefore subject to the criticism that the sporogonic cycle may have had an extrinsic origin. If so, what could this have been? *Culicoides* spp. have been shown to be the natural vectors of *Haemoproteus* in Canadian birds[4] and we found *Haemoproteus* in weaver birds in the mangrove swamps where we were working. Nevertheless, if the sporozoites had been of avian origin, the infection-rate in the controls should have been similar to that in midges fed on monkeys, but instead of 60 or 70 per cent it was 1·6 per cent, while in unfed wild-flies dissected on the day of collection, the incidence was 0·2 per cent. Keeping the flies for a week or longer would, of course, allow natural infections to mature and the rate would increase accordingly (that is, to 1·6 per cent). It is, therefore, probable that the natural rates represented true *H. kochi* infections acquired in the field, because monkeys are said to descend from the second growth forest, scrub or plantations to the mangrove swamps by the creeks, where they would be attacked by midges.

The rapid cycle of sporogony (5 days), the low production of sporozoites, their gradual release from oöcysts, and the small size of the oöcyst, are all features[5] of the developmental cycle of two other common members of the family—*Leucocytozoon* and *Haemoproteus*, and as *Hepatocystis* belongs to this family (Haemoprotedae) it is not surprising that it also should exhibit all or most of these features. Canadian workers took a long time to identify with certainty the oöcysts of both *Leucocytozoon* and *Haemoproteus*; they are much smaller than in *Plasmodium* and it is not altogether surprising that, so far, we have been unable to find them in *Hepatocystis*.

Slight differences can be seen in the sporozoites of the different genera as follows: *Leucocytozoon*, 9–13μ in length; more pointed at one end. *Haemoproteus* (*Culicoides* type), 10–14μ; ends similar.

Haemoproteus (*Lynchia* type), 9μ or less; more pointed at one end. *Hepatocystis*, 13μ; more pointed at one end. *Plasmodium*, 10–14μ; ends similar.

The morphology of the sporozoite found in *C. adersi* is another indication that they are not *Haemoproteus*. The vector of *Leucocytozoon* is a *Simulium* which, presumably, excludes this organism from the argument.

We were afraid at first that Koch's '*Plasmodium*' was not going to conform with his 'Postulates'; the monkeys which were inoculated with sporozoites from slide preparations of glands are still negative, but the monkey which was bitten by infected *Culicoides* has now, after 4 months, become positive. The tissue forms of *Hepatocystis* have a diameter of about 40 times that of a liver schizont of *Plasmodium*; the pre-patent period of the latter is 5–10 days, so it is scarcely surprising to find that it takes nearly that number of months for *Hepatocystis* to mature in the liver.

We report these findings in the belief that the midge is the vector of *Hepatocystis*, and in the hope that similar experiments will be made elsewhere in the Old World where species of *Hepatocystis* are common in various animals.

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Azulene Derivatives as Plant Growth Regulators

Among the many hundreds of compounds examined for effects in promoting cell elongation, very few having activity do not have a ring structure possessing aromatic character[1]. Thus it is logical to look for activity in compounds having 'non-classical' aromatic rings such as derivatives of azulene and ferrocene. The azulene compounds are of particular significance since this ring system occurs frequently in essential oils and plant tissue, and anti-inflammatory, anti-allergic, bacteriostatic, cancerostatic and anti-arthritic activities have been claimed for azulenes[2].

Ferroceneacetic acid, ferrocenediacetic acid and ferrocenepropionic acid are all completely inactive in cell elongation as shown by their lack of effect on 3-mm. segments of *Avena* coleoptiles[3]. However, as shown in Table 1, some azulene derivatives have high activity in promoting cell elongation. Since azulene compounds are isomeric with corresponding naphthoic compounds, it would be expected that a similar pattern of activity would be found in the two series and the lack of activity of azulene-1-carboxylic acid and 1-naphthoic acid in coleoptile tissue[4] is in agreement. However, many reports have assigned activity to 1-naphthoic acid in pea tissue[4–6] and it was, therefore, of interest to examine the effects of the azulene compounds on elongation of 5-mm. segments of stem tissue of pea. Azulene-1-carboxylic acid does not promote elongation in pea tissue although 1-naphthoic acid is very effective as shown in Fig. 1. This finding demonstrates a difference in the plant substrate in pea tissue and *Avena* coleoptile tissue. Whether the difference in activity of the azulene-1-carboxylic acid and 1-naphthoic acid is the result of a steric or electronic difference in the 7- and 6-membered rings remains to be established. Although azulene-1-acetic acid and 1-naphthoic acid at low concentrations have much less effect on elongation than indole-3-acetic acid or α-naphthaleneacetic acid, at high concentrations their effects become equivalent.

Table 1. Percentage Elongation of Coleoptile Segments in Solutions of Azulene Derivatives and Naphthoic Acid as compared with Percentage Elongation in Water

	Molar concentration			
	10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}
Azulene-1-acetic acid	0	+12	+22	+31
Azulene-1-acetonitrile	+8	+17	+16	+11
Azulene-1-carboxylic acid	0	0	0	−8
1-Naphthoic acid	0	0	0	0

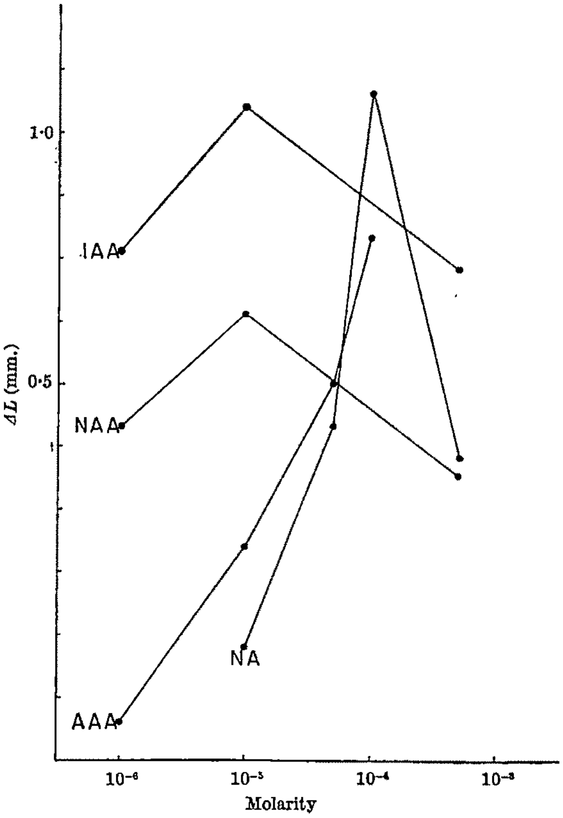

Fig. 1. Growth in length induced by indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA), azulene-1-acetic acid (AAA) and 1-naphthoic acid (NA) of segments of pea stem tissue

Azulene-1-acetonitrile is totally inactive in promoting elongation of pea tissue and is thus similar to indole-3-acetonitrile, which the pea tissue, unlike *Avena* coleoptile tissue, is unable to convert to the active acid[7].

It is difficult to decide how much activity should be expected for azulene-1-acetic acid in comparison with α-naphthaleneacetic acid. According to the two-point attachment hypothesis[1,3,6,8] such growth regulators react with an electron-rich point on a plant substrate through a position on the ring which is ortho to the carboxyl group of the regulator. It is well known that the 8-position in azulene is very susceptible to attack by electron-rich reagents, and high activity of azulene-1-acetic acid is therefore not surprising. Reactivity indices (approximate superdelocalizability) calculated by Koshimiza, Fujita and Mitsui[6] according to the frontier electron theory[8] indicate that the 8-position in 1-naphthoic acids is the site of reaction on the ring. Evidence for the involvement of the 8-position of azulene compounds in the reaction also exists in the sterically similar naphthalene situation where the 8-position seems more important than the 2-position, as Burstrom *et al.*[9] have shown 8-chloronaphthalene-1-acetic acid is much less active than α-naphthaleneacetic acid even though chlorine is normally an activating group. Whether or not the small activity at low concentrations is the result of the greater instability of the azulene ring or some basic structural unsuitability of the molecule is unknown.

The fact that the non-alternate aromatic system, azulene, is active in promoting elongation suggests that other such systems should be investigated.

We are indebted to Prof. Arthur G. Anderson, jun., of the University of Washington, for the azulene derivatives examined in this investigation, and to Prof. Kenneth L. Rinehart, jun., of the University of Illinois, for the ferrocene derivatives.

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ENTOMOLOGY

The Function of the Paleal Pegs of Corixidae (Hemiptera Heteroptera)

THE Corixidae are a family of aquatic bugs living on the habitat bottom. The front legs of both sexes are used for collecting food, the tarsi (or paleæ) being highly specialized for this purpose. The middle legs have retained their ambulatory form, and the long claws are used for anchoring the insect to the habitat bottom during feeding. The hind-legs are used for swimming, the tibiæ and tarsi being specially flattened for this purpose.

The front legs sweep a food-bearing water current towards the mouth by means of the longitudinal rows of setæ on the paleal edges (Fig. 1, *c* and *d*). In the males, the inner surface of each palea bears a longitudinal row of pegs, which is represented in the female by a row of short setæ (cf. Fig. 2 and Fig. 1, *b*). Kirkaldy[1] suggested these pegs were used for stridulation; but the work of von Mitis[2] has shown that sound is produced in other ways. The form of the male paleæ and the arrangement of the pegs is fairly constant for each species and these features have been used for species identification in British taxonomic works by Saunders[3], Butler[4], Macan[5] and by Southwood and Leston[6].

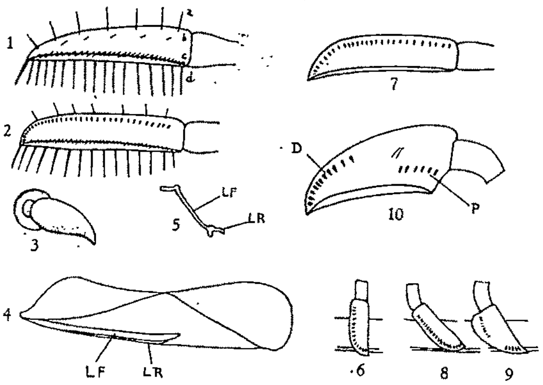

Fig. 1. Median surfaces of the tarsus and tibia from the front right leg of *Corixa punctata* (female). Fig. 2. Median surfaces of the tarsus and tibia from the front right leg of *Corixa punctata* (male). Fig. 3. Enlarged view on a paleal peg of *C. punctata*. Fig. 4. Hemielytron of *C. punctata*. Fig. 5. Enlarged view of a transverse section of the lateral flange to show outer ridges. Fig. 6. Left leg and lateral flange of *H. sahlbergi* during copulation. Fig. 7. Median surface of the tarsus and tibia of the front leg of *H. sahlbergi* (male). Fig. 8. View of left leg and lateral flange during copulation of a hypothetical species with a curved tibia. The paleal pegs would not grip the ridges of the lateral flange. Fig. 9. Lateral view of left front leg and lateral flange of *Sigara distincta* during copulation. Notice the distal row of paleal pegs is rotated to grip the flange ridges. Fig. 10. Median surfaces of the tarsus and tibia of *Sigara distincta* (male). (In Figs. 6, 8 and 9 the paleal pegs are drawn as though they could be seen through the palea.)

a, *b*, *c*, *d*, rows of paleal setæ; *D*, distal row of paleal pegs; *LF*, lateral flange; *LR*, longitudinal ridge of lateral flange; *P*, proximal row of paleal pegs

The true function of the paleal pegs may be observed during copulation. When mating, the male darts on to the back of the female which he holds by pressing his head against her pronotum—the frons of the male being flattened or even concave for this purpose. In addition the front legs are stretched sideways and the inner surface of each palea is extended over the lateral flange of the hemielytron on the corresponding side (Fig. 6). The paleal pegs are composed of a circular base on which is a short peduncle supporting a larger conical structure. The cone is not vertical, but bent towards the centre of the palea (Fig. 3). The lateral flange of the hemielytron (Fig. 4) is slightly concave in transverse section and the outer margin is raised into a longitudinal ridge, which in the region of the metathorax is enlarged and folded outwards to form a longitudinal grip (Fig. 5). When the front legs of the male are extended sideways, the distal pegs become caught under the curved longitudinal ridge of the lateral flange on the hemielytron and the two sexes are thus able to swim and surface while firmly attached to one another in this way.

In *Corixa punctata* (Illig.) the paleal pegs form a single row, which is curved distally to run slightly transversely across the palea (Fig. 2) and this appears to be a primitive arrangement in the Corixidae. Primitively the tibia is straight; but in the more specialized species tends to become curved inwards to aid food-collecting. If the paleal pegs retained their primitive arrangement, their effectiveness in holding the female would be reduced (Fig. 8), but changes in the tibia are associated with a broadening of the male palea and the row of paleal pegs is distally rotated so as to retain the original relationship with the hemielytral ridges. As a result of these changes, the row of paleal pegs tends to become broken into a distal functional row and a proximal row in which the pegs tend to become vestigial. These changes are well illustrated by a comparison of *Hesperocorixa sahlbergi* (Fieb.) (Fig. 7) and *Sigara distincta* (Fieb.) (Fig. 10).

At first sight the form of the male paleæ and the arrangement of the paleal pegs would appear to be non-adaptive; any one arrangement of paleal pegs being as effective as any other for gripping the female. But the front legs are used for other functions, particularly feeding, and a study of the functional morphology of the front legs shows that the form of the male paleæ and the arrangement of the pegs is of great functional significance, as well as being a valuable taxonomic feature at the species-level.

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Atypical Spermiogenesis in *Thermobia domestica*, a Thysanuran Insect

IN a recent communication, Bawa[1] has again put forward the hypothesis of a typical sperm in *Thermobia domestica*. However, I have shown[2], with some electron photographs of *Petrobius maritimus*, that the centriole and the acrosome are in their normal positions. The acrosome is a scoop-shaped body at the anterior end of the nucleus which forms a projecting tube. What Bawa has called the acrosome is probably another body, the centriole adjunct[3], which encloses a centriole and gives rise to the flagellum. In Thysanura, no division of the centriole occurs, and as such it is a fixed body on the posterior aspect of the nucleus. These observations are further strengthened by the detailed cytochemical reactions obtained by me, the results of which will be published elsewhere.

As regards the 'adnuclear fold' or the 'lateral fold', it is very clear from the electron photographs that the fold is not a continuation of the flagellum as it does not possess the typical fibrillar structure. On the contrary it is an intra-nuclear body of unknown significance. This body is an easily followed structure in X-irradiated *Petrobius*[4]. It also differs with the flagellum in chemical constitution.

Finally, I again stress that the sperm of thysanuran is a typical flagellate sperm and does not show any deviation from the normal patterns.

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MATHUR (preceding communication and ref. 1), in his communications, has again stressed that the thysanuran sperm is a typical flagellate one, having the acrosome and the centriole located at the proximal and distal end of the nucleus. I disagree with him and have the following comments to make in this context.

Mathur's[1] report is incomplete inasmuch as he does not mention (1) the name of the insect, (2) the stage of spermiogenesis electronmicrograph 1 represents and (3) the magnification of the two electron micrographs. I may add that I am unable to interpret his electronmicrograph 2. Furthermore, if I am interpreting correctly, Mathur's electronmicrograph 1 is a spermatid of a thysanuran. After scrutinizing scores of electronmicrographs of *Thermobia* spermatids[2], it is safe to point out that Mathur's acrosome *A* is actually the nucleus which shows nuclear pores at places, and his nucleus *N* is unmistakably a typical mitochondrial nebenkern revealing badly damaged cristæ. Mathur, however, does not mention anything about the mitochondria. It has been reported[2] that a typical mitochondrial nebenkern is formed in *Thermobia* and other thysanurans, and it remains an integral part of the mature spermatozoan. The structures F_1, F_2 and F_3 in Mathur's[1] electronmicrograph 1 do not reveal any structural detail to be identified as parts of the flagellum cut at different places. He also fails to make reference to bodies labelled as *M* in electronmicrograph 1. It is not clear whether bodies *F*, *M* identified by him in the upper right corner of electronmicrograph 1 are inclusions in the same cell referred to in the micrograph or are located in an adjacent cell. Structures *CA*, *M*, F_1, F_2 and F_3 probably are the microbodies or lipid elements, as such inclusions are invariably observed in the spermatids of *Thermobia*. So far as I can understand, the only structure identified correctly by Mathur[1] in electronmicrograph 1 is the Golgi material, *G*.

In *Pterobius* spermatid, Gatenby and Mathur[3] using light microscopy were unable to establish the correct nature of the 'adnuclear' or 'lateral fold'. This structure, according to Mathur, is the 'intranuclear' body of unknown significance. However, this 'intranuclear body' is seen to consist of the tail flagellum and the two mitochondrial nebenkern ribbons[1,4,5]. Electron microscopy[2] of *Thermobia* clearly indicates the composite nature of this intranuclear body. In favourably cut ultra-thin sections of *Thermobia* spermatids, one can discern tail filament complex and the mitochondrial cristæ in the nebenkern ribbons. It is important to mention also that the nucleus, mitochondrial nebenkern and the tail filament complex are seen to be disposed adjacent to each other. Such a unique spatial arrangement is especially clear in the cross-sections. Finally, there is evidence[2] that the acrosome occupies the basal end of the sperm head and that it is derived from the Golgi dictyosomes. The centriole is not embedded in the 'centriole adjunct' and it moves around the nucleus until it lies toward the apical end of the nucleus. Thus there is no reason to confuse the 'centriole adjunct' of Gatenby *et al.*[6] with the acrosome of *Thermobia domestica* spermatozoan.

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GENETICS

Mitotic Haploidization by Treatment of *Aspergillus niger* Diploids with *para*-Fluorophenylalanine

In genetic analysis based on mitotic segregation[1-4] an important step is the recovery of haploid segregants from heterozygous diploids. This has been done until recently by laborious or complicated techniques[4,5]. I am indebted to Dr. G. Morpurgo, of the Istituto Superiore di Sanità, Rome, for informing me and permitting me to make use of his discovery that treatment with *para*-fluoro-phenylalanine of diploids of *Aspergillus nidulans* leads to wholesale segregation (this discovery was made known in the privately circulated *Aspergillus News Letter*, p. 10, No. 2, Spring 1961). In an extensive work expanding the formal genetical analysis—via mitotic segregation—of the fungus *A. niger*[6], I have applied this treatment to diploids of this asexual species.

The amino-acid analogue DL-*para*-fluoro-phenylalanine was incorporated in standard complete medium[7] to a final concentration of 1/10,000 w/v and conidia from diploid strains heterozygous for a number of colour and nutritional markers were plated on the surface. At this concentration of the analogue, the viable counts remain the same as on standard complete medium, but the rate of growth is much slower (diameter of colonies after 5 days: 0·5–1 cm. instead of about 10 cm.) and sporulation is very poor. These colonies produced sectors, the rate of growth and sporulation of which was about the same as on normal complete medium. After 10 days, conidia of these sectors were streaked on complete medium, classified for ploidy on the basis of the diameter of their conidia and tested for requirements. All of them turned out to be haploid and of all possible recombinant types between independent markers.

As an example, a heterozygous diploid with black heads (wild-type) and prototrophic was synthesized from two haploid strains: one fawn (*a*) and the other olive (*o*) (two recessive non-allelic markers belonging to the same linkage group). The two strains also differed in four recessive nutritional requirements and the genotype of the diploid formed from them was:

$$\frac{a \qquad +}{+ \qquad o} \qquad \frac{+}{hist} \qquad \frac{+ \qquad gua_7}{lys_4 \qquad +} \qquad \frac{meth_7}{+}$$

The vigorously growing sectors produced by this diploid were either fawn or olive. To minimize clonal effects, only one sector of each colour (fawn or olive) was picked from a colony. The 74 sectors so isolated, 38 fawn and 36 olive, fell into the 8 possible classes of segregants and recombinants in respect of the four other markers belonging to three different linkage groups.

That haploidization, as a consequence of *para*-fluoro-phenylalanine treatment, occurs by successive losses of chromosomes—as found by Käfer[4] for *Aspergillus nidulans* in the case of 'spontaneous' haploidization—is shown by the following evidence: The results just mentioned are obtained by isolating vigorously and well-sporulating sectors from diploid colonies grown in the presence of the analogue for ten days. If small groups of hyphal tips are taken from diploid colonies only after 48 hr. of growth in the presence of the analogue and transferred to normal medium, they produce sectors in which more and more nutritional markers segregate as growth proceeds.

On *para*-fluoro-phenylalanine the amount of growth intervening between the original diploid mycelium and the completely haploid sectors is relatively small, indicating a selective effect against diploids and disomics. I interpret this to mean that loss of individual chromosomes in a haploid leads to no further divisions of that nuclear lineage, while in a diploid or disomic stunted growth may go on.

The availability of a treatment which produces wholesale haploidization is a very valuable addition to the techniques for genetical analysis via mitotic segregation.

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Hybrids between *Solanum bulbocastanum* and *Solanum cardiophyllum*

The Mexican diploid tuberous *Solanum* series Bulbocastana and Cardiophylla are important potential sources of genes for resistance to late blight of potatoes. Niederhauser and Mills[1] noted field immunity in certain clones of *S. bulbocastanum* in their field trials in Mexico. No investigator has hitherto reported hybrids between these series and the Tuberosa. A knowledge of the crossability relationships of the Mexican diploid species may be useful in overcoming their apparent isolation.

Extensive collections were made of *S. bulbocastanum* Dun. and *S. cardiophyllum* Lindl. throughout their ranges in Mexico during 1956 and 1957. The collections of *S. cardiophyllum* were comprised of *S. cardiophyllum sensu stricto*, *S. ehrenbergii* (Bitt.) Rydb. and *S. cardiophyllum* var. *endoiodandrum* Bitt. Seed set occurred from crosses between *S. bulbocastanum* (C-238) and *S. cardiophyllum* (C-297), and in the reciprocal cross between *S. ehrenbergii* (C-370) and *S. bulbocastanum* (C-194).

The hybrid plants from both crosses showed in their seedling stages the unifoliate characteristic of the *S. bulbocastanum* parent. As they approached the flowering stage, they presented the aspect of a compound-leaved *S. bulbocastanum*.

Although the hybrids produced a high percentage of stainable pollen, it was not always possible to obtain fruit and seed set by sibling pollinations within the families *C*-238 × *C*-297 and *C*-370 × *C*-194. The inter-family cross-pollinations attempted were successful in both directions. This observation suggests a repetition of the intra-family cross-incompatibility noted by Graham *et al.*[2] in *S. bulbocastanum*.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Tuesday, May 23

University of London (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. B. G. Neal: "Some Problems in Plasticity" (Inaugural Lecture).

Society of Chemical Industry, Plastics and Polymer Group (in the Film Theatre of Shell-Mex House, Strand, London, W.C.2), at 6 p.m.—Annual General Meeting. Mr. P. S. Adamson: Chairman's Address.

Society of Instrument Technology (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Annual General Meeting. 7 p.m.—Presidential Address.

Wednesday, May 24

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Anniversary Meeting. Prof. C. F. A. Pantin, F.R.S.: "On the Teaching of Biology" (Presidential Address).

Eugenics Society (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. C. D. Darlington, F.R.S.: "Cousin Marriage".*

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting, followed by Mr. T. McLachlan: "Some Historical Notes on Food Technology".

British Psychological Society, Medical Section (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 8.30 p.m.—Symposium on "The Saboteur in Group Psychotherapy". Speakers: Dr. Malcolm Pines, Dr. Robin Skynner and Dr. S. Resnik.

Thursday, May 25

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Sir Charles Darwin, F.R.S.: "The Gravity Field of a Particle. II". Mr. B. Thwaites: "The Aerodynamic Theory of Sails. I: Two-dimensional Sails".

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. K. R. Hyde, Prof. P. L. Robinson, Mr. M. J. Waterman, and Dr. J. M. Waters: "Reaction of Beryl with Sodium Fluorosilicate Used in Extracting Beryllium from the Mineral"; Dr. J. S. Tooms and Mr. S. Kaewbaidhoon: "Dispersion of Tin in Soil over Mineralization at Sungei Lembing, Malaya".

Royal Society of Arts, Commonwealth Section (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Mr. Wilfred Brining: "Bauxite and its Importance to the Commonwealth" (Henry Morley Lecture).

Institution of Electrical Engineers, Utilization Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. A. Broughall: "Electric Traction".

Institut Français du Royaume-Uni (at Queensberry Place, London, S.W.7), at 6.15 p.m.—Dr. Tomatis (Paris): "Phonation et Audition".*

Friday, May 26

British Psychological Society, Occupational Psychology Section (in the Department of Psychology, Birkbeck College, Malet Street, London, W.C.1), at 1 p.m.—Mr. Denis McMahon: "An International Study of Military Training".

Scientific Publications Council (at the Ciba Foundation, 41 Portland Place, London, W.1), at 6 p.m.—Discussion Meeting on "Time-Lag in the Publication of Scientific Manuscripts".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or **Assistant Lecturer in Bacteriology**—The Registrar, The University, Leeds 2 (May 27).

Lecturer (with an honours degree) **in Physics**—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (May 27).

Research Assistant (with a good degree in chemistry or biochemistry and preferably some research or industrial experience in the lipid field) **in Lipid Chemistry**—The Registrar, Brunel College of Technology, Woodlands Avenue, Acton, London, W.3 (May 27).

Demonstrator (Assistant Lecturer) in Physiology—The Registrar, The University, Leeds 2 (May 29).

Lecturer in Mathematics—The Principal, Borough Road College, Isleworth, Middlesex (May 29).

Assistant Lecturer in Physics—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 31).

Lecturer (graduate with medical qualifications) **in Anatomy**—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 31).

Lecturer (with special interests in physical or inorganic chemistry) **in the Department of Chemistry**—The Secretary, The University, Edinburgh (May 31).

Senior Lecturer/Lecturer in Geography at the University of New England, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 31).

Reader in Mathematics at Westfield College—The Academic Registrar, University of London, Senate House, London, W.C.1 (June 8).

Lecturer or **Assistant Lecturer** (medically qualified or otherwise) **in the Department of Physiology**—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (June 19).

Chair of Geography at University College, Ibadan, Nigeria The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 20).

Lecturer (preferably with a special interest in either vertebrate zoology or genetics) **in the Department of Zoology**, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 20).

Assistant Under-Librarian (graduate in science or with experience of library administration)—The Librarian, University Library, Cambridge (June 22).

Assistant Professor (Poultry Genetics) (with a Ph.D. or equivalent in genetics, and preferably training in biochemical genetics) **in the Department of Animal Science**—Head, Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada (June 30).

Chair of Education in the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, June 30).

Reader in Fuel Science in the Newcastle Division of the University—The Registrar, University Office, 46 North Bailey, Durham (June 30).

Research Fellow (Organic Chemist) in the Department of Medical Chemistry, John Curtin School of Medical Research, Australian National University, Canberra, to work with Prof. Adrien Albert or Dr. D. J. Brown on problems in the pyrimidines, pteridines or other hetero-aromatic series—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

Research Fellow (with a Ph.D. degree) and a **Research Student** (with an M.Sc. or an honours degree) **in the Department of Physics**, to participate in the research of a small group working on problems of brittle fracture and on the influence of absorbed substances on the strength of solids such as silica and tungsten carbide—Prof. F. R. N. Nabarro, c/o The Registrar, University of the Witwatersrand, Milner Park, Johannesburg, South Africa (June 30).

Senior Lecturer or **Lecturer in Physics** at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 31).

Senior Lecturers and **Lecturers** (with qualifications in either pure or applied mathematics) **in Mathematics** at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, August 9).

Assistant Lecturer in Organic Chemistry—The Registrar, The University, Manchester 13.

Assistant Professor (Ph.D. or M.B.) **in Pharmacology**—Prof. M. F. Murnaghan, University of Ottawa, Ottawa, Ontario, Canada.

Biochemist, to supervise all chemical procedures and to conduct research in fields related to blood transfusion in consultation with the Director—The Director, Red Cross Blood Transfusion Service, 409 Adelaide Street, Brisbane, Queensland, Australia.

Chemist or **Biochemist** (with an honours degree, a good knowledge of general chemistry and an interest in protein chemistry), to investigate the constituents of animal connective tissue together with related enzyme systems—The Director, Low Temperature Research Station, Downing Street, Cambridge.

Chief Laboratory Technician (with the City and Guilds Final Certificate in Laboratory Technology (or accepted equivalent) and previous experience in physiology, pharmacology or biochemistry, including the care and maintenance of equipment and apparatus) **in the Department of Physiology and Pharmacology**—Head of the Department of Physiology and Pharmacology, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

Curator (preferably with experience in museum administration, as well as general scientific knowledge, especially in biological fields) of the Coryndon Museum, Nairobi, Kenya—The Secretary, Coryndon Museum, P.O. Box 658, Nairobi, Kenya, East Africa.

Junior Biochemist, Basic grade (with an appropriate science degree or associateship of the Royal Institute of Chemistry) **in the Chemical Pathology Laboratory**, Nottingham General Hospital—The Group Secretary, Nottingham No. 1 Hospital Management Committee, General Hospital, Nottingham.

Master to teach Mathematics throughout the school—The Headmaster, Malvern College, Malvern, Worcs.

Research Students and **Assistants in the Department of Physics**—Prof. D. J. E. Ingram, Department of Physics, University College of North Staffordshire, Keele, Staffs.

Senior Mathematics Master—The Headmaster, Adams' Grammar School, Newport, Salop.

Technical Research Assistant in the Botany Department, to work with Prof. Street on the establishment of free cell cultures from higher plants—The Registrar, University College of Swansea, Swansea.

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ACADEMIC FREEDOM IN SOUTH AFRICA

WHATEVER view may be taken as to whether South Africa should continue to be a member of the Commonwealth while her Government pursues the policy of apartheid, the withdrawal of South Africa from the Commonwealth can only be regarded, as the Prime Minister said in the House of Commons on March 22, as a very sad event; "sad because of what seems to us a tragically misguided and perverse philosophy which lies at the root of apartheid; sad because of the many people in South Africa . . . who would like it at least tempered and made more elastic and more humane; sad because this event marks the end of an association of our countrymen for more than a hundred years with Colonies formed in Capetown, Natal and elsewhere; sad because it is the end of a fifty-year connexion which began with a decision then hailed as an outstanding example of magnanimity after victory; sad because it makes a breach in a community which has a great part to play in the world".

The issue of apartheid in connexion with academic freedom has, of course, been discussed several times in these columns (see *Nature*, June 6, 1959, p. 1551). It has already created difficulties with which some scientists have been particularly concerned. It has to some extent jeopardized the contribution which South Africa could make to the work of the Scientific Council for Africa South of the Sahara and of the Commission for Technical Co-operation in Africa South of the Sahara, and it has struck a serious blow at the South African universities. With the best will in the world, it is unlikely that those difficulties will diminish in a South Africa outside the Commonwealth. As Mr. Duncan Sandys, the Secretary of State for Commonwealth Relations, rightly said in the debate in the House of Commons, we must not let it be thought that it makes no difference whether one is in or out of the Commonwealth. This Mr. Sandys reaffirmed on April 24 in introducing the Republic of South Africa Temporary Provisions Bill. In his recent speeches, Dr. Verwoerd has been giving the impression that South Africa's withdrawal will make no difference; but while there will be a general wish to maintain close relations with South Africa and particularly to do anything that may be possible to help those South Africans who cherish their ties with Great Britain and are opposed to Dr. Verwoerd's policy and action, the position has been greatly changed by that withdrawal.

The effect of withdrawal on scientific research in South Africa and on South Africa's co-operation in scientific and technical matters was not discussed in either House of Parliament; but in the debate on South Africa and the Commonwealth in the House of Lords on March 23, Lord Beveridge referred briefly to the universities. Lord Hailsham, in opening the debate, had pointed out that, while it would be idle to pretend that the withdrawal from the Commonwealth would not affect relations between the two countries, time alone would show how far-reaching that change would be. For that reason the Government had decided to introduce a standstill measure, so that we might have time to think in an atmosphere of friendliness and toleration exactly what our course should be.

Lord Hailsham referred particularly to our obligations to our South African fellow citizens in High Commission Territories, and said that we must maintain relations with the South African Government which permit us to discharge them honourably. In this connexion, however, it must also be remembered that the educational policy of the South African Government has already made it more difficult for the needs of those territories to be met by our existing arrangements, especially in respect of higher education. Questions of status and citizenship will also, as Lord Hailsham noted, require discussion in an atmosphere of calm.

Lord Beveridge, who stressed the importance of considering the interests of people of British descent and British outlook in South Africa, referred to the implications of the South African Government's policy of introducing racial discrimination into the open universities of Cape Town, the Witwatersrand and Natal. He regarded it as vital to the future of peace between coloured people and white people that these universities should be made open and self-governing and free from racial discrimination. Under the Extension of University Education Act of 1959, these universities cannot continue to accept non-white students without the written consent of the Minister, and up to the present, even while the non-white university colleges are being developed, the Minister has refused all but 2 out of 153 applications from Bantus.

That Act is a serious infringement of university autonomy which in itself could have disastrous consequences for the universities as well as for science and learning generally in South Africa. These consequences could perhaps be exaggerated, for a university is inevitably influenced by the kind of community in which it is situated, and university autonomy cannot be maintained in a community which does not understand its nature or seek to preserve it, however firm the safeguards against interference by Government; though it should be remembered that there is a considerable volume of opposition to the South African Government's policy within South Africa. No less serious, however, is the extent to which the Act shatters the South African university tradition by subjecting the whole of a university staff to appointment by a Minister and to control in all their university activities by Ministerial regulations and by a statutory code of discipline which stifles the academic conscience.

The South African Government's proposals were opposed at the time by the universities with noteworthy dignity and cogency, and in their opposition they had the unequivocably expressed support of the universities of the world. The South African Government, however, was quite unmoved by this opposition, and with the withdrawal of South Africa from the Commonwealth it must become increasingly difficult to assist the South African universities to sustain their old and high traditions of learning and investigation, even if they are still able to participate unhindered in the meetings of the universities of the Commonwealth. Only time can show to what extent they will find it more difficult to recruit staff of high standing, for either teaching or research. It is at least unlikely that outstanding investigators will be ready to enter institutions where such Ministerial restrictions on freedom of investigation and utterance are current; if the full effect is only felt as existing members of the universities reach retiring age, the ultimate result will be no less disastrous.

Leaving out of the discussion the needs of the coloured peoples of South Africa, which the South African Government proposes to meet by measures described by Lord Beveridge as utterly unworthy of being termed university education, it is a matter of doubt whether the South African universities, under these conditions, will be able to supply the scientists and technologists which the country needs to-day. Obviously they will be unable to supply the numbers required, for the exclusion of coloured races means that they can offer no contribution to the immense numbers of trained Africans that are required to deal with the urgent problems of development in Africa to-day. Any contribution to the expansion visualized at the Commonwealth Education Conference is likewise barred, and it is more than doubtful whether they will long continue to supply the white scientists and technologists of the high quality required. A system which strikes at the root of the free intercourse and communication on which scientific and technical advance depends, and forces South African workers to go to Britain or America to meet Africans of university standard and ascertain their views, as Dr. H. J. Grenville-Wells pointed out at the conference on university apartheid in November 1957, carries the seeds of its own decay.

The problem, however, is essentially to find some means of inducing the advocates of apartheid to realize the implications of its application in the universities as regards scientific and technical work, or professional work generally, and of considering the possibility of relaxation to avoid those untoward consequences. The English-speaking universities have protested with dignity and determination against this closure of their open doors, and the University of the Witwatersrand, in particular, not content with affirming the principle that a university is a place where men and women, without regard to race or colour, are welcome to join in the acquisition and advancement of knowledge, has dedicated itself to the maintenance of this ideal and to the restoration of its autonomy. Its members are fully aware that a university can only make its proper contribution to national life when it is assured of freedom to teach, to investigate, to discuss and to criticize, and that when deprived of any one of these the quality of its intellectual life will decline, creative work be stifled, research discouraged or discontinued, and ultimately the quality of its teaching become that of the school class-room. Nor are they under any illusions that time is on their side: unless their autonomy is regained before the new Act has its full insidious effect, their university status will be lost, perhaps irretrievably.

Sir Eric Ashby, who in *The Guardian* last year described some of the protests made by the English-speaking universities, finds some hope in the liberalism to be found among some Afrikaans-speaking intellectuals, although the four Afrikaans-speaking universities, the policy of which has been to enrol white students only, in public and officially subscribe to the policy of apartheid. Prof. B. B. Keet, of the University of Stellenbosch, and Prof. P. V. Pistorius, of the University of Pretoria, have both incisively condemned the Government policy, and probably the book, *Our Responsibility*, by the Hon. H. A. Fagan, formerly Chief Justice of South Africa, is even more influential. While it is unlikely that criticism even from such quarters will have much influence on the present South African Government, at this juncture of South Africa's withdrawal from the Commonwealth it is well to remember that the policy of apartheid finds its critics in South Africa among people of Dutch as well as of British stock.

The Act which strikes at the very existence of the South African universities has another effect. So far from extending university education, its immediate effect is to put higher education out of the reach of hundreds of non-whites. It is true that it established three so-called university colleges for Bantus and one for other non-whites, but so far they are out of reach of thousands of the students for whom they are professedly intended, and, apart from the legitimate doubts expressed by Lord Beveridge as to their academic calibre, there is no evidence that the South African Government is sincere in these constructive proposals of the 1959 Act. To all intents and purposes, the Act has wrecked any immediate prospect of the coloured peoples of South Africa being trained to a level permitting them to make the contribution to the development of Africa which they could justly expect to make.

That aspect will undoubtedly deeply influence the attitude of members of the United Nations to the South African Government, and it may well come to affect in due course South Africa's membership of such bodies as the Scientific Council for Africa South of the Sahara, though it must be remembered that the members of that Council are not representatives of Governments or responsible individually to the country from which they are drawn. The Council is rather a body of independent scientists, the primary allegiance of which is to the advancement of science for the benefit of Africa. The Commission for Technical Co-operation in Africa South of the Sahara, on the other hand, is an inter-Governmental organization, and it remains to be seen to what extent it will be possible for the Commission to continue to provide a forum for the discussion of technical matters of importance to the continent while the South African Government maintains its present views on racial questions.

Just what the universities in the Commonwealth can do to offset the weakening of the South African universities by the action of the Government of the country is not easy to say. It is, of course, important that the Commonwealth universities should not confuse the South African universities with the South African Government, and if they can only give moral support, that should be given without stint and at every possible opportunity. The stand which the English-speaking universities of South Africa are making for academic freedom is writing a noble page in the history of the universities of the world. It is important that the academic world as a whole should be fully aware of what is happening in South Africa: of the courage of those who, in spite of sanctions, maintain their disapproval of the new Act; of the ostracism of members of the staff of Afrikaans-speaking universities who defend academic freedom. The withdrawal of South Africa from the Commonwealth has not changed such things, nor has it diminished the claims on the whole academic and scientific world for the support of those who in South Africa are striving so valiantly to maintain the conditions on which scientific advance and academic life depend.

ANALYSIS OF AGRICULTURAL MATERIALS, FOODS AND DRUGS

Official Methods of Analysis of the Association of Official Agricultural Chemists

Edited by William Horwitz. Ninth edition. Pp. xx+832. (Washington, D.C.: Association of Official Agricultural Chemists, 1960.) 18 dollars.

THE Association of Official Agricultural Chemists was founded at Philadelphia, U.S.A., in 1884 by a group of chemists interested in the analysis of fertilizers and their application to growing crops. The earlier work of the Association was concerned with devising methods of analysis for fertilizers and other agricultural materials the composition of which was the subject of State laws; results obtained by these methods were accepted for enforcement purposes. Later, as further legislation controlling food, drugs, insecticides and fungicides was introduced, the interests of the Association widened to include these items. Eventually, in 1920, the methods were published in book form under the title *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, and, by that time, the range of contents had become extensive, covering beverages, foods and related substances, in addition to agricultural materials and drugs.

Methods are continually being tested by the Association in order to keep pace with new legislation, and the established methods are constantly reviewed in the light of developments in analytical technique. Any changes or additional methods are published first in the Association's journal, and are eventually incorporated into the book of methods, which is revised every five years for this purpose. The present, ninth, edition therefore represents the accumulated work of 76 years.

Except for the omission of the words "and Tentative", the title of the book has remained the same during these nine editions, and, to those readers who are not familiar with the work of the Association, it could be misleading, as it suggests that agricultural analyses form the main subject. The table of contents shows this is not so: of the 43 chapters, only seven are directly concerned with agriculture. Indeed, the chapter on soils which was included in previous editions has now been omitted because of the specialized nature of that subject. In effect, the operative word in the title is "Official", and it is to analysts who are concerned with the examination of foods, beverages (both alcoholic and non-alcoholic), drugs, fertilizers and feeding stuffs that this book must prove most useful. As a source of standard methods it holds a unique position, and it is widely quoted for this purpose.

The changes occurring in the new edition are extensive. Classical methods tend to remain unaltered, although a colorimetric method for phosphorus is now included in the chapter on fertilizers, and a useful addition to the Kjeldahl method for determination of nitrogen is a means of standardizing the digestion heaters.

The work of the Association, however, is naturally directed more to modern problems; the danger to public health of pesticide residues on foods, for example, has emphasized the need for methods of determining trace amounts of these newer organic materials. The ninth edition includes methods applicable to certain crops for the extraction and determination of many such pesticides, including aramite, BHC, captan DDT, malathion, methoxychlor, parathion, piperonyl butoxide, sulphenone and thiram.

Also in connexion with the presence of trace amounts are the methods for determining drugs and antibiotics in feeding stuffs. This subject has been extended considerably and now occupies a separate chapter. The chapter covering drugs in general contains many new methods and numerous revisions.

In developing the newer methods, the technique of chromatography on columns is used extensively for the isolation of the desired compounds; the use of paper chromatography is also made official for the first time in this edition, for example, for detecting commercial glucose in honey, other plant materials in vanilla extract and for the differentiation of the BHC isomers.

The examination of foods for "extraneous materials" has been of interest to the Association for many years, and the chapter on this subject, although little different from that in the previous edition, is particularly valuable. Many changes have been made in the section entitled "Nutritional Adjuncts", which now includes methods for the determination of vitamin A in margarine and in mixed feeds, and for the fluorometric determination of vitamin B_1 in bread. The individual methods for the microbiological determination of the B-group vitamins have been replaced by a single general method with appropriate modifications for particular vitamins. A method for the biological evaluation of protein quality is also included in this section.

The actual amount of material in this book is continually increasing, and in order, therefore, to keep the size within reasonable bounds the style of this new edition has been changed to a larger page divided into two columns. The new format is easier to read, and I welcome the change, especially as it means that further abbreviations of the text have thus been avoided. H. V. HART

CONTROLLED THERMONUCLEAR REACTIONS

Plasma Physics and Thermonuclear Research

Vol. 1. Edited by G. Longmire, James L. Tuck and W. B. Thompson. (Progress in Nuclear Energy, Series XI.) Pp. vii+612+xii. (London and New York: Pergamon Press, 1959.) 105*s*. net; 15 dollars.

Nuclear Fusion

Edited by Prof. William P. Allis (Second Geneva Series on the Peaceful Uses of Atomic Energy.) Pp. viii+488. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1960.) 12.50 dollars; 94*s*.

Controlled Thermonuclear Reactions

An Introduction to Theory and Experiment. By Samuel Glasstone and Ralph H. Lovberg. Prepared under the auspices of the Office of Technical Information, United States Atomic Energy Commission. Pp. xvi+523. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1960.) 5.60 dollars; 42*s*.

THE opening in September 1958 of the second International Conference on the Peaceful Uses of Atomic Energy will go down in scientific history as the day the dam burst. It was then that the veil of secrecy was removed from some six years of intensive study in the United States, the United Kingdom and the U.S.S.R. of what is now generally called nuclear fusion. The views and results of hundreds of scientific workers were suddenly thrown open for free discussion, and were supplemented by an exhibition of working experimental equipment on an unprecedented scale.

The first of these three books provides the edited proceedings of this part of the Second Geneva Conference. It is divided into surveys of progress in the chief countries working in this field, followed by reviews of the main experimental devices, the *Stellarator*, the magnetic mirror, the plane shock and the pinched discharge.

These are succeeded by a series of more general theoretical papers on plasma physics and finally by proposals from the U.S.S.R. and the United States for more esoteric methods of stabilizing hot plasma, such as the *Astron*.

Valuable though this collection is, the papers are not easy reading; hence the second book, by Prof. Allis, which is successful in providing most of the information in a more easily digested form, is welcome.

The third work, by Glasstone and Lovberg, carries the process of digestion still further, and is an excellent advanced introduction to the whole subject. It is a graduate text-book carefully thought out to meet the needs of experimentalists rather than of theoreticians, though theoreticians will find it a valuable guide through the thicket of experimental results and devices. Particularly good is the second chapter. Here are discussed the physical conditions which have to be met for thermonuclear reactions to occur at all, as well as those needed to achieve a net gain of power in any practical device.

It is now clear that the difficulties in the way of the attainment of economic power by fusion of the isotopes of hydrogen are formidable. The fuel (deuterium gas or a deuterium–tritium mixture) must be confined in a space isolated from material walls and maintained in the ionized state at an extremely high temperature (some tens or hundreds of millions of degrees) for recurrent periods each lasting about a second. In nearly all proposals this confinement involves the use of powerful magnetic fields and in the forms most worked with—the simple pinch and the *Stellarator*—they have so far been found to fail because the confined plasmas are basically unstable. Instabilities may take several forms; to discover means of curbing them calls for a much deeper understanding of their nature than exists at present, and of the way they build up from small perturbations to large disturbances and so cause the plasma to escape.

The authors deal in as clear a way as seems possible to-day with this matter, as well as with radiation losses from a very hot plasma, including 'cyclotron' radiation-loss at high temperatures, a spectre raised by Russian workers two years ago and not yet fully laid.

In his foreword to the book, Dr. A. E. Ruark expresses the hope that it will command the respect and gratitude of a world-wide audience. He can be assured that it will.

These three works show that the achievement of economical controlled thermonuclear power presents an exceptional scientific and technical challenge, one so difficult that it may indeed never be possible to meet. It is far too early to be pessimistic about the outcome of this great enterprise, but it is now clear that success will not be easily attained nor will progress be so rapid as with power from nuclear fission. BASIL SCHONLAND

CARBOHYDRATE CHEMISTRY

The Carbohydrates

By S. F. Dyke. (The Chemistry of Natural Products, Vol. 5.) Pp. v + 232. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 36*s*.

THIS book is one of a series of monographs designed to supplement the general text-book of organic chemistry with more detailed descriptions of the main groups of natural products. It gives a balanced account of the various facets of carbohydrate chemistry, and the text is adequately supplied with references to reviews and original papers. The author assumes little or no previous knowledge of the subject, but the treatment is sometimes sketchy and some of the material is presented in the form of notes with little commentary. One feels that the student would have been better served if the evidence for recent developments in the field had been treated more fully, and if greater emphasis had been given to the present growing points of the subject. In these respects this volume does not reach the high standard set by some earlier volumes in the series.

The first three chapters of the book deal with the basic chemistry of the monosaccharides, their general reactions, stereochemistry, and ring structures. In the two following chapters, derivatives of monosaccharides are discussed, both those used in synthesis and structural studies, and those of natural occurrence, including the branched-chain sugars and deoxy sugars. It is a pity that, apart from two rare sugars found in antibiotics, sugars containing more than six carbon atoms are not discussed, and that no mention is made of the aldoheptoses found as constituents of polysaccharides from micro-organisms, or of the naturally occurring heptuloses and heptitols, or of the sialic acids with nine carbon atoms. The next chapter provides examples of *O*-glycosides of plant origin, but no space is found for any discussion of the chemistry of *N*-glycosides, for example nucleotides. The chapter on oligosaccharides gives examples of both chemical synthesis of disaccharides and of the determination of structure of di- and tri-saccharides. There follows a brief survey of polysaccharide chemistry, including an account of general methods of structural determination and a description of some representative groups. Although the full potential of enzymes as tools for structural investigation of polysaccharides has yet to be realized, one feels that their use in the elucidation of the fine structure of starch and related polysaccharides deserves more specific mention. The book is concluded with a chapter on carbohydrate metabolism with sections on glycolysis, photosynthesis, and the biosynthesis of monosaccharides and polysaccharides.

At present-day prices the book is reasonable value. There are comparatively few errors, and the general standard of production is good.

G. O. Aspinall

HUMAN NUTRITION

Recent Advances in Human Nutrition, with Special Reference to Clinical Medicine

By Prof. J. F. Brock. Pp. xii+454. (London: J. and A. Churchill, Ltd. 1961.) 50*s*. net.

PROF. BROCK has contributed considerably to our knowledge of human nutrition by a large amount of important research. Reluctantly, one must conclude that he has not contributed anything like so impressive a book.

To begin with, it is impossible to decide the sort of person for whom it was written. Certainly not for the average clinician, for he would have no idea of what is meant by "*cis-trans isomerism*". The nutritionist, on the other hand, would not accept that "there is no specific sign of riboflavin deficiency in the human". Moreover, the nutritionist would have to be very magnanimous to forgive the misspelling of one of the world's outstanding nutritionists as "Bam".

The first eighteen chapters of the book are written by Prof. Brock himself. The remainder is written by a number of other nutritionists, and, as is so common with multiple authorship, the result is uneven. Indeed, it is difficult to understand why some parts were included at all, since they have little or no bearing on nutrition. An interesting feature is that several chapters describe the nutritional work in particular areas; much of this information will be new even to many nutritionists.

It is understandable that Prof. Brock and his colleague Dr. Bronte-Stewart, who have done a great deal of original and important work on the subject, should stress the possible role of dietary fat in the causation of coronary disease. But in a book of this sort one would have liked to see rather more mention of the contradictory evidence in the alleged connexion between the two. Many would not agree, for example, that "only the dietary fat intake remains consistently in parallel with the mortality experience of this disease".

Again, one is disappointed to find that the myth is still perpetuated that the cause of obesity "remains a mystery". It is just not true that "there is still a fortunate majority who can dispose of excess calories with impunity and an unfortunate minority who have to count every calorie".

There are very few books dealing generally with the subject of nutrition, so that this book, by Prof. J. F. Brock, was eagerly opened and read by me. It is disappointing to have to report that the book does nothing to enhance the reputation of a most distinguished clinical nutritionist.

J. Yudkin

GRASSLAND PHILOSOPHY

The Grass Crop

Its Development, Use, and Maintenance. By Dr. William Davies. Second, revised edition. Pp. xv+363+16 plates. (London: E. and F. N. Spon, Ltd., 1960 45*s*.

THE second edition of William Davies's *The Grass Crop*, which has been brought up to date and somewhat enlarged, is again a welcome addition to the literature of the subject. This is not a textbook in the generally accepted sense nor yet a purely practical guide for farmers, for it is something much more profound and wider in its scope. It is, in fact, a clear exposition of grassland philosophy, and threaded throughout its pages is the philosophy of one who has studied grassland in all its facets and in many parts of the world. This is well illustrated by one sentence: "The world at large must recognize that the soil is its one heritage which it must pass on unspoilt and untrammelled to the generations to come".

How true this is and yet how often forgotten in the mælstrom of technical agricultural development to-day. Throughout the book, however, the author is at pains to emphasize the fertility-building properties of the ley as well as its contribution to reclamation and to solving the livestock feeding problems for milk and meat production which face us to-day. Two new chapters, "Farming Systems" and "Extending the Grazing Season", pin-point the remarkable changes that have occurred in our farming of recent years and outline something of the vast potential which the grass crop still has to offer.

A cautionary word, too, is uttered on the too slavish adoption—a modern trend—of statistical analysis of new techniques before they are advised for incorporation into farm practice. Each farmer should interpret experimental results for himself and try to relate them to his own specific conditions. He should try new methods which, in the light of his experience, offer advantages. The trial may fail, but it is far better to fail on the chance of succeeding than not to try out a new technique simply because in trial plots the results did not attain the desired level of significance to suit the statisticians.

Future prospects are very ably summarized in a final chapter when problems facing both research workers and farmers are clearly stated. The problems cited do not simply concern grass as a crop, but also the animals which consume the grass. Animal nutrition is as important as crop nutrition, and only of recent years has research indicated that there are fundamental differences in herbage plants which dictate the reactions of animals to them in the digestibility and production secured from them when eaten by ruminants. Parasitism raises a multitude of fresh problems which indicates the need for team work between physiologists, veterinarians and agronomists. Then, too, the underground parts of the grass plant are frequently as important, if not more so, than the aerial ones.

Since the first edition of this book appeared in 1952, our knowledge of the grass crop has greatly increased and the full appreciation of its vital role in British agriculture is now more widely acknowledged. The author incorporates throughout the text recent results from experiments at the Grassland Research Institute for Great Britain as well as from elsewhere.

All students of agriculture at all levels should make a point of not only reading this book carefully, but also of studying it seriously. An excellent bibliography and a serviceable index add greatly to its value for students. It has in my view, however, a wider value, and biologists, geographers, geologists and indeed all interested in the countryside will find profit and fascination in its pages.

IAN MOORE

CRYSTAL GAZING

Computing Methods and the Phase Problem in X-ray Crystal Analysis

Report of a Conference held at Glasgow, August, 1960. Edited by Ray Pepinsky, J. M. Robertson, and J. C. Speakman. (International Tracts in Computer Science and Technology and their Application, Vol. 4). Pp. viii+326. (London and New York: Pergamon Press, 1961.) 63*s*. net.

Crystal-structure Analysis

By Prof. Martin J. Buerger. Pp. xvii+668. (New York: John Wiley and Sons, Inc.; London: John Wiley and Sons, Ltd., 1960.) 18.50 dollars; 148*s*.

About a decade ago there was held at Pennsylvania State College a conference on "Computing Methods and the Phase Problem in X-ray Crystal Analysis". The main purpose of this meeting was to exhibit the new X-*RAC* computer designed by Ray Pepinsky, and the volume which described the conference and the machine is one of the important landmarks in the literature of the subject.

Of the machine itself little need be said except that it forms the most powerful tool for Fourier synthesis which has so far been produced. The other papers in the earlier volume were chiefly concerned with the central problem of X-ray analysis, the determination of phase.

In the new book under review, we see how the hopes and aspirations of the workers have developed. The X-*RAC* is still the most powerful machine for its special purpose, but, as Pepinsky points out, it is "an anomaly among computers", an analogue machine which is more economical and capable of greater accuracy for its special purposes than any digital machine yet on the market. Nevertheless, the machine will become of less importance in the future because of the development of new techniques and of the essentially versatile nature of digital machines.

The main portion of the book is, however, concerned with two aspects of modern structure analysis: digital computation and phase determination. Of the former it may be said that all the principal machines and programmes are described. The papers vary from the mere catalogue of programmes for an *IBM* '650', provided by Jeffrey and Shiono, to the extensive description of *Pegasus* and its programmes given by Cruikshank. To say that these papers were dull would be unfair, but they are unlikely to be of great interest to anyone who does not have access to one of the machines described. An exception should perhaps be made in favour of Robertson's paper, which describes a charming little machine having the code-name *Rufus*, an indication that in these days of high-speed electronics there is still place for mechanical ingenuity.

The papers which deal with phase determination and theoretical crystallography are revealing. They range from the extremely useful analysis of least-squares techniques by Sparks, to the disappointingly short paper on the later history of direct methods of phase determination by Hauptman. Experimental methods of phase determination also receive attention and the phase part of the book may be summed up by the statement that, with complex structures at least, if no experimental method of phase determination is available the theoretical methods are unlikely to be of much help.

The book is well produced, and authors, editors and publishers alike must be congratulated on producing it so soon after the conference. It will be an essential book for all serious crystallographers.

Buerger's book, while dealing with many of the topics covered by the specialists in the previous work, is a systematic study of the whole process of crystal structure analysis. It starts with a description of the fundamental processing of X-ray diffraction, explains the experimental techniques which are used to obtain X-ray data, and then derives the various expressions which are needed to reduce these data to a form suitable for Fourier analysis.

The latter part of the book is a compendium of Fourier methods, from practical procedures of computation to theoretical techniques of phase determination. Although the book contains nearly 700 pages the whole subject of vector space is barely mentioned—it is the subject of an earlier book by Buerger—and one gets the impression of increasing compression of treatment towards the end. This terseness is, however, relieved by the excellent lists of references which end each chapter, and, were it not for the extremely high price, every student of crystallography would find the book an essential part of his library.

A. D. BOOTH

Tin and Its Alloys

Edited by Dr. Ernest S. Hedges, with the collaboration of Prof. J. W. Cuthbertson, Prof. E. C. Ellwood, Dr. W. E. Hoare and W. R. Lewis. Pp. viii+424. (London: Edward Arnold (Publishers), Ltd., 1960.) 126*s.* net.

THIS book of more than four hundred pages is more in the nature of an encyclopædia than a text-book, and no significant aspect of tin and its uses is overlooked. Prepared by experts in the different aspects of the subject, all of whom are, or have been, connected with the Tin Research Institute, the treatment is authoritative. Despite the wide scope of the ground covered, there is nothing superficial in the discussions, and the 187 figures are well reproduced and pertinent.

After an introduction by Dr. E. S. Hedges, tin in its cast and wrought forms is dealt with, and it will come as a surprise to many to learn that several tons of the metal are to be seen in the enormous pipes in the organ at the Albert Hall in London. Two chapters are concerned with the physical metallurgy and chemical behaviour of tin and its alloys. The approach, however, is really to the user of the metal, and the treatment of the scientific aspects is subordinate to the more directly practical. It is, however, adequate both for the purposes of the manufacturer and for the engineer, chemical or otherwise, who uses the product. Where so much is good, it is almost invidious to single out Prof. Cuthbertson's account of the metallography of the bronzes for special mention.

The electrodeposition of tin and its alloys, hot-tinning, tin-plate, tin in bearing alloys, die-casting alloys, solders and bronzes are all treated at sufficient length to be really informative. The extent of the treatment is vast, and readers interested in the tomb of the Empress Maria Theresa, in the tinning of cast iron or in Babbitt's original patent of 1839 will all find what they seek.

Within the obvious limitations of the space available, it is difficult to see how this publication could have been bettered. F. C. THOMPSON

The Moon

Our Nearest Celestial Neighbour. By Prof. Zdeněk Kopal. Pp. vii+131+15 plates. (London: Chapman and Hall, Ltd., 1960.) 25*s.* net.

FOR many years the Moon was neglected by all except a few enthusiastic amateur observers. The recent developments in space research have led to a welcome change of attitude, and professional astronomers also have begun to pay attention to lunar studies. The latest book on the subject, by Prof. Z. Kopal of the University of Manchester, is written at an elementary level, and may be followed throughout by the layman; its greatest appeal will probably be to younger readers. It is attractively produced, and the photographs, many of which have been taken at the Pic du Midi Observatory, are excellent.

Beginning with fundamental facts, the author goes on to describe what he calls "the Strange World of the Moon", and considers the various theories of crater formation. On this controversial subject he is wisely cautious, and believes that both vulcanism and meteoric impact may have played a part in the moulding of the surface. The actual nature of the surface is further discussed in the next two chapters, after which a section is devoted to the observation of an outbreak inside the crater Alphonsus, reported in 1958 by the Russian astronomer N. A. Kozyrev. The final chapter is entitled "Destination Moon", and is frankly speculative, though the author carefully refrains from any flights of fancy.

A few minor criticisms may be made. In particular, some of the important studies of recent years are not mentioned; a notable omission is any reference to G. Fielder's investigations with regard to the lunar grid system. There are also one or two over-simplifications, and a number of misprints, such as 'air' instead of 'aid' on p. 19. The general impression is, however, of a very pleasant and readable little book. PATRICK MOORE

Practical Organic Chemistry

By Dr. Frederick George Mann and Dr. Bernard Charles Saunders. Fourth edition. Pp. xix+585. (London: Longmans, Green and Co., Ltd., 1960.) 25*s.* net.

FOUR editions within twenty-five years testify to the success of this book in a highly competitive market. They also mark the authors' concessions to the claims of evolving techniques. In this latest edition increased emphasis is given to semi-micro methods, both of preparing and characterizing organic compounds; there is a section—all too brief—on chromatography, including the use of ion-exchange resins; the scope of qualitative analysis is enlarged over that of previous editions; and the variety of standard syntheses is increased by new examples. Spectroscopy is not included, and perhaps this is wise although, even in organic teaching laboratories, automatically recorded spectra are now increasingly invoked. Certainly, in spite of judicious pruning, the new edition is larger by more than a hundred pages and further increase must bring into question the advisibility of combining within a single volume both elementary and more advanced techniques. Meanwhile, "Mann and Saunders" maintains the style and character which have given it wide acceptance. J. D. LOUDON

Qualitative Organic Analysis

By B. Haynes. Pp. 239. (London: Cleaver-Hume Press, Ltd., 1961.) 17*s.* 6*d.*

THIS book is designed for students in the range from National Certificate to university-level. It is self-contained in that detailed instructions for each step towards identifying a simple substance or mixture are combined with reference tables of melting points and boiling points for a generous selection of compounds and their useful derivatives. It gives the author's approach to teaching the subject and bears his emphases and preferences. Stress is placed on preliminary tests, chemical formulæ and equations are deliberately omitted, and supplementation by lectures is implied. A good standard of lucid description is maintained throughout, but the order in which classification tests are arranged will not appeal to all tastes, and more generally, one feels that the student may have difficulty in penetrating the mass of detail to the principles underlying the analysis. However, the book is rich in sound, useful information and fully merits consideration by teachers of the subject.

J. D. LOUDON

STRONTIUM-90 IN FALL-OUT AND IN MAN IN AUSTRALIA, JANUARY 1959–JUNE 1960

By Dr. F. J. BRYANT
Atomic Energy Research Establishment, Harwell

L. J. DWYER, D. J. STEVENS, Prof. E. W. TITTERTON, C.M.G.
Australian Atomic Weapons Tests Safety Committee
AND

and J. R. MORONEY
Department of Supply, Melbourne

EARLY in 1956 a network of stations established by the Atomic Weapons Tests Safety Committee commenced to monitor radioactivity in fall-out over Australia from weapon tests at the Monte Bello Islands and at Maralinga, as well as from those held overseas[1–7]. The number of stations operative, as well as the type of sampler employed in this network, has varied from time to time. Many more stations were used during periods of weapon tests in Australia, and the sensitivity of monitoring techniques has been improved as fall-out levels decreased. Early in 1957 the monitoring programme as a whole was extended to include the determination of strontium-90 in materials representative of the Australian environment, and this has become a major feature of the programme. The results from this strontium-90 survey to the end of 1958 have already been reported[8], and since that time the number of samples collected has been increased considerably. Thus, 744 specimens of total precipitation, soil, powdered milk, cabbages, wheat grain, and sheep and human bones were collected during 1959, and 1,230 during 1960. In order to avoid unnecessary analyses, specimens of the same type were sometimes combined together to provide a single representative sample, and as a result a total of 480 analyses are involved. The analytical effort is being provided by the following laboratories: (1) Human bone; by the Woolwich Outstation of the Atomic Energy Research Establishment, Harwell (under the direction of one of us—F. J. B.), and the Lamont Group at Columbia University; (2) precipitation samples: by the Health and Safety Laboratory of the U.S. Atomic Energy Commission, as part of a world-wide 'high wall pot' programme; (3) wheat grain: by the U.K. Agricultural Research Council Radiobiological Laboratory; (4) all other material : by the Woolwich Outstation of the Atomic Energy Research Establishment, Harwell.

Although analysis of all samples for the period is not yet complete, extensive data for human bone, from Woolwich, and for precipitation, from the Health and Safety Laboratory, are to hand for the 18-month period January 1959–June 1960. Because of their importance and interest, they are reported in this article. Later this year the bone data from Lamont will be published with all other data.

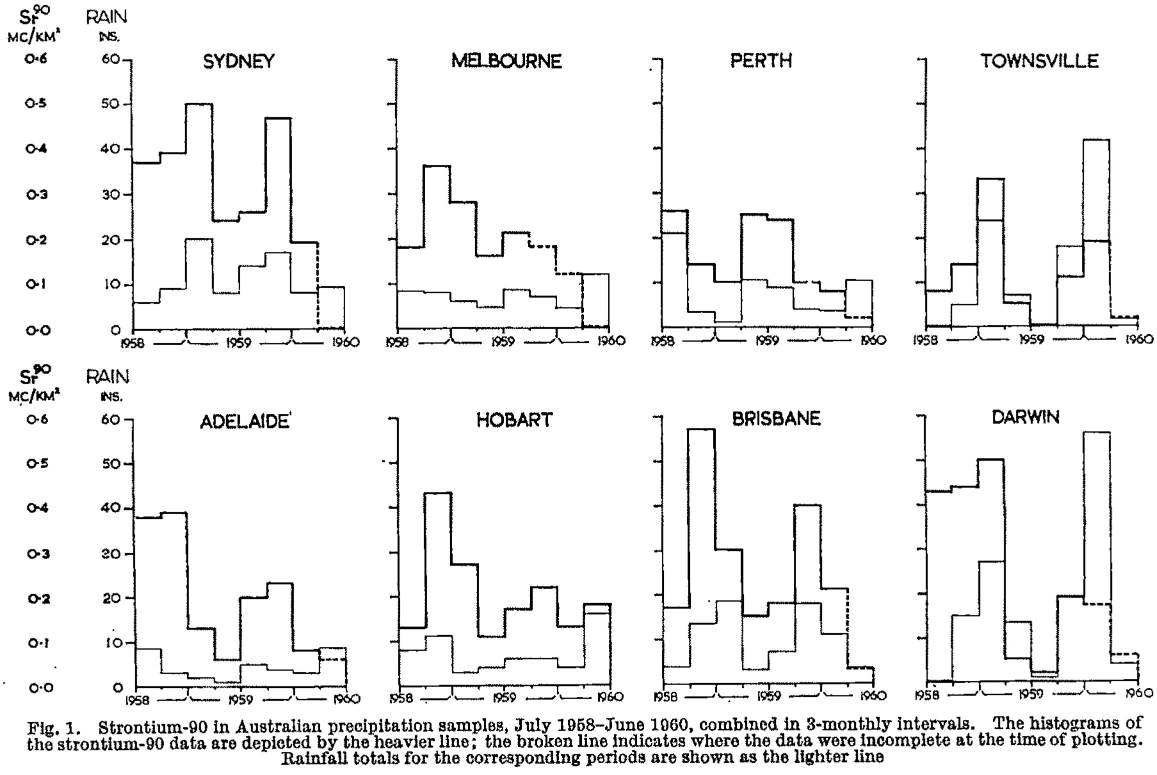

Fig. 1. Strontium-90 in Australian precipitation samples, July 1958–June 1960, combined in 3-monthly intervals. The histograms of the strontium-90 data are depicted by the heavier line; the broken line indicates where the data were incomplete at the time of plotting. Rainfall totals for the corresponding periods are shown as the lighter line

Table 1. (*a*) STRONTIUM-90 IN AUSTRALIAN HUMAN BONE, 0–59 MONTHS, JANUARY 1959–JUNE 1960
The age of each specimen contributing to a sample is given; where there is more than one specimen of a particular age, the number is shown in brackets. An asterisk indicates that no specimens were available in the age-group, although in some cases †, because the available specimen mass was insufficient for analysis, the specimens were combined with adjacent age-groups

Origin	January–June 1959			July–December 1959			January–June 1960		
	Age (months)	No. of specimens	^{90}Sr μμc./gm. Ca	Age (months)	No. of specimens	^{90}Sr μμc./gm. Ca	Age (months)	No. of specimens	^{90}Sr μμc./gm. Ca
0–1 month									
Perth	*	*	*	*	*	*	*	*	*
Adelaide	0 (2)	2	0·65	0 (13)	13	0·50	0 (14)	14	0·45
Melbourne	0 (7)	7	0·8	0 (6)	6	0·6	0 (6)	6	0·65
Sydney	0 (4)	4	0·55	0 (3)	3	0·6	0 (10)	10	0·45
Brisbane	*	*	*	*	*	*	*	*	*
1–23 months									
Perth	0†, 1, 13, 24†	4	0·85	0†, 1 (2), 3, 4 (2), 5, 7 (2), 8, 16, 20	12	0·95	0†, 7 (3), 45†	5	1·1
Adelaide	3, 6, 14	3	1·2	0†, 1 (10), 2 (5), 3 (2), 5 (4), 6, 7, 8 (2), 12, 14, 24†	29	1·15	1 (7), 2 (2), 5, 6 (2), 8, 12, 14, 18	16	0·9
Melbourne	1, 2, 3, 7, 8, 10 (2), 11, 18	9	1·35	1 (4), 2 (4), 3 (2), 8 (2), 20	13	1·0	1 (3), 2 (2), 3 (2), 4, 5 (2), 6, 8	12	0·9
Sydney	1 (4), 2, 4 (2), 7, 9, 14, 18	11	1·25	3, 4, 6, 8 (2), 9, 10 (2), 11, 15, 22	11	0·7	1 (8), 2 (3), 3 (4), 4, 9, 10 (2), 15, 17, 19	22	0·9
Brisbane	0†, 1, 3, 4, 18	5	0·65	2, 4, 15	3	0·9	5, 9, 18, 24†, 48†	5	0·95
24–59 months									
Perth	*	*	*	24, 30, 36	3	1·4	*	*	*
Adelaide	36, 48	2	0·65	24, 31, 34, 48, 56	5	0·95	24, 27, 30, 42, 48	5	1·3
Melbourne	*	*	*	24, 34	2	1·9	24, 25 (2), 33, 48, 55	6	0·9
Sydney	24 (2), 40	3	0·85	30, 36, 42, 48	4	1·35	24 (2), 30, 36, 46	5	0·95
Brisbane	24, 36 (2), 44, 48	5	0·65	*	*	*	*	*	*

Table 1 (*b*). STRONTIUM-90 IN AUSTRALIAN HUMAN BONE, 5–19 YEARS, JANUARY 1959–JUNE 1960

The bracketed figures give the number of specimens from which the samples were derived for analysis

Origin	Jan.–June 1959	July–Dec. 1959	Jan.–June 1960
	^{90}Sr μμc./gm. Ca	^{90}Sr μμc./gm. Ca	^{90}Sr μμc./gm. Ca
Perth	0·5 (2)	0·55 (7)	0·45 (3)
Adelaide	0·60 (9)	0·50 (12)	0·65 (7)
Melbourne	0·50 (10)	0·60 (5)	0·75 (4)
Sydney	0·50 (9)	0·55 (13)	0·75 (17)
Brisbane	0·6 (5)	0·50 (9)	0·50 (14)

Table 1 (*c*). STRONTIUM-90 IN AUSTRALIAN HUMAN BONE, MORE THAN 20 YEARS, JANUARY 1959–JUNE 1960

Comparisons between cities can only be made where the bone types are identical; normalization to a skeletal mean has not been attempted. For convenience, the specimens collected in each capital city during 1959 were analysed as one group. The bracketed figures give the number of specimens from which the samples were derived for analysis

Origin	January–December 1959		January–June 1960	
	^{90}Sr μμc./gm. Ca	Bone	^{90}Sr μμc./gm. Ca	Bone
Perth	0·35 (24)	vertebræ	0·47 (26)	vertebræ
Adelaide	0·40 (8)	ribs	0·48 (2)	ribs
Melbourne	0·14 (72)	crania	0·12 (37)	crania
Sydney	0·35 (148)	vertebræ	0·43 (52)	vertebræ
Brisbane	0·30 (75)	vertebræ	0·40 (55)	vertebræ

Sampling Methods and Results

(*a*) *Human bone tissue.* Between January 1, 1959, and June 30, 1960, 890 specimens of human bone tissue were collected through the agency of pathologists in the five capital cities—Perth, Adelaide, Melbourne, Sydney and Brisbane. Each of these specimens was ashed at 800° C. and then weighed. The ashed specimens were divided into three groups corresponding to the periods: January 1–June 30, July 1–December 31, 1959, and January 1–June 30, 1960. For each of the five capital cities specimens collected during these periods were divided into five age-groups: less than one month, 1–23 months, 24–59 months, 5 years–19 years and 20 years and older. In a few cases there were gaps due to specimens for the relevant period and age-groups being unavailable. Within each age-group an equal mass of ash was taken from individual specimens, and these equal masses were combined to form the sample for analysis. The resulting 59 samples were analysed by the Woolwich group.

The reported, near-uniform distribution of strontium-90 through skeletons of age up to 20 years[9,10] allowed samples representative of the four age-groups to be prepared by mixing different bone types from the appropriate collection periods. However, in view of the known non-uniformity of strontium-90 concentration in the adult skeleton, the survey of adult tissue was restricted to a single bone type for each capital city.

The results are presented in Tables 1*a*, *b* and *c*; the age of each infant specimen is included in Table 1*a*.

(*b*) *Total precipitation.* Stainless-steel high-walled pots, 12 in. diameter and 12 in. high, were continued in operation at the eight stations: Perth, Adelaide, Melbourne, Hobart, Sydney, Brisbane, Townsville and Darwin. The monthly collections from these pots were analysed at the Health and Safety Laboratory[11]. The analytical results from July 1958 until June 1960 have been accumulated in 3-monthly intervals and plotted as histograms in Fig. 1. Rainfall data have been arrayed in a corresponding manner.

Discussion

Fig. 1 indicates an overall increase in strontium-90 fall-out over Australia in late 1958 and early 1959. During 1959 the level decreased markedly before increasing somewhat in most localities towards the end of the year. The relatively short period covered by the data in Fig. 1 precludes a constructive analysis of any relationship between fall-out and precipitation in Australia. Further seasonal variations in deposi-

Table 2. STRONTIUM-90 DEPOSITION IN AUSTRALIA, UNITED KINGDOM AND UNITED STATES

Location	Deposition in mc. ^{90}Sr/km.^{2}			
	July–Dec. 1958	Jan.–June 1959	July–Dec. 1959*	Jan.–June 1960*
Australia				
Adelaide	0·77	0·19	0·43	0·19
Brisbane	0·74	0·45	0·58	0·33
Darwin	0·87	0·55	0·21	0·23 (5)
Hobart	0·56	0·38	0·39	0·31
Melbourne	0·54	0·44	0·39 (5)	0·16 (4)
Perth	0·40	0·35	0·34	0·19
Sydney	0·76	0·74	0·73	0·34
Townsville	0·22	0·38	0·11	0·22
United Kingdom				
Milford Haven	2·94	4·12	1·61	0·94
United States				
Los Angeles	0·25	2·38	0·27	0·64
New York	2·56	7·86	0·96	1·22

* Not all analyses of monthly samples from these periods are complete. When the set is incomplete, the bracketed figure gives the number of months represented; a crude approximation to the level for the six-month period can be made by scaling appropriately.

tion of strontium-90 are to be expected in the future, and an analysis of these may be profitable at a later stage if there is no further large-scale injection of strontium-90 into the atmosphere. For purposes of comparison, Table 2 presents the data combined in 6-monthly periods, together with results from measurements in the United Kingdom[12,13] and the United States[11]. The British station is that at Milford Haven, where measurements have been made since 1954, and for which the most detailed data exist. The American station at Los Angeles is selected as being representative of the west coast of the United States, where fall-out levels are, in general, low; the region of higher fall-out, on the east coast, is represented by New York.

The deposition of strontium-90 in Australia in the period July 1958–June 1959 ranged from 0·6 to 1·5 mc./km.^{2}, with a mean value for the eight stations of 1·0 mc./km.^{2}. The 1·0 mc./km.^{2} is one-third of the deposition at Los Angeles, one-tenth of that at New York and one-seventh of that at Milford Haven, in the corresponding period. The results for Australia do not show the marked rise in deposition of strontium-90 evident in the northern hemisphere during the period January–June 1959, which was mainly due to weapon tests during October–November, 1958.

The strontium-90 content of bone tissue is known to vary between individuals of a single age-group and from one group to another. Some difference between the results for the capital cities in individual age-groups is therefore to be expected, particularly when the number of specimens in the sample is small. The observed differences are not systematic throughout all age-groups, and the results in each age-group have been combined to give weighted mean values for Australia. These are shown in Table 3, which also includes the results of measurements on bone tissue in the United Kingdom[14–18] for comparison.

For all age-groups the mean level of strontium-90 in Australian human bone increased during 1958–59; but for younger tissue this rise was not carried into 1960. For the 0–1 month and 1–23 month age-groups, the increase was about two-fold; but it was followed by a downward trend into 1960. The level in the 24–59 month age-group rose later in 1959. The 5–19-year and over 20-year age-groups showed a slow rise over this period, which is to be expected with the turnover of pre-existing bone and, in the case of children and adolescents, the accretion of new bone tissue.

A similar, approximately two-fold rise in the strontium-90 level of infant human bone tissue also occurred in the United Kingdom in the period 1958–59. In general the levels of strontium-90 in Australian human bone are one-half to one-third of those in the United Kingdom[14–18]. Data for the United States, published by the Lamont Group[19], indicate levels 2–3 times higher than those in Australia.

Conclusions

(1) Mean levels of deposition of strontium-90 in Australia were about one-fourth of those in the United Kingdom and eastern United States during the period July–December, in both 1958 and 1959. The marked increase in fall-out rate in the northern hemisphere during the first half of 1959 was not experienced in Australia. It is probable that the mean annual deposition of 1·0 mc./km.^{2} recorded in Australia for the 12-month period July 1958–June 1959, will not be exceeded.

(2) The measurements on Australian infant bone tissue indicate that the level of strontium-90 in newly formed bone passed through a maximum during the period January–June 1959, and showed a downward trend to mid-1960. As expected, the levels in child, adolescent and adult bone tissue continued to increase gradually. At all ages the levels in Australian human bone tissue are one-half to one-third those in the United Kingdom and the United States.

(3) The Medical Research Council in Britain recently[20] accepted a recommendation of the International Commission on Radiological Protection that the maximum permissible level of strontium-90 in bone, averaged over the whole population, should be 67 μμc./gm. calcium, and for any individual in the population, 200 μμc./gm. calcium. At the same time, the Council revised its previous cautionary level at which a re-assessment of the situation would be required, from 10 to 33 μμc. strontium-90/gm. calcium. It will be seen that the levels found for

Table 3. STRONTIUM-90 IN AUSTRALIAN AND BRITISH HUMAN BONE, JANUARY 1958–JUNE 1960 (μμc. ^{90}Sr/gm. CALCIUM)
The bracketed figures give the number of specimens on which the result is based

Period	0–1 month		1–23 months		24–59 months		5–19 years		More than 20 years	
	Australia	U.K.	Australia	U.K.	Australia	U.K.	Australia	U.K.	Australia*	U.K.†
Jan.–June 1958	0·39 (8)	0·55 (92)	0·66 (27)	1·74 (23)	0·68 (10)	1·28 (11)	0·34 (20)	0·72 (20)	0·24 (43)	0·45 (7)
July–Dec. 1958	0·2 (2)	0·85 (34)	0·51 (4)	1·74 (5)	0·5 (1)	1·23 (3)	0·32 (5)	0·87 (5)	0·20 (6)	0·40 (4)
Jan.–June 1959	0·70 (13)	1·16 (49)	1·13 (32)	2·74 (35)	0·71 (10)	2·14 (9)	0·54 (35)	0·93 (15)	0·33 (247)	0·58 (33)
July–Dec. 1959	0·54 (22)	1·14 (152)	1·00 (68)	3·27 (77)	1·30 (14)	1·90 (13)	0·53 (46)	0·89 (16)		
Jan.–June 1960	0·49 (30)		0·92 (60)		1·04 (16)		0·64 (45)		0·43 (133)	

* Vertebræ only.
† United Kingdom results for adult femora have been converted to the equivalent vertebral value using a factor vertebra/femur: 4·5.

strontium-90 in Australian human bone are very much lower than the cautionary level.

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L. EULER, THE PRINCIPLE OF RELATIVITY AND THE FUNDAMENTALS OF CLASSICAL MECHANICS*

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SINCE they received their definite form through Newton, the significance of the fundamentals of classical mechanics has always remained a controversial subject, at least up to 1905. Where Newton had spoken of absolute space and time, and referred to experiments, some of his successors pretended that they did not need such hypotheses, or even went so far as to reduce his famous second axiom to a mere definition. Thus the boundary between mathematics and physics as an experimental science was strongly displaced at the expense of the latter, from which the so-called pure mechanics was separated.

Others, on the contrary, insisted on the basically experimental character of this axiom. There have been many attempts to display the long series of arguments from all sides of this very involved discussion. It is the aim of this article to direct attention to a single point of this process, namely, the instant where probably for the first time there emerged a completely new point of view: the confrontation of mechanics with optics. It will be shown that the result of this confrontation was the recognition that the principle of relativity, closely connected with the fundamentals of mechanics, was called in question. It is therefore appropriate to dedicate this article to the memory of Wolfgang Pauli who, forty years ago, in December 1920, published his famous article on relativity theory in the *Mathematical Encyclopedia*.

The discovery that the principles of classical mechanics are entangled with the principles of optics was made by Euler in 1739. Or, as we can put it more precisely, he discovered that the decision between a wave and a corpuscular theory of light reflects on the validity of the principle of relativity. (Already in his *Mechanica*, published three years earlier, he had devoted the first chapter to an ample explanation of the difference between relative and absolute motion, cf. "Mechanica sive motus scientia analytice exposita", *L. Euleri Opera Omnia*, Ser. Sec. 1, 13.) In a paper of that year, he examines the aberration, discovered by Bradley in 1727, mainly in order to calculate corrections for astronomical use. The aberration is a systematic error in observing the stars, of the magnitude of 20″, which occurs through the Earth's movement in relation to the stars. Presuming that the fixed stars stay at rest, then three movements remain, of which the influence on the observation may be calculated: the daily rotation of the Earth, the yearly rotation of the Earth, and the movement of the planets. But, it is characteristic of Euler that he is not only interested in the special problem to which this examination gives rise, namely, the calculation of the required correction, but he rather attacks the question in its fullest generality as a physical problem. The astronomical calculation is only the starting point for investigating basic questions in mechanics, where the astronomical language is a mere fashion of presentation. Euler distinguishes four cases, which he examines one after the other: (1) the observer and the object are at rest; (2) the object moves; (3) the observer moves; (4) object and observer move.

For the second case he then derives the aberration formula. In the course of this, he notes that one may ask whether the velocity of light depends on the velocity of the radiant object (always measured in the rest system of the observer). But he himself gives the answer that this is not the case, since the propagation of light is a wave process in the stationary ether, which is independent of the movement of the emitting body, and he finds for the correction (I use Euler's notation):

$$\mathrm{tg}\alpha = \frac{ms}{c - \mu s}$$

where α is the angle between the observed and the true location of the object, c and s are the velocities of light and the object respectively, m and μ sine and cosine respectively of the angle between the light ray and the velocity of the object. He applies his formula to the simplest forms of movement (movement on a straight line and on a circle). Thereby he reaches the conclusion: to an observer at rest, the speed of a body in uniform and rectilinear motion seems to diminish more and more with increasing distance.

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* This article is an enlarged version of a chapter of the author's introduction to the forthcoming volume containing the papers referring to physical optics of *L. Euleri Opera Omnia*. Euler's article, "Explicatio Phaenomenorum quae a motu lucis successivo oriuntur", was published in the *Commentarii Academiae Scientiarum Imp. Petropolitanae*, Tomus XI ad annum MDCCXXXIX, but appeared only in 1750.

Now the third case, which for practical application is the most important: the object rests and the observer moves. Since Euler was one of the few authors who do allow the reader to participate in his investigations and to watch closely his paths, it might be worth while to report his reasoning in detail. For here it is possible for once to observe singly the steps through which a fundamental discovery is reached and to pinpoint as closely as possible the exact moment at which a crucial insight is gained.

He starts from the remark that one may reduce this case to the previous one by giving the system observer–object a speed which is equal but opposite to the original velocity of the observer. Therefore one may take over the formulæ derived above (that is, those derived in case 2) and apply them also to this case:

$$\operatorname{tg}\alpha = \frac{mr}{c - \mu r}$$

where r is now the velocity of the observer and m, μ are the sine and cosine respectively of the angle between the light ray and the direction of the observer's motion. This formula seems to settle the problem.

Euler, however, is not yet satisfied and wishes, as he says (section 20), "to investigate the effect which the light ray exerts on the eye in motion and the difference between the true and the apparent place according to the rules of motion". This investigation is essentially independent of the former and therefore a new derivation of the correction formula.

The velocity of light in the ether and the velocity of the observer are now added according to the rules of kinematics. Euler explains hereby his short trigonometric calculations amply with the help of his vector diagrams. But now one is surprised to find the astonishing result—the correction computed with this method differs from that found before: one gets:

$$\operatorname{tg}\alpha = \frac{mr}{\sqrt{c^2 - m^2r^2}}$$

instead of:

$$\operatorname{tg}\alpha = \frac{mr}{c - \mu r}$$

Euler asks: From where does this difference arise and which of the two answers, both the result of what appear to be rigorous derivations, is the correct one? First he remarks: it is clear that the difference arises from the fact that in the first derivation because of the reduction to case 2 we had implicitly assumed that the light velocity remains c, independent of the speed of the observer, whereas in the second derivation the light velocity becomes $y = \mu r + \sqrt{c^2 - m^2r^2}$.

The decision between the two correction formulæ therefore is reduced to the question, Which is the correct velocity of light? Euler now observes, the system under consideration comprises not only object and observer, but also the ether, which hence ought also to have received the same speed, which of course is excluded. In other words, the ether distinguishes an absolute system, and cases 2 and 3 are not equivalent but basically different. Equivalence between both cases, however, would hold in a corpuscular theory, as he points out, for in such a theory no ether distinguishes an absolute co-ordinate system and the principle of relativity (Euler says: "haec hypothesis . . . *motus compositionem* recipit", Section 28) prevails. (This is the point of departure for W. Ritz's attempt at corpuscular optics, in order to explain the negative result of the Michelson experiment. Cf. W. Pauli's article in the *Mathematical Encyclopedia.*) Therewith, Euler found what he had looked for; an 'experimentum crucis' which permitted at least in principle a decision between the two theories, wave and corpuscular optics. However, the difference is too small, as he notes explicitly, to be susceptible to experiment—in this case the astronomical measurement. Euler therefore calculates in his further investigations with his simpler formulæ which follow from the corpuscular hypotheses, since they coincide with those of the wave theory to within a difference of the second and higher order in v/c, that is, he makes full use of the principle of relativity (linear addition of the velocities).

This piece of work seems to me to be one of the most beautiful that Euler ever wrote; perhaps one may regard it as one of the classical papers in physics. This is not only because of its clarity and elegance, but also much more so for the peculiar manner in which a seemingly simple question gives rise to a discussion of the most basic principles of physics. At first one believes to have caught Euler with an error and one is then even more surprised by the infallibility with which he aims at the point which more than a hundred years later was to become the centre of discussion about the fundamentals of mechanics. How surprised, on the other hand, he would have been to learn that the decision had been made in favour of the principle of relativity and against the ether, and that this decision was made just on the grounds of the wave optics confirmed meanwhile by the Michelson experiment which is closely related to aberration! This might be another example of what W. Pauli used to call "the life of the ideas", "showing", as he formulates it in the preface to the second edition of his article, "how a fundamental scientific discovery, sometimes even against the resistance of its creator, gives birth to further fruitful developments following its own autonomous course". Though perhaps in this case one rather should say "even in a direction opposite to the intention of its creator. . . ".

From the following remark quoted from Section 35 of his paper, it is clear that Euler was well aware of the significance of the so-called systems of inertia as well as their peculiar problems: "licet enim salvis legibus et regulis *mechanicis* universo cuidam systemati corporum motum aequabilem in directum imprimere, eta, ut nil in phaenomenis mutetur, at vel motum inaequabilem vel curvilineam tribuere minime licet". This demand, namely, to regard the question not only as a kinematical but also as a physical one ("legibus et regulis *mechanicis*" . . .) should have saved him from being decried as having only a rather formal attitude towards physics. In this particular fashion of looking at those problems he remains the "true son of Newton", to quote C. A. Truesdell (cf. the introduction to vol. 8, Ser. Sec. of *L. Euleri Opera Omnia*, p. cxviii). However, just the mathematicians and sometimes even the physicists later on often overlooked this point, whereas Euler thought of the foundations of mechanics as subject to experimental examination. His explanations on how the speed of a moving object appears to an observer at rest are a first tentative prelude to Einstein's later criticism of time measurement.

There is an amusing question to be asked concerning the response to this paper. It was written by Euler in 1739, but its publication by the Academy of St. Petersburg was delayed until 1750. Meanwhile, he had published in 1748 a continuation to it in the *Mémoire de l'Académie des sciences de Berlin* (Mémoire sur l'effet de la propagation successive de la lumière dans l'apparition tant des planètes que des comètes, 1746. The volume was printed, however, in 1748.) There, however, he did not enter into the question: wave or corpuscular optics, but applied without further explanation the simpler formulæ of corpuscular optics as an approximation in the above-mentioned sense. A careful reader could have objected that Euler was in contradiction to the principles of his own wave theory, proposed and discussed in detail in his *Nova Theoria Lucis et Colorum* published in 1746.

Was there such a careful reader ?

I received much encouraging criticism and various stimulating suggestions from Prof. A. Koyré. I am indebted to Dr. G. Barton for his linguistic advice. I wish also to express my gratitude to Prof. J. R. Oppenheimer and the Institute for Advanced Study for the generous hospitality offered to me.

THE BREEDING OF IMPROVED MILKING COWS THROUGH ARTIFICIAL INSEMINATION*

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THE story of commercial artificial insemination in Great Britain is quite a short one, spanning, in fact, less than twenty years, for it was as recently as November 1942 that the first commercial artificial insemination (A.I.) centre was opened at Cambridge. In 1944, the Milk Marketing Board took over its first centre—Beccles—from the North Suffolk Cattle Breeders, and subsequent expansion was so rapid that by December 1958, only some fourteen years after acquiring its first centre, the Board's organization had inseminated a total of 10 million cows[1]. Now the 23 Milk Marketing Board and 6 non-Board centres, which together provide the artificial insemination service in England and Wales, inseminate more than 2 million cows a year, or approximately 60 per cent of the national herd. The Milk Marketing Board centres alone inseminate more than 1½ million cows a year, of which about 1 million are with semen from dairy and dual-purpose bulls.

In addition to the growth in the use of artificial insemination which is still continuing, there have been technical developments in the dilution and storage of semen, so that now one bull may be used for upwards of 10,000 inseminations a year, a figure which seemed impossible a short while ago, and the method is therefore well suited to the exploitation of a superior bull. Further, semen can be stored apparently indefinitely at a temperature of − 79° C.; this facilitates transport and makes possible better utilization of semen.

The aim of the artificial insemination organization of the Milk Marketing Board is to breed cows capable of giving, economically, relatively high yields of milk of satisfactory quality as regards composition. Since in Britain at the present time milk is paid for on the basis of quantity, milk yield is a characteristic of major importance; but the milk produced must satisfy certain minimum standards of composition and therefore the quality must also be taken into account in selection. It is also necessary that the cows should be capable of economic milk production; in other words, their conformation must be such that they are capable of a long trouble-free life in the herd, they must have a satisfactory temperament, be easy to milk and so on. These factors which go to make an economic cow can only be assessed subjectively, so here we will discuss only milk yield and fat percentage, which are recorded in National Milk Records, the official milk-recording scheme in England and Wales.

The production characteristics, milk yield and fat percentage, are only expressed in the female, and, moreover, the heritability of milk yield is low, being of the order of 0·3. In this situation it is clear that the breeding worth of a bull must be assessed by means of a progeny test, even though as a result the generation interval is increased; for although a bull can be used when he is a year old, he is about six years of age before the result of a progeny test can be obtained even in favourable circumstances. A further problem in assessing a bull's genetic merit for milk yield is that as much as 90 per cent or even more of the differences in level of production between herds are due to environmental factors. This means that the average yield of a bull's daughters may be a reflexion of the environment under which they were milked rather than of the breeding worth of the bull. It was to combat this situation that the contemporary comparison method of bull evaluation was introduced[2-4]. This entails a series of comparisons within herd-years between the average first lactation milk yield of the daughters of the bull and the average yield of their contemporary heifers by other bulls, each comparison being weighted according to the number of daughters and contemporaries. The weighted differences from the separate herd-years are then combined to give a single figure for the bull, which is the mean weighted difference between the first lactation yields of the daughters of the bull and their contemporaries. The total weight on which it is based is a measure of the accuracy of the test. The accuracy of a contemporary comparison can, in fact, be expressed in terms of the total weight (W) and the heritability of single records (h^2) as $\frac{W}{W + (4 - h^2)/h^2}$, which reduces to $\frac{W}{W + 12\cdot33}$ when a heritability of 0·3 is assumed[5]. This fraction may vary from zero for no information to 1 for complete information about the bull's breeding worth, and it increases as W increases.

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* Substance of a paper read at the 135th meeting of the Genetical Society on March 23.

The weighting is not, however, the only factor to be taken into account in assessing a bull for milk yield. The general applicability of a contemporary comparison rests on the assumption that the contemporary heifers are genetically average for their generation. This may reasonably be assumed to be the case where a bull has been used in artificial insemination and his daughters are consequently scattered through a large number of herds where the contemporary heifers are by many bulls; and it has been found that in these circumstances the contemporary comparison behaves in practice more or less as would be expected in theory. A natural service bull, however, usually has most of his daughters in a single herd, where the contemporary heifers are likely to be by very few, perhaps even by only one, sire. Although the resulting contemporary comparison may not be generally applicable, the method is not useless in such a situation. Indeed, it probably has its greatest value when the daughters of a bull milk mainly in one herd, since it is in these conditions that the environment is likely to have its greatest effect on the average production of a bull's daughters.

In the case of fat percentage, the situation is simpler. The heritability is higher than for milk yield, being about 0·5 as compared with that for milk yield of 0·3; more important, however, is the fact that environmental differences between herds do not affect fat percentage to the same extent as they affect milk yield, so that the average fat percentage of a bull's daughters may be used to assess his breeding merit for this characteristic. The accuracy of a progeny test for fat percentage is $n/(n + 7)$, where n is the number of daughters of the bull. Therefore, 7 daughters will give a progeny test for fat percentage with an accuracy of 0·5, whereas a weighting of 12·33 or approximately 18 daughters are required to give a progeny test for milk yield of the same accuracy.

A bull in artificial insemination can generally be tested through his progeny more accurately than a bull in natural service, both by virtue of his having a greater number of daughters and because these daughters are scattered through a large number of herds. He will also generally be younger when his progeny test assessment is first available. In order to make the best use of the superior bulls which are found, they must be used not only to breed females but also to breed bulls to enter the artificial insemination stud for progeny testing in their turn. In other words, the artificial insemination population must be genetically closed, at least to some extent. The Milk Marketing Board has for some time been using its best bulls to breed sons out of cows with at least four lactations which have proved to have outstanding individual performances in their own herds and which have been approved for conformation, temperament, etc., by a panel of breeders. It has been shown[6] that a large number of young bulls bred in this way will on average be of greater genetic merit than the population as a whole, and by progeny testing it is possible to select the best from this superior group. However, such selection within a closed population is inevitably accompanied by inbreeding, and the question arises of how far one can go in closing an artificial insemination population or in limiting its size.

In practice, because of the long generation interval, the problem is not pressing ; but nevertheless it is one which merits early consideration, and in order to obtain a consensus of opinion, the proposition that "2 million dairy cattle may be bred annually to 200 sires of known breeding merit without genetic deterioration" was put to a number of geneticists, both in Britain and overseas[7]. Their replies were generally favourable.

In considering the problem, two facts must be borne in mind. First, that the artificial insemination population is divided into a number of breeds (the Milk Marketing Board gives service from nine dairy and dual-purpose breeds) varying in importance from Friesians, where about 735,000 cows a year are inseminated, to Northern Dairy Shorthorns, where just over 1,000 cows a year are inseminated. Secondly, milk records are kept for only a minor proportion of cows bred by artificial insemination; the proportion varies with breed and area of Britain, but averages between 15 and 20 per cent, which reduces substantially the effective size of the female population. The consequences of inbreeding in dairy cattle are a reduction in yield, viability and fertility, and Robertson[8] has estimated that there is a fall of 0·32 per cent of the original yield for every 1 per cent increase in the inbreeding coefficient. A small degree of inbreeding is therefore unlikely to have serious consequences, but steps must be taken to ensure that the coefficient of inbreeding does not rise to too high a level. There are three main ways in which this can be done:

(1) A degree of diversity can be maintained by limiting the number of sons by any one bull. This would certainly result in a low rate of inbreeding, but it would also limit the amount of selection which could be applied, and is therefore not a very attractive proposition.

(2) Alternatively, bulls could be introduced from outside the system from time to time but not used extensively until their breeding worth was known. The Milk Marketing Board has been doing this since the inception of its artificial insemination service and will certainly continue to do so for a variety of reasons. However, if, as it should, the average genetic merit of the artificial insemination population rises more quickly than that of the natural service herds, it will become increasingly difficult to find superior bulls from outside the artificial insemination group.

(3) A third possibility is to divide the population into several isolated or semi-isolated groups, so that strains within the breed are formed. This system certainly has a number of genetic advantages, one being that although the different sections of the artificial insemination population would be moving towards the same goal, they would not necessarily be travelling by the same route, so that a degree of genetic diversity would be maintained.

Again, because of the long generation interval in cattle, the exhaustion of genetic variability as a result of continued inbreeding is a secondary problem; but it is possible to safeguard against it by maintaining supplies of frozen semen, so that if necessary some of the genetic variation which has been lost can be re-introduced. In doing so, however, some of the superiority in economic characteristics built up by selection would be lost.

Another danger of inbreeding is that of increasing the frequency of undesirable recessive genes in the population. A bull may be tested for simple recessives either by mating him to 23 of his own daughters, when there is a 95 per cent chance of detecting the 'carrier' bull, or by checking on any abnormal calves resulting from approximately 600 outbred matings when, provided that the frequency of the recessive gene in the population is 1 per cent, there is again a 95 per cent chance of detecting the heterozygous bull.

For the progeny test of young bulls, the Milk Marketing Board carries out 500 inseminations in recorded herds and 500 in non-recorded herds, and these should result in approximately 600 calves being born. Thus if the existing reporting system can be improved, it should be possible to detect the majority of those bulls carrying deleterious recessive genes of importance in the population without resorting to sire–daughter matings. This is the procedure which is favoured. It is true that sire–daughter matings will enable the detection of any recessive gene whatever its frequency, but they are difficult to organize in practice, and therefore it is proposed to carry them out only in exceptional cases.

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OBITUARIES

Academician A. F. Joffé

The death on October 14, 1960, of A. F. Joffé, one of the pioneers of physics in the U.S.S.R., member of the Russian and many other Academies, is a severe loss for science. Joffé took a most active part in the remarkable and violent development of physics from the dawn of the twentieth century up to the present time. To the last day of his life he never ceased experimenting with his own hands.

Joffé's early work (1907–13) was devoted to experimental substantiation of the quantum theory set out by Einstein in 1905 while investigating the photoelectric effect and photochemical phenomena. The theory provoked at first adverse comments on the part on many eminent physicists (Ladenburg and others). In 1907 Joffé undertook a theoretical treatment of experimental results obtained by Ladenburg and others, and proved convincingly that Einstein's photon theory was not contrary to, but on the contrary justified by, all experimental data, including those of Ladenburg.

Joffé's experiments on the photoelectric effect exhibited in metal dust suspended in the electric field of a condenser provided direct confirmation for Einstein's theory. Joffé showed that under illumination a dust particle loses its electric charge by portions corresponding to the electron charge, the nature of the process being statistical and in keeping with that of the photon theory. The same experiments supported the atomistic theory of electricity. The conditions of Joffé's experiments were somewhat similar to those of R. A. Millikan, who followed the behaviour of oil droplets suspended in an electric field and showed that all fluctuations in droplet charges were multiple integers of the elementary electric charge. The experiments of Millikan and Joffé were conducted simultaneously and independently, and were published in 1911 (Millikan) and 1912 (Joffé).

In 1913 Joffé published his results on the magnetic field of the cathode beam. The occurrence of a magnetic field around the cathode beam was still uncertain at the time. The mere fact was denied by many scientists. Besides providing confirmation for the occurrence of the magnetic field, Joffé succeeded in showing that the field intensity was akin to that of an equivalent current in a metal conductor.

At the beginning of the 'twenties, the advent of quantum mechanics, Joffé reverted to his 1912 experiments and found that metal dust particles irradiated by X-rays emitted electrons. These experiments, carried out with Dr. Dobronravov, gave convincing proof that the energy of electrons emitted by dust particles corresponds to that of X-ray quanta.

However, the main trend of Joffé's theoretical and experimental works throughout his life was in the direction of electrical and mechanical properties of dielectrics and semi-conductors. He may with full justice be considered among the pioneers of the physics of solids. His earliest scientific investigations dealt with elastic and electric properties of quartz. In 1922, while investigating the plastic strain in crystals, Joffé and Kirpicheva discovered that the spots of the Laue diffraction pattern were extended into radial stripes as soon as the elastic limit was exceeded. This was evidence that plastic strain leads to the breaking up of a crystal into differently oriented blocks.

Joffé's disciples extended his investigations to a great variety of crystal bodies, and their work obtained world-wide appreciation. A new trend in the physics of metals arose and was successfully developed.

Another line of experimental investigation pursued by Joffé provided a basis for the theory of crystal strength. Prior to these experiments there was a large discrepancy between the values of crystal strength obtained experimentally and those calculated on the crystal lattice theory. For example, the theoretical limiting value for rocksalt crystals was found to be several hundreds of kgm./cm.^{2}, whereas experiment gave a limit of hundreds of gm./cm.^{2}. Measurements of rocksalt crystals immersed in water and similar experiments carried out with balls of rocksalt cooled in liquid air which were immersed in boiling water or molten tin showed that under these conditions the limiting strength was close to the theoretical order. The low limit observed before was ascribed to microcracks and other crystal defects.

Another important discovery was made by Joffé when investigating the mechanical properties of crystals. He found that the nature of brittle or plastic destruction of crystals at a given temperature was determined by the relation between the temperature-dependent yield strength and the ultimate tension which is only slightly dependent on temperature. This relation, established later for a number of industrial materials, led to many conclusions of great practical value.

PHYSICS DEPARTMENTAL LIBRARY

Of extreme importance were Joffé's investigations on electric properties of dielectrics. Prior to his experiments, all electric phenomena displayed by dielectrics were considered as a sort of agglomeration of anomalies. Joffé found that thin polarization layers were formed at the electrode surfaces during the passage of current through a dielectric crystal. The potential difference of these layers appeared to be enormous. By allowing for polarization, Joffé showed that the flow of current through a crystal was in strict obedience to Ohm's law. Joffé was the first to discover (in 1916) that the conductivity of the majority of ionic crystals was of an electrolytic nature and due in part to lattice-occluded impurities and in part to thermal dissociation of the lattice.

Joffé and his disciples have conducted a vast amount of work on dielectric breakdown, in particular on breakdown stress as a function of the thickness of the dielectric material. These investigations were of great importance for the technology of insulating materials.

From 1930 Joffé's interests began to concentrate on semi-conductors. Even then, Joffé was aware of the importance of semi-conductors for engineering problems. Joffé himself and his co-workers were very successful in this field and discovered a number of fundamental facts in the physics of semi-conductors. First of all considerable attention was paid to the working out of techniques for obtaining physical values characteristic of semi-conductors, such as the concentration of carriers, their charge, mobility, etc. Most important was the discovery of the effect of impurities on electric properties of semi-conductors. It was shown, moreover, that an excess or a deficiency in some component may exhibit an effect similar to that of an impurity. It appeared, for example, that lead sulphide containing an excess of lead atoms was a conductor of the n-type, whereas a sulphur excess would make it p-type. Joffé suggested that these facts were due to impurity or excess atoms either creating additional electron sources or picking up electrons with consequent formation of holes. The concept of electron-or hole-semi-conductors, now defined as n- and p-type, seems to have originated from Joffé's laboratory.

Towards the end of the 'thirties Joffé, together with his wife, proposed a mechanism of rectification which is now widely accepted. It implies that the barrier-layer in rectifiers is formed at the p-n junction. The flow of current is due to electrons and holes approaching each other and recombining at the junction. When the electrons and holes drift apart, the resistivity of the layer adjacent to the junction is raised and the latter consequently becomes a barrier-layer.

Of the many practical applications of semi-conductors Joffé considered as paramount the utilization of their thermoelectric and photoelectric properties for conversion of heat and light energy into electric energy. Joffé's theory of thermoelectric generators (and thermoelectric refrigerators) opened a clear path for physicists and engineers working in this field. The idea of plasma thermoelectricity, now being developed in many countries, was also set forth by Joffé.

His great achievements in this domain were emphasized by the eminent American scientist J. Bardeen at the International Conference on the Physics of Semi-Conductors in Rochester, 1959. In his inaugural speech Bardeen said: "The first real scientific study of electron mobility, thermal conductivity, and other factors important for thermal elements, and the way they depend on composition was made by Joffé and his co-workers. Results they have obtained clearly demonstrate the virtue of the scientific over the purely empirical approach. . . . . Research on thermoelectric effects and on thermal conduction in semi-conductors has yielded a number of interesting phenomena, involving phonon scattering, phonon drag, and heat transfer by ambipolar diffusion".

A profound and eager concern for all problems of physics and adjacent fields was very characteristic of Joffé as a scientist. A great number of Joffé's ideas, developed later by his disciples, date back to the 'twenties and 'thirties; they laid the foundation for the development in the U.S.S.R. of modern trends of physical science. Joffé acknowledged his authorship only in investigations that were carried out with his own hands. He was never prone to put his name to papers dealing with investigations that were carried out by his numerous disciples, even though the ideas and guidance were his. This makes his contribution to science, embracing a great variety of physical fields (nuclear physics, chemical physics, the theory of metals, physics of heat, etc), even larger than it would seem at first.

He created a widely spread school of Soviet physicists dealing with most diverse trends in physics and related sciences. His gift for inspiring his colleagues, especially young ones, with a passionate interest for science was truly remarkable.

Sixteen new institutes were founded under Joffé's sponsorship. In 1918 he created the Physico-Technical Institute of Leningrad, which played an exceptionally important part in the development of physics. He was director of this Institute for more than thirty years, but his activities reached far beyond its scope. He took part in the development of physics all over the country, and his was the idea of a widely spread system of physico-technical institutes and laboratories that would serve as a scientific basis for the growing industry. He contributed largely to the organization of physical institutes in Kharkov, Dnyeporpetrovsk, Tomsk, Sverdlovsk. Groups of able scientists were chosen by him from his staff for work in each of these institutes, thus forming the nuclei of future scientific bodies. The Institute of Chemical Physics in Moscow and the Electro-Physical Institute in Leningrad sprang up from the Leningrad Physico-Technical Institute. The Institute of Physical Agriculture, so far the only one of its kind, was also founded by Joffé. An outstanding Physico-Mechanical Department of an entirely novel type was created by Joffé in the Leningrad Polytechnic Institute. Its programme combines deep penetration into theory with most lively actual practice. Engineers coming from this department possess a profound knowledge of modern physics and are carrying the latest scientific achievements to all fields of industry.

Joffé's remarkable personality, his immense scientific prestige and wide erudition, his unquenchable passionate love for science, his gift for inspiring his disciples, his rare simplicity and affability won him respect and affection among the widest circles of scientists and engineers of the Soviet Union, deep friendship on the part of his disciples, including the present writers. The strongest bonds of friendship tied him with eminent scientists of many countries. It will be hard for all who knew him to accept the fact that he is no more, that the hearty man who seemed unaware of age, so full of the joy of life and far-reaching projects, has gone, that the tireless, wonderful intellect has ceased to exist.

N. N. Semenov
V. N. Kondratiev

Prof. C. W. Dannatt

Prof. Cecil William Dannatt, who died at his home in Blackheath on April 9, was born on August 2, 1892. He was educated at Colfe's Grammar School and the Royal School of Mines, where he graduated in metallurgy in July 1914. In August of the same year he enlisted in the Queen's Westminster Rifles. He received a commission in 1915 and served in France, Salonika and Palestine. On being demobilized in 1918, he joined the Ministry of the Interior, Cairo, as assistant director of the Technical Section of the Public Security Department. After two years of very varied experience in this post, he went to Trinidad as geologist to an oil company, but in 1923 he returned to the Royal School of Mines, to work as a research student in the Metallurgy Department. A few months later he joined the teaching staff of the Department, and the work was so much to his liking, and he so well fitted for it, that he remained on the staff for thirty-four years, first as demonstrator, then lecturer, reader, acting director and finally, from 1945 until his retirement in 1957, professor and head of the Metallurgy Department.

Throughout his long connexion with the Royal School of Mines and Imperial College, and particularly after his appointment as professor, he devoted himself to the advancement of the Department, and the training and education of his students, in every one of whom he took a personal and sincere interest. Under his leadership, the Department added continuously to its reputation as a centre of learning and research, and when he handed over to his successor in 1957 he was able to do so in the knowledge that its standing had never been higher. Although his interests and knowledge extended to all branches of metallurgy, and he was a vigorous opponent of specialization within his Department, his greatest interest was in fact in the scientific and technological aspects of the extraction of metals from their ores, and the achievement that afforded him more gratification than any other was the formation of the Nuffield Research Group in Extraction Metallurgy. He was largely responsible for the establishment of this group in his Department in 1950, since when, with his active support, it has developed into the leading academic research school devoted to the study of metallurgical processes at high temperatures.

Prof. Dannatt had a charming and gracious personality, and he was an unsparing worker. He was always anxious to help, and always reluctant to refuse any request for advice or information, or demand for his services. Scientific societies, professional institutions, educational and examining bodies, and Government, college and university committees made many such demands not just on account of his position but also because his wide knowledge, alert mind and ability to express himself lucidly and concisely were powerful assets in committee work. He particularly valued his long association with the Institution of Mining and Metallurgy, of which he was president during 1956–57, the Institution of Metallurgists, of which he was vice-president during 1953–57, the Sir John Cass College and Woolwich Polytechnic, as member of their governing bodies, and the City and Guilds of London Institute, as member of the General Advisory Committee and Moderating Committee for Metallurgical Subjects.

Shortly after his retirement, the title of professor emeritus of metallurgy was conferred on him by the University of London, and in 1960 he became a Fellow of the Imperial College. Although incapacitated by a prolonged and severe illness, he retained his interest in metallurgy to the end. He is survived by his widow and daughter.

M. S. Fisher

Prof. J. Bordet, For.Mem.R.S.

Jules Bordet, who died at his home in Ixelles in Belgium on April 6, was a pioneer in the science of microbiology and the allied one of immunology. The last twenty-five years of his life—he died at the age of ninety—were spent in retirement and he had used the early years of this period of leisure to prepare a second edition of his excellent text-book, *Traité de l'Immunité dans les Maladies Infectieuses*, which appeared in 1939. But in the closing years of the past century and the first thirty years of this one, Bordet's brilliant mind and experimental skill led to discoveries of fundamental importance and a flow of scientific papers which established for him an indisputable claim to fame.

Bordet was born in Soignies, Belgium, on June 13, 1870. Electing to enter the profession of medicine, he studied at the University of Brussels, graduating M.D. in 1892. Choosing a laboratory career, he went to the Pasteur Institute, Paris, in 1894, and worked there for seven years, being recalled in 1901 to become first director of the newly founded Pasteur Institute, Brussels. Six years later he was appointed to the chair of bacteriology in the University of Brussels, holding this post until his retirement in 1935.

Bordet's inquiring mind led to his study of many aspects of microbiology, among others, anaerobiosis, bacterial agglutination, the pleuropneumonia group of micro-organisms and the bacteriophages, on all of which he made valuable original observations. It was, however, his investigations into the ætiology of whooping-cough and his elaboration of the complement-fixation test, in both of which he had the assistance of his colleague, Gengou, that stood out above the rest. It was he and Gengou who, in 1906, discovered the causal agent of whooping-cough—*Haemophilus pertussis*—a discovery which has made possible the specific prophylaxis of this disease; and it was at the end of the last century, in 1895 to be precise, that he published his first paper on bacteriolysis, showing that lysis of a bacterium by an immune serum was the work of two serum components, specific heat-stable antibody and non-specific heat-labile complement. It was from this observation, of course, that the complement-fixation test emerged (1901), with its wide application in the diagnosis of disease.

Recognition of the value of these researches brought Bordet many honours. In 1911 he was given the Prix de la Ville, Paris, and two years later the Swedish Medical Society awarded him the Hansen Prize and the Pasteur Medal. He was made a foreign member of the Royal Society in 1916, and in 1919 he was given the Nobel Prize for medicine and physiology; the University of Cambridge conferred on him the honorary degree of M.D. Bordet was married and had three children, a son and two daughters.

S. P. Bedson

NEWS and VIEWS

Medical Statistics in the London School of Hygiene and Tropical Medicine: Prof. A. Bradford Hill

PROF. A. BRADFORD HILL retires from the chair of medical statistics at the London School of Hygiene and Tropical Medicine at the end of the current academic year. As a son of the late Sir Leonard Hill he has inherited perspicacity and sharpness of wit, but he has made his own very distinctive contributions to medicine and to statistics. He joined the staff of the Medical Research Council and its Industrial Health Research Board in 1923, and soon immersed himself in problems of industrial sickness. His surveys of sickness in the cotton spinning, cotton weaving and printing industries immediately became standard references, and early in this period he read to the Royal Statistical Society a classical paper on the effect of internal movement of population on local mortality. In 1933, Hill became reader in epidemiology and vital statistics in the University of London and set himself to understand doctors and their problems and to teach them what he calls "arithmetic with logic". He was pre-eminently successful in both tasks. In 1937, he published *Principles of Medical Statistics*, a text-book which still occupies an unchallenged place, is in its sixth edition in English and has been translated into other languages. Since 1945, Hill has been professor of medical statistics in the London School of Hygiene and Tropical Medicine and director of the Statistical Research Unit of the Medical Research Council. His interest and experience in the conduct of the controlled clinical trial are probably unique, and he has set very high standards for his unit. Any research worker who survives Hill's devil's advocacy can be sure his experiment is properly designed; he can also be sure of warm encouragement and help. Hill has been widely honoured, being elected a Fellow of the Royal Society (1954), president of the Royal Statistical Society from 1950 until 1952 (to which he gave much time and energy as honorary secretary during 1940–50) and its gold medallist in 1953; president of the Section of Epidemiology, Royal Society of Medicine 1953–55, and member of the International Statistical Institute.

Prof. P. Armitage

DR. PETER ARMITAGE who, at thirty-seven, succeeds Prof. Bradford Hill in the chair of medical statistics is a mathematical graduate of Cambridge. His studies were interrupted by the War and he went to the Ministry of Supply Statistical Advisory Department during 1943–45, and later to the Mathematics Division of the National Physical Laboratory. In 1947 he joined the Statistical Research Unit of the Medical Research Council. Dr. Armitage has contributed much to recent advances in mathematical statistics, especially in their application to prophylactic and therapeutic trials. In particular he has developed the sequential method as a highly effective tool and has recently published an excellent text-book, *Sequential Medical Trials*. Dr. Armitage is, like his predecessor, unstinting in his efforts to further the cause of the Royal Statistical Society; he has been a member of the Council since 1954 and an honorary secretary from 1958.

Pharmacy at the Royal College of Science and Technology, Glasgow: Prof. J. P. Todd

PROF. JAMES P. TODD, who retires at the end of this session, was appointed to the newly constituted School of Pharmacy in the Royal College of Science and Technology, Glasgow, in 1921 as its sole lecturer. The growth of the School to its present proportions is a remarkable tribute to his foresight and able direction. The first courses offered were those leading to the examinations of the Pharmaceutical Society, but in 1924 the first student was enrolled for the degree of B.Sc. in pharmacy of the University of Glasgow, which had been instituted in 1907, and teaching for this degree began in the School under the Ordinance of Affiliation. Todd was awarded a Ph.D. in 1932, and in 1937 was appointed professor of pharmacy, this being the first chair of pharmacy in the United Kingdom. His responsibilities also included the Departments of Botany and Bacteriology, and the Scottish School of Bakery. Much of the credit for the development of these disciplines to the status of full departments with separate chairs in food science and in applied microbiology and biology belongs to Prof. Todd. No record of his achievements would be complete without mention of his work during the War years, when infusion fluid and blood transfusion units were set up in the College under his direction. The permanent West of Scotland Region Blood Transfusion Service stands as a lasting reminder of this period of his career. His studies of bacterial pyrogens, which later became a major interest, stemmed directly from this work, and he is now recognized as a leading authority on this subject.

Prof. J. B. Stenlake

DR. JOHN B. STENLAKE, who is to succeed Prof. J. P. Todd, was educated at Ealing Grammar School. He then served an apprenticeship in pharmacy before proceeding on a Jacob Bell Memorial Scholarship to the College of the Pharmaceutical Society, where he qualified as a pharmaceutical chemist in 1941 and was awarded the Pereira Medal. After war service in the Royal Air Force, he joined the teaching staff of the School of Pharmacy of the University of London, and during this period graduated B.Sc. with honours in chemistry. In 1950 he was awarded a Ph.D. degree by the University of London and a D.Sc. (London) in 1960, for work in the field of medicinal and pharmaceutical chemistry. Since 1952 he has held the appointment of senior lecturer in pharmaceutical chemistry in the School of Pharmacy of the Royal College of Science and Technology. He will take up his new post on September 1.

Applied Microbiology and Biology at the Royal College of Science and Technology, Glasgow: Prof. E. O. Morris

DR. ERNEST O. MORRIS has been appointed to the new chair of applied microbiology and biology at the Royal College of Science and Technology, Glasgow, and will take office as from September 1. Dr. Morris was educated at the Handsworth Junior Technical School, Birmingham, and, during 1929–39, worked as a laboratory technician in a hospital and in the Departments of Cancer Research and of Pathology and Bacteriology in the University of Birmingham. During this time he qualified as an associate of the Institute of Medical Laboratory Technology. In 1939 he was mobilized with the Territorial Army and served with the Royal Army Medical Corps in France, Assam and India. Later he served on the staff of General Headquarters at the Central Military Laboratory, Poona, where he assisted in the training of army personnel in medical laboratory technology. After demobilization he matriculated at the University of Birmingham, where he graduated with honours in bacteriology in 1950. In 1952 he was awarded a Ph.D. at the University of Birmingham for work on the bacterial flora of the mouth in relation to dental caries. Following this he became head of the Microbiological Division at the Brewing Industry Research Foundation under the directorship of Sir Ian M. Heilbron, a post he held until 1956. During this period his own research interests ranged from fundamental studies of the physiology of yeasts to the development of techniques for use in the fermentation industries. In 1956 he was appointed lecturer in the Microbiological Division of the School of Pharmacy, Royal College of Science and Technology, and principal lecturer in that Division in 1959. When the Divisions of Microbiology, Biology and Biochemistry in the School of Pharmacy were formed into the Department of Applied Microbiology and Biology, Dr. Morris was appointed head of the new Department.

Zoology at the Banaras Hindu University: Prof. S. P. Ray-Chaudhuri

Dr. S. P. Ray-Chaudhuri has been appointed to the chair of zoology at the Banaras Hindu University, Varanasi, India. He obtained his Ph.D. in the University of Edinburgh, working with Prof. H. J. Muller on the effects of radiation on the fruit-fly *Drosophila*, and has been engaged in teaching at the University of Calcutta since 1941. Dr. Ray-Chaudhuri was elected a Fellow of the National Institute of Science of India in 1950, and was the president of the Zoological Section of the Indian Science Congress in 1960. He is well known for his contributions to radiobiological research.

Commonwealth Technical Training Week

In Great Britain, Commonwealth Technical Training Week is to be held during May 29–June 3, 1961. In Aden, Hong Kong, Nigeria, Malaya and Pakistan the week will have been celebrated before this date and will have carried appropriate messages from H.R.H. the Duke of Edinburgh. In the United Kingdom support is almost universal. In England and Scotland possibly seven areas may not take part, but both Wales and Northern Ireland have each achieved 100 per cent participation. A special exhibition at the Royal Exchange, London, is being arranged by the City and Guilds of London Institute in co-operation with the City of London, the City Livery Companies and the London County Council. The exhibition will illustrate the opportunities for training and education in industry, commerce and the professions. Most of the exhibits will be provided by organizations which are nationally representative of the major groupings in industry and commerce, while the City Livery Companies will provide exhibits illustrating the historic background of training and craftsmanship. The Duke of Edinburgh has recorded a short address for use at the opening ceremonies. His Royal Highness, speaking from the dairy at Windsor Castle, has also contributed a lively introduction and epilogue to the film "Training To-day for To-morrow".

U.S. Antarctic Research Ship *Eltanin*

The U.S.N.S. *Eltanin*, an ice-strengthened cargo ship, is to be equipped as a sea-going scientific laboratory for the U.S. Antarctic Research Programme. It is expected that the first research cruise will begin in the later autumn of 1961. The ship will be fitted to accommodate numerous disciplines, including meteorology, upper atmosphere studies, marine and terrestrial biology, physical oceanography, submarine geology, and geomagnetic studies. The cost of conversion will be borne principally by the National Science Foundation; the Military Sea Transportation Service will supervise the ship's modification. The *Eltanin* will continue to be owned and operated by the Military Sea Transport Service. The National Science Foundation, as sponsor of the ship, will be responsible for the scientific programme and will designate a senior scientist aboard. It is expected that the ship will work in Antarctic waters at least ten months a year. Individual cruises will vary from one to two or more months in length, depending on the research in progress. During refuelling calls at southern hemisphere ports, scientists will be able to change equipment for experiments.

British Leather Manufacturers' Research Association : Open Days

The work exhibited during the 1961 open days (May 2–4) in the laboratories of the British Leather Manufacturers' Research Association at Milton Park, Egham, mostly showed developments from last year's programme (see *Nature*, 186, 1024; 1960). Work on the mechanical properties of collagen and leather fibres has continued. An automatic amino-acid analyser (Bender and Hobein) has been installed for work on the protein constituents of skin. A synthetic approach to tanning chemistry, involving the preparation of phenolic glucosides and sugar esters, has been commenced. Considerable progress has been made in the use of enzyme preparations in place of the traditional milk of lime plus sodium sulphide bath, for loosening hair or wool in preparing skins for tanning. The work (sponsored by the U.S. Department of Agriculture) on the deterioration of leather by perspiration and warm, damp conditions has also advanced considerably, and some aspects of the problem have been solved. In the section on dyeing, two topics were illustrated: improving regularity of shade by finding how to control the dyeing process more precisely and improving the products from the users' point of view, for example, the problem of loose colour in suede leather. Development of performance trials for assessing the reliability of laboratory tests of physical properties has continued, particularly in the work on waterproofing leather. The 'effluent liaison' section, which advises on the application of existing knowledge to the effluent problems of the leather industry (*Nature*, 187, 992; 1960) is now in full operation and the apparatus used by the mobile laboratory was shown. A substantial part of the Association's effort is devoted to liaison activities (member services) of various kinds, and between 700 and 800 production problems are investigated each year for member firms.

British Hat and Allied Feltmakers Research Association

The thirteenth annual report of the Director of Research, British Hat and Allied Feltmakers Research Association, covering the year ended August 1960, refers to considerable effort in the production of control charts for members (Pp. 20. Droylsden, Manchester: British Hat and Allied Feltmakers Research Association, 1961). Work on the storing of carroted rabbit skins was continued, and a literature survey of information published on the carroting of fur during the past ten years was issued to members. Work also continued on the possible applications to fur felt of classes of dyes other than the acid-levelling type, and acid-milling and the 1 : 1 and 1 : 2-premetallized colours have been applied successfully on a small-scale cone-dyeing machine with the aid of proprietary dyeing assistants, giving improved fastness properties. Work on the effect of pressure, moisture and temperature on pressing performance has led to the definition of limits for each, and the limits of accuracy required in the dimensions of blocks and press-dishes to give specified hat sizes have also been studied and defined. A start has been made on the measurement of the coefficient of friction of wool felt with other materials when immersed in water and aqueous solutions, and results obtained in measurements of the shock absorption behaviour of felt against other materials, such as sponge rubber and foam plastics, are being analysed to resolve differences which are believed to be fundamental to the problem of shock absorption.

Protection of Workers against Unsealed Radioactive Substances

Requirements for the protection of persons employed in factories (and other places to which the Factories Act, 1937, applies) against ionizing radiations and other hazards arising from the use of unsealed radioactive substances are set out in a recent publication issued by the Ministry of Labour (*Unsealed Radioactive Substances Regulations* (*Preliminary Draft of Regulations*). Pp. 28. (London: H.M.S.O., 1961.) 1*s*. 3*d*. net.) This draft, called the 'Unsealed Radioactive Substances Regulations', is a complementary code to the statutory draft of regulations recently published dealing with the protection of workers from dangers arising from the use of 'sealed sources' of radioactive substances, and from machines which produce ionizing radiations. It has been prepared by the Ministry of Labour with the help and advice of an *ad hoc* committee appointed to advise the Chief Inspector of Factories on the subject, by the Advisory Panel on Radiological Problems in Industry, and by the General Purposes Committee of the Radioactive Substances Advisory Committee. The proposed regulations will apply to the whole range of radionuclides used in industry, including those used in nuclear reactors, and they will, in time, replace the 'luminizing regulations' made in 1947. Unsealed radioactive substances are defined by the regulations to include nuclear fuel-elements. Maximum permissible doses, based on the recommendations of the International Committee on Radiological Protection, and supported by the Medical Research Council, are laid down. Provision is made for reliance to be placed on working arrangements that provide intrinsic safety, for the instruction of workers concerned with the hazards involved and the precautions to be taken, and also for medical supervision. The Ministry invites observations on the draft regulations. These should be sent to 8 St. James's Square, London, S.W.1.

Rise in Population in Britain

The *Quarterly Return* of the Registrar General for the fourth quarter of 1960 gives the provisional figures for that year. Births, at 783,000, were 32,000 up on the previous year and 118,000 higher than in 1955, since when the figure has been steadily rising. The crude birth-rate at 17·1 per thousand was the highest since 1948, and the figures will present considerable problems for educational planners later in the 1960's. Infant mortality, at 21·7 per 1,000, is the lowest on record, and the nineteen-fifties have seen a fall in this index of one-third. Neo-natal mortality and peri-natal mortality have also fallen. The continued rise in births is an interesting phenomenon, and it will be necessary to await the detailed statistics to see whether the rise implies an increase in family size or merely a shift in timing. The population projections which are a feature of the *Quarterly Return* have been modified to take account of the rising number of births, and imply an increase in the population by 10 millions to 56 millions by the end of the century.

Water Research

Recent issues of the periodical published by the Water Research Association contain articles surveying two widely different subjects of interest to all concerned with the provision of public water supplies. It is well known that water supplies containing undue concentrations of nitrate are dangerous to infants because of a reaction between the nitrate and hæmoglobin in the blood. It is clear from the article in *Water Research News* (No. 10) that considerable research is required before the mechanism of this reaction can be fully understood. It does not appear likely that the nitrate can oxidize the hæmoglobin directly, and it is suggested that its effect is due to blocking of an enzyme system. No. 11 of *Water Research News* is devoted largely to a discussion of the mechanism of the coagulation process, which is of fundamental importance in the production of clear water supplies. In this case the article contains an account of the results of research work, and is primarily concerned with the effect of *p*H value on coagulation by aluminium sulphate; it is a summary of fuller technical papers on the subject.

The American Type Culture Collection

The American Type Culture Collection, Washington, D.C., has been given a grant of 865,500 dollars by the National Science Foundation towards the construction of a new building to house its collection of micro-organisms used in research and teaching. The Collection at present includes more than 5,000 strains of bacteria, yeasts, moulds, algae, protozoa and animal, plant and bacterial viruses. These materials, besides being of great importance for biological, medical, agricultural and industrial research and for training of personnel in the biological and medical sciences, also serve as standards for comparison with micro-organisms isolated from natural sources, and are employed for the assay and production of drugs, enzymes and other chemicals. During 1960, more than 12,000 cultures were distributed to various institutions. The new building, which, according to preliminary plans, will be of about 30,000 sq. ft., will provide working space for about sixty people, including scientists, technicians, research assistants, etc. The estimated cost of the new building is 1,046,000 dollars. The Collection has received a grant of 90,278 dollars from the National Institutes of Health, and, in addition, about 150,000 dollars has been raised from private industry.

Co-operation between University and Industry in Manchester

THE Manchester Joint Research Council, established in 1944 as a co-operative effort between the University of Manchester and the Manchester Chamber of Commerce, now regards its most useful function as the extension of direct contact with local firms, particularly those small and medium-sized firms which depend on science and technology. As a basis for the necessary liaison work the Council has published two booklets. One of these is entitled *An Account of Some of the Scientific and Technological Facilities which Manchester University and the Manchester College of Science and Technology can offer to Industry*, and includes an indication of some of the research work carried out by the different departments (Pp. 19. Manchester: Manchester Joint Research Council, 1960). The other booklet is entitled *Sources of Scientific and Technical Information Available to Local Industry* (Pp. 21. Manchester: Manchester Joint Research Council, 1960). This appears to be less satisfactory than the first booklet: while there is a list of the research establishments of the Department of Scientific and Industrial Research, and of the Research Associations, and a list of some organizations which will name consultant experts in particular fields, publications are not covered, and under library services the only references are to the Manchester Technical Library and to Aslib.

Journal of Atherosclerosis Research

ATHEROSCLEROSIS is being studied nowadays by workers in a wide diversity of fields, and the results of their studies are appearing in many different journals. To try to co-ordinate publications on the disease, a new journal has been started (*J. Atherosclerosis Res.*, Elsevier, Amsterdam, bi-monthly, price £6 5*s*. a year). The editorial board contains members from more than a dozen countries, and the journal will contain review articles, research reports and short communications—usually in the English language. Communications on subjects related to atherosclerosis (for example, thrombosis) will also be accepted. The first part (January–February 1961) includes a review on morphological aspects of the disease by Prof. T. Crawford, and one on the thyroid and atherosclerosis by Dr. G. S. Boyd. Manuscripts may be sent to the editorial secretary, Dr. F. P. Woodford, Witte Singel 36, Leyden, The Netherlands, or to any member of the editorial board.

Life-History of the Green-veined Orchid

THE vast field of work for amateur naturalists is brought out by the continuous efforts of a schoolboy, J. F. H. Doulton, over the past three years, in the Report of the Rugby School Natural History Society for 1960 (Pp. 16. (94th issue). Rugby: George Over, Ltd., 1961). Doulton investigated the life-history of the green-veined orchid, *Orchis morio*. A quadrat was marked out, and, each year, all the plants of *O. morio* were marked on a map, together with a note of whether they were producing leaf or flowers. It is hoped to continue this work in the future to trace the life-histories of individual plants over a number of years, but it is already possible to draw tentative conclusions about the sequence of leaf and flower production. The general conclusions are that plants which have flowered do not do so again, at least in the first or second year after flowering; they are more likely to die after flowering than after being in leaf; their average life is four years; they are less likely to flower in their first year than in subsequent years. Several additional points must be taken into account when assessing these results. First, it is difficult in many cases to be sure that the investigator is following the same plant over several years because plants may come up in slightly different places, and the place of one plant may be taken by another. Secondly, there are five cases in which a plant has apparently remained dormant for a year; and it is possible that this behaviour may be more widespread, in which case some of the above conclusions may need modifying. Lastly, the observations refer only to plants producing conspicuous leaves or flowers and not to any previous year's growth as mycorhizome. Experiments on the removal of flower heads suggest that removal of flower heads before seed can be set makes it quite likely that the plant will flower again in the next two years.

Primitive Rock-Shelter Art in Australia

THERE is no doubt that interest in the Australian rock-shelter art is booming. Barely have notes on one publication been written before another is to hand. The *Journal of the Royal Society of Western Australia* (43, Pt. 4, 1961), among other articles, contains an account by P. E. Playford of rock paintings in the West Kimberley region of Western Australia. The article is illustrated with six full-page reproductions of the paintings. The local tribes know of the painted localities, but, while in some cases they tell stories about them, they say that they are the work of aborigines long ago. The paintings themselves include crude figures of animals and human beings, frogs, serpents, lizards, emus, crocodiles and kangaroos. The descriptive section of the article not only records localities but also attempts to explain some of the groups in terms of the myths obtained by questioning the local inhabitants. The article is one which will be welcomed by all students interested in this 'prehistoric' yet not very ancient rock-shelter art of Australia.

Termites and Coal Seams in Angola

IN England coal miners looking for outcrops often used to examine rabbit holes for signs of coal dust; a German firm of consulting engineers is seeking to assess Angola's resources in brown coal by studying the ant-hills built up by colonies of termites (*Science Afrique*, No. 22; October 1960). The theory is that termites prefer to build on lignite foundations and use the lignite as building material because its low-heat conductivity "keeps the house cool". They take the local light-coloured clay only as an outer covering to reflect away the heat of the sun. If the theory should prove to be sound, Angola with its countless termite hills may have big deposits of lignite. The nearest hard-coal mines are more than 500 miles away in Rhodesia, and locomotives on the country's railways still use wood as fuel. The assumption is that this might, with advantage, be replaced by briquettes made from brown coal.

Geology of British Borneo

AN important monograph on *The Geology of Sarawak, Brunei and the Western Part of North Borneo* has recently been published by the Government Printer in Sarawak, sponsored by the Directorate of Overseas Geological Surveys. Since the discovery of the Miri oilfield in 1910, very large sums have been spent by the Royal Dutch Shell Group in geological explorations throughout British Borneo, culminating in an expenditure of close on £20 million in the decade 1949–58. The geology of the territories, no greater in size than England and Wales, is thus known in considerable detail. A Government Geological Survey was established in 1949, to which the reports of the oil companies were at once made available; and it is a reflexion of the happy relationships between Government and industry that this official monograph has been compiled by Dr. P. Liechti, of "Shell", in association with Dr. F. W. Roe and Dr. N. S. Haile, of the Geological Survey. The accompanying tectonic map (1: 1,000,000) and geological maps (1: 500,000) have been excellently colour-printed in the Netherlands. Congratulations are due to the authors, to their parent organizations, and to the Government Printer in Kuching for the production of a lucid description of a region of outstanding geological interest (Bulletin 3. Vol. 1 (Text): Pp. xvii + 360 + 73 plates. Vol. 2 (Portfolio). Table of Rock-Stratigraphic Columnar Sections. Tectonic Map. (Kuching, Sarawak: Government Printing Office; London: Overseas Geological Surveys.) 1960. 21 Malayan dollars; 49*s*. the two volumes).

Beit Memorial Fellowships

THE Trustees of the Beit Memorial Fellowships for Medical Research, in their report, announce that Prof. Alexander Haddow has been elected a member of the Advisory Board. The following elections have also been made: *Junior Fellowships*, to S. P. R. Rose, to investigate the properties and localization of certain enzymes associated with the active transport of ions across cellular membranes in nervous tissue, at the Department of Biochemistry, University of Oxford; Dr. R. Laverty, to investigate the action of drugs on catechol-amines in the central and sympathetic nervous systems, at the Agricultural Research Council's Institute for Animal Physiology, Babraham; Dr. Ruth F. Itzhaki, to investigate the radiosensitivity of deoxyribonucleoprotein under various conditions with the view of explaining the action of radiation on malignant tissues, at the Department of Radiotherapeutics, University of Cambridge; C. W. F. McClare, to investigate energy transfer in nucleic acids, at the Department of Radiotherapeutics, University of Cambridge; S. S. Hayreh, to investigate experimentally the pathogenesis of papillœdema, at the Institute of Ophthalmology, London. *Fourth-year Fellowship*, to Margarethe M. A. Holzbauer-Sharman, to enable her to continue investigations into the mechanisms involved in the increase in the secretion of aldosterone following hæmorrhage.

American Academy of Arts and Sciences: Grants-in-aid

GRANTS-IN-AID, ranging between 500 dollars and 1,500 dollars, are available from funds administered by the American Academy of Arts and Sciences for research in any recognized scientific field, including mathematics, physics, biology and the social sciences. Applications must be received before September 1 for grants to be made in October. In general, applications from individual scientists rather than from institutions are favoured, although this is not an inflexible policy. Support is not usually given for work aimed primarily at fulfilling the requirements of an academic degree. Further information and application forms can be obtained from the American Academy of Arts and Sciences, Committees on Research Funds, 280 Newton Street, Brookline Station 46, Boston, Mass.

Lederle Medical Faculty Awards

THE Lederle Laboratories Division of the American Cyanamid Company is making available a number of medical faculty awards which will begin with the academic year 1962–63. These awards are intended only for individuals who share in all departmental responsibilities, particularly in the teaching of undergraduate medical students. They are not intended to be research fellowships, or for the support of individuals whose only or major function and interest is in the conduct or supervision of research activities. Also, these awards are not intended for assistance of research departments or divisions of postgraduate medical schools. The awards are primarily intended to assist medical schools in providing for their major clinical disciplines individuals who, having demonstrated ability and interest in teaching and research, will devote their major efforts to the teaching of clinical medicine with the view of integrating the knowledge and principles of the biological sciences, as applied to man. Further information can be obtained from the Lederle Medical Faculty Awards, Office of the Secretary, Pearl River, New York.

Graduate Course in Electrical Machines

THE University of Birmingham is organizing a graduate course, lasting twelve months as from October 1, on "Electrical Machines". The course is available to honours graduates in electrical engineering who have, preferably, had a year or two of engineering experience outside the university. The syllabus for the course consists of five major sections: (1) electromechanical energy conversion; (2) analysis of rotating machines; (3) mathematical techniques; (4) either electrical properties of materials, or electronic computers (analogue and digital); (5) automatic control systems. The fee for this course will be £81. Further information can be obtained from the Graduate Course Supervisor, Electrical Engineering Department, the University, Edgbaston, Birmingham 15.

The Tenth International Botanical Congress, Edinburgh, 1964

AT a meeting of the ninth International Botanical Congress in Montreal in 1959 it was suggested that the tenth Congress might be held in the United Kingdom in 1964. On the recommendation of the British National Committee for Biology, the Royal Society of London on behalf of all host organizations suggested that the tenth International Botanical Congress should be held in Edinburgh during August 3–12, 1964. Representatives were invited from universities, research institutes and biological societies to a meeting in the Senate Hall of the Old College, Edinburgh, on March 22, 1961. Prof. H. Godwin, of the University of Cambridge, has been elected president of the Congress. Joint secretaries of the Executive Committee, which is to be concerned with the preparation and planning of the Congress, will be Prof. G. E. Fogg, Department of Botany, Westfield College, Hampstead, London, N.W.3, and Dr. A. J. Brook, Department of Botany, University of Edinburgh, at the Royal Botanic Garden, Edinburgh 3.

The Night Sky in June

New moon occurs on June 13d. 05h. 17m. U.T. and full moon on June 28d. 12h. 38m. The following conjunctions with the Moon take place: June 2d. 18h., Saturn 3° S.; June 3d. 06h., Jupiter 3° S.; June 9d. 09h., Venus 2° N.; June 18d. 10h., Mars 2° N.; June 18d. 20h., Regulus 0·5° N.; June 30d. 00h. Saturn 3° S.; June 30d. 12h., Jupiter 3° S. In addition to these conjunctions with the Moon, Mars is in conjunction with Regulus on June 27d. 08h., Mars being 0·7° N. Mercury is an evening star, visible during the first few days of the month low in the west after sunset; later in the month it is too close to the Sun for observation. Venus is a morning star, rising at 2h. 15m., 1h. 50m. and 1h. 25m., on June 1, 15 and 30, respectively. Its stellar magnitude on June 15 is $-4·0$; the visible portion of the apparent disk increases from 0·379 to 0·555 during the month, and its distance from the Earth increases from 51 to 73 million miles. Mars is an evening star, but conditions for observation are becoming less favourable. It sets at 23h. 30m. on June 15. Jupiter rises at 23h. 40m., 22h. 45m. and 21h. 40m. on June 1, 15 and 30, respectively. It is in Capricornus; its stellar magnitude on June 15 is $-2·2$ and its distance from the Earth on that date is 400 million miles. Saturn rises at 23h. 20m., 22h. 20m. and 21h. 15m. at the beginning, middle and end of the month, respectively. It is near the border of Sagittarius and Capricornus; its stellar magnitude on June 15 is $+ 0·5$ and its distance from the Earth is 850 million miles. There is one occultation of a star brighter than magnitude 6 visible at Greenwich: June 3d. 1h. 42·2m., ρ Cap. reappearance. The summer solstice occurs on June 21d. 16h.

Announcements

Mr. M. J. Lighthill, director of the Royal Aircraft Establishment, Farnborough, has been awarded the Hopkins Prize of the Cambridge Philosophical Society for his contributions to fluid mechanics. The Prize has been awarded every three years since 1867 for work in mathematical-physical or related fields; the present award relates to the period 1957–60 and is of the value of £200.

Mr. Charles A. Marshall, editor of *British Communications and Electronics*, has been appointed honorary secretary of the Television Society in succession to Mr. Geoffrey Parr, who has been elected to an honorary life fellowship of the Society in recognition of his services during the past fifteen years. Mr. Parr will continue to edit the Society's *Journal*.

A summer school in quantum chemistry is being organized by Prof. C. A. Coulson and the Delegacy for Extra-mural Studies at St. Antony's College, University of Oxford, during September 9–23. Further information can be obtained from the Secretary, Extra-mural Delegacy, Rawley House, Wellington Square, Oxford.

The Institution of Engineering Inspection and the Society of Non-Destructive Examination are jointly organizing a conference at Oxford during September 5–8. Further information can be obtained from the Oxford Conference Secretariat, Institution of Engineering Inspection, 616 Grand Buildings, Trafalgar Square, London, W.C.2.

A conference is to be organized jointly by the University of Southampton and the Timber Development Association on "Timber Engineering", at the University of Southampton, during September 18–23. Further information can be obtained from Dr. H. G. Allen, Department of Civil Engineering, University of Southampton.

A symposium on North Atlantic biota and their history is being arranged under the sponsorship of the NATO Advanced Study Institutes Programme and will be held in Reykjavik during the first two weeks of August. Further information can be obtained from Dr. Áskell Löve, Institut Botanique de l'Université de Montréal, 4101 est, rue Sherbrooke, Montreal.

The Netherlands University Foundation for International Co-operation is organizing a summer school in science, which will be devoted to "Fundamental Problems in Statistical Mechanics", at Breukelen, The Netherlands, during August 1–16. Further information can be obtained from the Registrar, Netherlands University Foundation for International Co-operation, 27 Molenstraat, The Hague, The Netherlands.

A symposium on "Photomultiplier Tube Applications" is to be held at E.M.I. House, Manchester Square, London, W.1, during September 13–15. The symposium is being organized for the exchange of information on the application of photomultiplier tubes in a wide variety of scientific and industrial fields by E.M.I. Electronics, Ltd. A small nominal fee will be charged. Further information can be obtained from Mr. B. Johnson, Press Officer, E.M.I. Electronics, Ltd., Hayes, Middlesex.

A new series of publications, the *Annual Review of Astronomy and Astrophysics*, is being arranged by Annual Reviews, Inc., Palo Alto, California. The first volume will appear in the autumn of 1963, under the editorship of Dr. Leo Goldberg, Higgins professor of astronomy, Harvard University.

Translations into English of the Russian optics and spectroscopy journal, *Optika i Spektroskopiya*. have been arranged by the Optical Society of America. Special terms have been arranged for societies wishing to join the translation scheme and non-members and libraries. Further information can be obtained from the Assistant Secretary, OSA. 1155 16th Street, N.W., Washington 6, D.C.

Vol. 4, Part 5, of Scientific Institutions and Scientists in Latin America, issued by the United Nations Educational, Scientific and Cultural Organization, Montevideo, contains information received during the printing of the three former volumes, to which it is an appendix (Pp. i + 707–767. Montevideo : Unesco; Centro de Co-operacion cientifica para America Latina, 1960).

Hilger and Watts, Ltd., announce that Wray (Optical Works), Ltd., have come into association with them. Mr. A. W. Smith, a former president of the Scientific Instrument Manufacturers' Association, and chairman of its Education Committee, is continuing as managing director of Wray (Optical Works), Ltd.

MAN AND THE SOIL

A SYMPOSIUM, arranged jointly by the London Branches of the Institute of Biology, the Royal Institute of Chemistry, and the Institute of Physics and the Physical Society, was held on March 15 at the Senate House, University of London. The general title was "The Soil : Problems raised by its Use and Misuse", and the seven main speakers dealt with this question from the different points of view of biologists, chemists and physicists. Often a meeting of this kind, no matter how carefully planned, becomes in effect a series of unrelated papers. On this occasion the different contributions were clearly interrelated, and each contributed much to the understanding of the others.

The first speaker was Sir John Russell, who spoke on "Soil as Nature left it—Man's Use and Misuse". Sir John has, in an active working life as a soil scientist of more than sixty years, contributed more to our knowledge of the soil than any other person. He has always taken a broad view of the subject, and although he started as a chemist much of his work has elucidated problems of soil biology. On this occasion his paper did much to draw together the themes of the subsequent speakers. He showed how the equilibrium of a natural soil is upset by cultivation, and how a new equilibrium may be reached. Man may destroy the soil, but he may also prevent this destruction. Perhaps the most serious problem facing mankind to-day is whether research will be continued and applied in under-developed countries, now self-governing, so that the fertility of their soils can be maintained.

Mr. E. Crompton next spoke on "Soil Formation". He described the five soil-forming factors—parent material, climate, organisms, relief and time—and described how they combine to condition certain physical, chemical and biological processes occurring in the soil itself. The processes, which are the immediate mechanisms producing profile differentiation, he summarized as erosion/deposition, weathering, translocation and the organic cycle. These processes may produce many combinations, reflecting the geographical variety of the soil-forming factors.

"Physical Properties of Soil" was the title of the paper by Dr. H. L. Penman. He showed that soil physics has three aspects, for the emphasis of a technical problem may fall on the soil as a particulate system, as a porous system, or as a quasi-homogeneous system. Particle problems arise in weathering of soil, in clay mineralogy, in studies of soil structure. Pore-space problems are very important, for the biotic part of the soil needs living space, with water laid on, and some degree of air conditioning. In thermal studies, soil may be treated as homogeneous. However, ultimate explanations must go back to a study of particle properties, pore properties or both together.

Dr. G. W. Cooke spoke on "Some Chemical Aspects of Soil Fertility and Crop Production". He defined 'soil fertility' as the capacity of a soil to produce the crop desired. Fertility is not absolute, for a soil fertile enough to support a good forest may be so poor that farm crops fail on it. The term 'fertility' integrates physical and chemical factors that influence supplies of water, air and nutrients to roots; in addition, a fertile soil must anchor plants satisfactorily and be easy to cultivate and penetrate by roots. Chemical aspects of fertility are concerned mainly with nutrient supplies and reserves, but they also involve physical factors that affect root growth and penetration. The essential nutrients obtained from soil are calcium, potassium, magnesium, nitrogen, phosphorus and sulphur (the major nutrients) required in quantities of pounds or hundredweights per acre, and the much smaller quantities of micro-nutrients (from a few grams to a few ounces per acre are needed) boron, chlorine, cobalt, copper, iron, manganese, molybdenum and zinc. The rational use of different chemical fertilizers was indicated, and the importance of available or non-available reserves was discussed. Chemical analysis of soils, and the relation of the results to agriculture, is still a subject requiring much research.

The first primarily biological paper was on "The Interaction of Agricultural Practice and the Soil Fauna", by Dr. K. Mellanby. He stressed our ignorance of the soil fauna, and the difficulties in studying it. No complete census of the soil fauna of any field exists. Where soil animals are pests and destroy crops, these forms have been more thoroughly studied, and successful control measures devised, but the effects of these control measures on the rest of the fauna are unknown. Soil animals are believed to affect soil structure, the breakdown of organic matter and its incorporation in the soil, but few accurate quantitative data exist. Even earthworms, which often form more than 50 per cent of the total weight of the soil fauna, are animals the biology of which we still do not understand.

Dr. S. D. Garrett spoke on "Farming Patterns and Soil-borne Diseases of Crops". Crop rotation has, for centuries, been the main method of controlling soil-borne disease, as well as of controlling many insect pests and weeds. The efficacy of crop rotation depends on the host specialization of most parasites. Unfortunately many soil-borne diseases are caused by fungi with long-lived resting bodies, and so complete control requires rotations of undue length. 'Decoy crops' and other methods may eliminate some of the resting spores. Crop rotation alone cannot often eliminate a pest, but may keep parasite populations down to an acceptable level. Modifications of crop husbandry can prevent parasites from causing serious crop losses.

The last paper, by Mr. G. V. Jacks, was on "Climate and Erosion". He showed that four main factors determine the amount of man-accelerated soil erosion. These are land utilization, relief, erodibility of the soil, and the intensity of rain or force of wind. Land utilization is the only one of these factors controlled by man; it is also the most important, for natural erosion is generally important only in regions so arid that few crops could be grown. The importance of devising means of maintaining a complete plant cover in seasons when rain or wind would be likely to cause erosion was stressed. When this is done, the danger of erosion can be greatly decreased, and fertility restored in land already seriously affected by erosion.

KENNETH MELLANBY

SOLAR SEEING

WHILE the role of the atmosphere in degrading optical images has received considerable attention in respect of night-time observations, the question of day-time seeing has been almost completely ignored. In both cases temperature inhomogeneities cause the damage, though diurnal heating of the lower atmosphere results in conditions much worse by day than by night. Largely with the aim of stimulating interest in a subject of extreme importance to the selection of solar observatory sites and the design of solar telescopes, the Consiglio Nazionale delle Ricerche, with the co-operation of the Rassegna Internazionale Elettronica e Nucleare, organized a small symposium on "Solar Seeing" in Rome, during February 20–25.

In his opening address, Prof. M. G. J. Minnaert (Astronomical Observatory, Utrecht) differentiated between the blurring, dancing and scintillation (or intensity variation) of stellar images, and reviewed measurements of them. In general, there is little correlation between scintillation and blurring because the former results from the focusing effect of distant inhomogeneities only, whereas blurring and dancing can originate anywhere in the light path. The very few observations of stellar scintillation in the daytime have suggested that this is at most a factor of 1·5–2 stronger than at night, confirming its origin in the high atmosphere where changes due to diurnal heating are relatively small. Prof. Minnaert referred to the waviness of the limb on the solar image, pointing out that if the disturbed atmospheric layer is localized and if the direction of wave motion is parallel to the wind, a combination of the apparent velocity along the limb with that of shadows on the objective seen through a focal plane slit can give the layer height and velocity. As the subsequent controversial discussion demonstrated, a clear description of the phenomenon of limb waviness is as yet lacking, and its causes are still uncertain.

Prof. K. O. Kiepenheuer (Fraunhofer Institute, Freiburg) described seeing conditions at a wide number of sites in Europe. Wendelstein (1,840 m.), the highest mountain peak in its neighbourhood, has the best seeing conditions known in Germany, particularly with high-pressure systems, low wind velocities and snow-covered ground; but the total amount of sunshine is small. At Kanzelhöhe (1,560 m. and 1,900 m.), on a mountain covered with green woods, seeing is good only for a brief period in the morning, and possibly late afternoon, though it remains good longer at the greater height. Here, too, seeing is better in high-pressure conditions. At Zugspitze (3,000 m.), the seeing is good unless the wind is too strong. Freiburg, in the Rhine Valley, and Schauinsland (1,200 m.), nearby in the Black Forest, can differ greatly in seeing, but not preferentially. With easterly winds, seeing is bad presumably because the trees cause turbulence, while with westerly winds a turbulent 'Fern wind' descends from the Vosges mountains, causing bad seeing. However, very good seeing is again associated with high-pressure systems. At Lake Locarno (500 m.), the seeing improves continuously until early afternoon, being best when there is a general westerly wind, the nearby mountains funnelling it northwards over the Lake towards the observatory. Conditions deteriorate as the wind stops. At Anacapri (160 m.), seeing is good only in the morning and with winds from the sea, lasting longer in winter than in summer. With off-shore winds from the Apennine Mountains, seeing conditions are bad. In summary, Prof Kiepenheuer stated that sea breezes bring good seeing, siroccos bring bad seeing. In general, high-pressure systems are superior to low-pressure systems, presumably because with the former there is air slowly descending, while with the latter there is ascending air which has moved in over the heated ground. During summer, an increase of 10 m. in the height of the observatory above the ground can bring 2–3 more hours of good seeing.

Dr. G. E. Moreton (Lockheed Solar Observatory, Burbank), too, reported general correlations of seeing with meteorological conditions at the Lockheed Observatory. The Observatory (500 m.), open overhead and having a wooden floor with gaps, overhangs a hill and catches up-slope breezes. Definition is generally good with low inversion layers and poor with high inversion layers. At the Pic-du-Midi, Dr. J. Rösch (Observatoire du Pic-du-Midi, Bagnères-de-Bigorre) has found that good definition, though possibly with a little distortion, holds for an hour or so after sunrise. It is interesting that the time of optimum seeing differs for different instruments, clearly depending on the onset of turbulence associated with quite local heating. Miss Torelli (Rome Observatory) outlined observations of seeing conditions which indicate that in Rome image blurring is least in summer, though on the contrary the waviness of the limb is least in spring, late autumn and early winter. Finally, Dr. R. G. Giovanelli (Commonwealth Scientific and Industrial Research Organization, Sydney) described results obtained by Dr. Bray and Mr. Loughhead near Sydney. Recording limb-unsteadiness photoelectrically, they have found that on a day of good seeing there may be 50–200 occasions of good definition, each lasting a few seconds. In these, the limb steadiness first improves very rapidly (in a few seconds or even less than a second), and afterwards deteriorates to a more average value equally quickly; Dr. Priestley had suggested that this might possibly be correlated with a similar intermittancy in temperature fluctuations in the lower atmosphere, due to convective plumes, the rising air in which shows inhomogeneities of the order of 1 deg. C., the air between the plumes being uniform to better than 0·1 deg. C.

The design of solar telescopes to avoid internal seeing problems has interested a number of astronomers in recent years. Distinction may be made between long focal-length telescopes and short focal-length instruments where there is strong heating near the prime focus. In both cases, however, the height above the ground is important. Prof. D. H. Menzel (Harvard College Observatory) described the precautions being taken at the National Observatory, Kitt Peak, by water-jacketing the tower telescope so as to obtain homogeneous air in the long light-path within the instrument. As an aid to deciding the height of the tower, preliminary observations had been made on the temperature fluctuations at various heights in the atmosphere. Typically ΔT was approximately 2–3 deg. C. a foot or so above the ground, 1 deg. at 10 ft., 0·2 deg. at 55 ft. and 0·1 deg. at 180 ft., with a resolving time of about 1 sec., these results according with those of various micrometeorologists. A few preliminary observations made in Sydney of deviations produced in horizontal light beams at various heights were described by Dr. Giovanelli, the root mean square deviations falling off exponentially in the first 20 ft. with a scale-height of about 11 ft. These scanty results had been used to fix the height of the axis of the 5-in. equatorial photoheliograph at 9 ft. Mounted in the open, the use of air suction devices near all surfaces of this instrument exposed to direct sunlight, including the prime focus, seems to have eliminated completely any air inhomogeneities in or immediately outside the telescope.

Prof. Kiepenheuer showed a model of a new photoheliograph, to be made by Zeiss (Oberkochen), with the objective 10–14 m. from the ground. This instrument is also to be mounted in the open, but a special feature is a completely separate wind shield surrounding the telescope tube. To reduce heating, the supporting tower is to be faced with white tile, the heat reflectance of which is reputedly extremely high. A rather different approach to telescope design is the new Pic-du-Midi instrument, where Dr. J. Rösch has the telescope tube projecting well through the slit of a dome, the slit being sealed to prevent the mixing of internal and external air. Whatever be the relative merits of these various schemes, it is clear that, by bold but careful planning, considerable progress has been made in reducing or eliminating internal seeing problems. Moreover, it was welcome to hear from Prof. Menzel that proper thermal insulation of the large Sacramento Peak spectrograph had enabled it to yield definition as good as that of the vacuum spectrograph.

Whatever improvements are made, ground-based observations will almost certainly remain limited by atmospheric seeing conditions. Two devices to assist in the best use of moments of excellent seeing are the seeing monitor, which automatically selects these instants, and the electronic camera, which enables exposure times to be reduced so as to use them best. In Sydney, a seeing monitor operating on the steadiness of the solar limb has been used successfully by Dr. Bray and Mr. Loughhead to obtain, among other things, sequences of observations of facular granules; while a second device developed by Dr. Ramsay and Mr. Kobler is ready for field trials on stellar images, and with slight modification can be used for deciding movements of greatest granule contrast. Dr. Rösch has been experimenting with a somewhat similar device, recording the intensity fluctuations as a pinhole is scanned rapidly across the solar image; even at the best of times, successive scans show significant differences due to seeing.

Photographs have been obtained by Prof. G. Wlerick (Observatoire de Meudon, Paris) and Dr. Rösch and Mlle. Dupré on nuclear emulsions with a Lallemand electronic camera. The great speed (approximately 100 times that of normal photography) has already been used to obtain improved photographs of double stars and planets, while high-resolution solar spectra have been photographed in less than 0·1 sec. There is little doubt that, combined with a seeing monitor, the electronic camera will yield spectra showing the full angular resolution of the telescope.

A group of papers on meteorological aspects of turbulence left one with the feeling that the association between solar seeing and turbulence has been given much too little attention; the field is wide open for exploration. This was the general feeling of participants also, who at the final session passed the following resolution: "The members of the symposium are convinced of the necessity for more extensive research on seeing problems, particularly with the view of application to the selection of observatory sites; and therefore recommend that basic studies be made of the optical properties of the atmosphere". R. G. Giovanelli

TROPICAL ECOLOGY IN INDIA

A SYMPOSIUM on "Ecological Problems in the Tropics" was held at Allahabad during February 3–5, under the auspices of the National Academy of Sciences (India). The meetings were attended by more than one hundred scientists from all parts of the country. Dr. M. S. Randhawa, vice-president of the Indian Council of Agricultural Research, New Delhi, inaugurated the symposium and in his opening address specifically stressed the importance of ecological research in various development projects that the Government of India has taken in hand for the proper survey, development and exploitation of natural plant wealth of the country and for the increase in the production of food, fodder, fuel and fertilizers.

Dr. G. S. Puri, director of the Central Botanical Laboratory, Allahabad, who had organized the symposium, pointed out the progress so far made in Indian ecology and the need for a co-ordinated research programme in ecological studies in the tropics. He emphasized that the vegetation and animal life in the tropics raised problems entirely peculiar to these climatic conditions. He explained the concept of the forest ecosystem and recommended the use of *The Forest Ecosystem Proforma* published by the International Society for Tropical Ecology for use by the scientists in ecological studies and the application of the data to agriculture, forestry, soil and water conservation, desert immobilization, flood control, land use planning and wild life conservation, etc.

Forty-five papers were contributed to the symposium.

The paper by Sir Harry Champion on tropical ecological problems and by J. H. Davis were discussed with a number of those present. Prof. N. R. Dhar spoke on the value of nitrogen and phosphorus in Indian soils. Interesting papers were presented on the following subjects: tropical soils by Dr. G. S. Puri and K. Lakshminarayanan; fish ecology by Dr. S. M. Das and D. B. Saxena; arid zones by Sri S. K. Jain; relationships between habitat conditions and genome by Dr. R. P. Patil; distribution of perennial plants by Dr. J. K. Choudhury; soils of Madhya Pradesh by Dr. S. C. Pandeya; grasslands by Dr. S. S. Ramam; mangroves by Sri S. S. Sidhu; ecological problems in the Western Himalayas by Sri M. K. Wali; *Aspergilli* by Dr. B. S. Mehrotra; humid tropics by B. S. Ahuja and K. P. Singh; sal leaf litter by Sri N. K. Jain.

At the end of the symposium the following recommendations were made:

"Ecologists working on the tropical region, particularly Indian ecologists, should undertake co-operative studies to fill up the lacunæ in our knowledge as soon as possible. For achieving the purpose it is desirable to launch a vigorous and intensive programme of research with a view to obtaining data of practical and immediate applicability to agriculture, forestry and soil, water and wild life conservation. It is felt that *The Forest Ecosystem Proforma* prepared and published by the International Society for Tropical Ecology will be found of extensive utility. The same with a suitable amendment can be conveniently used for studies of animal and human ecology."

A committee to implement and expand the results of the symposium and to raise necessary funds needed for supporting the project in co-operation with the International Society for Tropical Ecology was formed. Further information concerning this project can be obtained from Dr. G. S. Puri, general secretary, International Society for Tropical Ecology, 10 Chatham Lines, Allahabad. *The Forest Ecosystem Proforma* can be obtained from the Society, price Rs. 5.

THE WEST AFRICAN RICE RESEARCH STATION

THE West African Rice Research Station, which is situated at Rokupr in Sierra Leone, is supported by the four West African territories of Nigeria, Ghana, Sierra Leone and Gambia. It is now ten years since a free grant from the United Kingdom under the Colonial Development and Welfare Act enabled the existing experimental station of the Sierra Leone Department of Agriculture to be transformed into a major inter-territorial research station, and five years since completion of buildings and establishment enabled a full-scale research programme to be started. It is hoped that this year will mark a further stage in development and that the Station will attain the status of an independent institute.

Rice production and consumption is expanding rapidly in West Africa, not only in Sierra Leone and adjacent parts where it has long been the staple food of the population but also in all other territories where it is of more recent cultivation. Present production in the whole of West Africa is estimated to be about one and a half million tons of paddy per annum, of which just more than a quarter of a million tons is produced in Sierra Leone. In view of the important part that rice seems destined to play in the economy of West Africa as a major food crop and in efficient utilization of the land, all Governments gave support to the establishment of the Station, the basic policy of which is to carry out fundamental research on rice as a crop under West African conditions, and to advise territorial Ministries, who remain responsible for local application and extension work, on their programmes of rice development and production.

The main station at Rokupr, in addition to an area of upland for administrative, laboratory and farm buildings and staff quarters and for experimental work on upland rices, consists of a 66-acre swamp rice-farm contiguous with the estuary of the Great Scarcies River, from which it is irrigated by tidal flooding with fresh water throughout the growing season. In addition, the Station maintains small areas on other types of soil in different parts of Sierra Leone. The laboratories, which were built in 1955, are well equipped and have recently been supplemented by a series of cabinets with controlled illumination and temperature for work on growth, an open-sided greenhouse for pot-work with soils and culture media, and a deep-water tank for experimental work with floating and flood-resistant rices. An air-conditioned building is provided for storage of the seed of the West African varietal collection, which is maintained by the Station, the responsibilities of which also include the maintenance of nucleus pure seed stocks of established and recommended varieties for seed multiplication schemes of the contributing territories.

At present the cultivation of rice in West Africa is carried out under a wide range of natural water conditions, from those of coastal mangrove swamps to the seasonal flooding of riverain plains in the lower-rainfall areas. There are extensive and widespread areas, potentially useful for rice growing, at present undeveloped. The attention of the Station has thus been concentrated on the investigation of the rice plant in relation to its habitat and on the production of rice varieties to suit natural conditions, together with work on soils and the changes that occur in them when modification of conditions may take place during development.

Selection from local and introduced varieties of rice has given excellent results in the improvement of yields in the past, but the stage has been reached when further advances can only result from a breeding programme which includes hybridization. The primary object of the present programme is the provision of high-yielding acceptable rice varieties for all the major West African habitats, including deep-flooding areas and rain-fed uplands. While consideration is given to factors such as disease-resistance, straw-strength and milling quality, none of these at present is of such importance as to warrant specific breeding programmes, and they are considered merely as they reflect on yield. Recent series of trials in various parts of West Africa indicate, however, that in some areas effects of light and, possibly, temperature are limiting in preventing existing established varieties from attaining their full potential, and a specific breeding programme for early maturing varieties insensitive to photoperiod is being undertaken. In conjunction, physiological investigations have been started on the effects of light and temperature on duration and yield.

Other physiological work to date has been largely designed to produce useful techniques for the plant breeders. Work on viability and dormancy has resulted in improved storage of seed and speeding up the breeding programme, while investigations into hydroponics and culture media have led to a method of clonal multiplication of considerable value in experimental use. Work on milling quality—in particular with the use of parboiling, the recommended and accepted method in West Africa—has indicated that modifications in established milling practice would probably increase the efficiency and output of the commercial mills to an appreciable extent.

Research on soils has been concentrated, during the past few years, on the chemistry and microbiology of mangrove soils; in particular, on the problems of their reclamation in areas where flood-waters are saline throughout the year. These soils, which are paralleled by vast areas of similar soils in many parts of the world, pose particular, problems due to their high content of sulphur and their potentiality for acid-development by microbial action on drying. Research work has now led to an understanding of the processes occurring in these soils and to the formulation of techniques of reclamation which are at present being tested in the field. More recently, attention has been paid to the changes in nutrient status of these soils—which are potentially highly fertile in their natural state—when they are reclaimed and cultivated.

Investigations are now being made into the relationship between the rice plant and soil conditions, in particular the oxidizing or reducing status of the soil and its poising by the balance of ferrous and ferric iron present. It is considered that this investigation will lead to useful techniques in the initial testing of soils for their suitability for rice growing, and for predicting the effect on them of specific modification of natural conditions.

Contributing Governments are kept informed of immediate practical recommendations, and an annual report of world-wide distribution is published to record briefly the work in progress; individual researches are reported more fully by contributions to technical journals. It is of note that within ten years of its inception the Station has been able not only to play its part in the general economic development of West Africa but also to contribute substantially to scientific knowledge of the world's major food crop.

H. D. JORDAN

TECHNICAL EDUCATION IN WALES

A RECENT report dealing with technical education in Wales* is as surprising for the boldness of its recommendations for training within industry as it is for its ambivalence about the resolution of educational and administrative problems within its purview. Within a brief sixty pages it first reviews the history of technical education in Wales with candid admissions of former shortcomings, then it surveys the great changes in industrial background. The present pattern of technical education is next reviewed, under three main headings, of courses and training for craft apprentices, student apprentices and graduate apprentices. Significant differences are noted from the rest of the British scene. In this way the report rapidly traverses the background to its terms of reference, which were "In the light of contemporary changes in the industrial pattern in Wales, to consider what educational provisions should be made to serve the best interests of industry and of those employed in it."

The fourth and final chapter of the report of the Central Advisory Council for Education (Wales) embodies the Council's observations and recommendations, some of which go well beyond the particular needs and circumstances of Wales. The report incorporates much contemporary discussion and exhortation, and thus joins in the now customary commendation of such developments as block-release courses, sandwich courses, pre-apprenticeship courses, group apprenticeship training schemes, a two-year Ordinary National Certificate course, the introduction of lower standard courses for new City and Guilds of London Institute examinations, and other ideas to be found in the Crowther Report and the second White Paper *Better Opportunities in Technical Education.*

A fundamentally new approach for industrial training in the United Kingdom is urged in the proposed establishment of a national craft apprenticeship system, especially to meet the needs of small firms which largely compose British industry, particularly in Wales. This would be administered by the State through the Ministry of Education and a National Apprenticeship Council, training being given from the age of sixteen years in apprentice training centres. The centres may or may not be attached to technical colleges, and would be financed by an apprenticeship levy on industry (though curiously this important point is not mentioned in the final summary of recommendations). This proposal constitutes a strong challenge to present Government policy, and to the existing Industrial Training Council; but the report sees no other adequate way of meeting the compelling needs of the greatly increased age-groups 16–19 years coming into industry from 1963 onwards, or of meeting the needs of smaller firms.

With regard to advanced courses, the report concludes that the overall provision greatly outruns the present demand, and urges that to avoid wasteful and inefficient duplication these courses should be confined to five technical colleges—Treforest, Newport, Swansea, Wrexham, *together with* the Welsh College of Advanced Technology in Cardiff. The words here shown in italics illustrate the Council's refusal to grasp the essential ideas of the four-tier structure of technical education, with preferential concentration of advanced courses in the colleges of advanced technology. This is related to its somewhat despairing view of the continued existence of the Cardiff College, of which it presents a very partial picture indeed (it has 186 in sandwich courses within four years of its designation out of a total sandwich course *and* full-time enrolment—not mentioned—of 826 students—compared with 732 students at the University College of North Staffordshire after 10 years of existence, not to mention the former's large part-time enrolment).

The report suggests that all five colleges should be administered jointly by an administrative council which might be the technical sub-committee of the Welsh Joint Education Committee. Never was there a better example of the new Benthamism—the greatest pacification of the greatest number of local education authorities, and consequently, the strongest possible built-in deterrent to the emergence of concentrated excellence at the College of Advanced Technology. Not surprisingly, the Council concludes that the proposal to seek closer association of the latter college with the University is not inconsistent with the purposes of the University. So little does it regard the Welsh Advanced College in its own right, that it sees the link with the University as unquestionably conferring status on technical studies in Wales, and that "A further and quite substantial advantage would be that a clearer and more easily recognisable line of demarcation than now exists could be drawn between the College of Advanced Technology and other technical colleges concerned with advanced courses". Under the Administrative Council, or under University governance, or both? What happens then to the new route in higher education complementary to that of the universities, and to the apex of the technical college system?

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* Ministry of Education: Central Advisory Council for Education (Wales). *Technical Education in Wales.* Pp. ix + 146. (In English and Welsh.) (London: H.M. Stationery Office, 1961.) 7*s*. net.

Instead of being doubled in size through bilingual publication, the report would have been better had it analysed more fully the consequences of its recommendations, and also dealt with some important omissions. Notable among the latter are some of the basic reasons why less than 2 per cent of eligible Ordinary National Certificate candidates go on to courses for the Diploma in Technology, and less than 7 per cent to Higher National Diploma courses. Do the other colleges accept a contributory role to the higher colleges? The resistance to change and support of new ventures, for example, by the schools and firms, are hinted at but not fully examined. Nowhere does the report deal candidly with the issues underlying "the delicate work of regionalisation of advanced courses . . ." which as an independent body it ought to have done; it is not surprising already to find it reported that the Flintshire authorities are taking steps to protect their right to advanced courses. A more penetrating and critical examination on the educational and administrative sides, as was done on the apprenticeship side, would have greatly increased the value of the report. The Minister of Education commends the report as a good one, and hopes that many of the recommendations will be put into practice as soon as possible: many, it may reasonably be hoped, but not some of its major ones, not at all before due consideration has been given to the report of the Committee of Enquiry into Higher Education, of which Lord Robbins is chairman.

P. F. R. VENABLES

ADDICTION-PRODUCING DRUGS

IN the eleventh report issued by the World Health Organization's Expert Committee on Addiction-producing Drugs* recommendations are given that the following substances should be subject to the provisions of the 1931 Convention in respect of addiction-producing drugs comparable to morphine: clonitazene, diapromide, diphenoxylate, etonitazene, hydromophinal, phenampromide and phenoperidine.

Evidence concerned with two analgesic benzodioxane derivatives and ethoheptazine indicated that these compounds were not to be regarded as addiction-producing drugs. Exemption from the provisions of international control was recommended in favour of two preparations containing diphenoxylate, a new constipating agent, but the Committee was unable to support the request for exemption in favour of dextro-phenomorphan.

The Committee was concerned with reports on the abuse of amphetamines and amphetamine-like substances contained in weight-reducing medicines, and emphasized the need for appropriate control measures on a national level to prevent such abuse. Attention was also directed to the need to watch closely the development of new psychically active compounds that might be abused by narcotic addicts, as has already occurred in the case of SPA((−)-1-dimethylamino-1,2-diphenylethane) in Japan. There is need for improvement in regard to the information given on the possibilities of addiction liability of new drugs, particularly where analgesic and anti-cough properties are claimed.

In response to an invitation from the Economic and Social Council, the Committee outlined the procedure by which the addiction-producing properties of a drug are established; but a precise code of practices cannot be drawn up at the present time.

The Committee was not of the opinion that there is at present any case for making *Cannabis* available for the extraction of antibiotic substances, and considered that the prohibition or restriction of the medical use of this plant should continue to be recommended by the international organs concerned.

With regard to the treatment of drug addicts, the Committee was in favour of a proposal that addicts should be committed to the care of a medical panel, as is done in the case of mental patients in general. This measure is intended to ensure adequate and complete treatment, but not to replace penalties for violations of the law.

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* World Health Organization: Technical Report No. 211. Expert Committee on Addiction-producing Drugs—Eleventh Report. Pp. 16. 1 Swiss franc; 1*s.* 9*d.*; 0.30 dollar. (Geneva: World Health Organization; London: H.M.S.O.) 1961.

CULTURAL SEQUENCES IN NORTH-WEST AMERICA

IN the past few years, evidence has been accumulating for a considerable variety in subsistence activities at an early date in North America. To the big-game hunters of the Plains have been added people who gathered seeds and plants and hunted small game in the Great Basin, and the archaic foragers of the East have proved to have begun earlier than we used to believe. The latest report* of Prof. L. S. Cressman's pioneer explorations in the north-western United States shows that yet another way of life was followed by people who settled at the strategic point where the Columbia River passes through the Cascade Range on its westward journey. In historic times this was a great trading centre and the Indian inhabitants were noted middle-men.

Trans. Amer. Phil. Soc.*, N.S., **50, Part 10: *Cultural Sequences at the Dalles, Oregon—a Contribution to Pacific Northwest Prehistory.* By Prof. L. S. Cressman, in collaboration with David L. Cole, Wilbur A. Davis, Thomas M. Newman and Daniel J. Scheans. Pp. 108. (Philadelphia: American Philosophical Society, 1960.) 3 dollars.

The construction of the Dalles Dam was the occasion of an archæological salvage programme, and Prof. Cressman, of the University of Oregon, undertook the work on the left bank of the river. A good deal of the evidence had been destroyed by previous building work, but Cressman succeeded in establishing a continuous succession from at least 8000 B.C. to recent times. (The date is based on a composite radiocarbon sample from the lowest stratum, and Cressman considers that it could indicate a beginning date of 9000 B.C. or earlier.) At an unknown time before this, the release of some 500 cubic miles of water by the breaking of an ice dam had produced a fantastic flood through the gorge, obliterating most of the signs which could indicate an earlier occupation, although Cressman produces some rather slender evidence that there might have been one.

The succession falls into three main divisions, dated by four carbon dates. There is an early one lasting until a few centuries after 6000 B.C., followed by a transition lasting to about 4000 B.C., leading to a late period covering protohistoric and historic times. Certain types of artefact are confined to the early period and others to the late, and there are two other groups, one of which decreases from an early maximum while the other increases to a late one. The period of greatest interest to readers outside North America is the early one, to which antler and bone artefacts, bolas stones, certain types of projectile point, and burins are confined. The assemblage is interpreted as indicating a great exploitation of the salmon run, the hunting of large scavenger birds (the condor, etc.) with the bolas, and the hunting of small animals and, to a lesser extent, elk and deer; in short a dependence on the river and its immediate surroundings. Of great interest is the use of burins to cut antler and bone, in exactly the same way as was done at Prof. Grahame Clark's Mesolithic site at Star Carr in Yorkshire at about the same time, though many of the resulting artefacts differ. With the end of the early period not only the characteristic artefacts but also the animal, bird and fish bones disappeared entirely from the archæological record, a loss which has not yet been satisfactorily explained.

G. H. S. BUSHNELL

TOTAL MAGNETIC FIELD MEASUREMENTS BETWEEN NEW ZEALAND AND ANTARCTICA

By DR. D. A. CHRISTOFFEL

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SOME results of a survey of the Earth's total magnetic field between New Zealand and Antarctica are presented in this article. No magnetic survey of this area has previously been made.

A proton magnetometer, of design similar to that described by Hill[1], was constructed at the Naval Research Laboratory, Auckland, New Zealand. It was installed aboard the Antarctic supply ship, H.M.N.Z.S. *Endeavour*, and was operated during this ship's Antarctic cruise between December 20, 1958, and February 15, 1959. One measurement was made each minute and the readings were recorded on a strip chart recorder to a significant value of 1γ.

Figs. 1 and 2 show charts of the area covered by the operation. The solid portions of the ship's track represent those parts over which measurements were obtained. The decimal figures beside the tracks give the values of the total field in gauss. These values have been smoothed, where necessary, to correct for local anomalies, but no corrections for time variations of the field have been made. The solid line contours have been drawn both from these measurements and also by reference to spot values obtained for various islands in the area and from the New Zealand Geomagnetic Survey maps[2]. The dotted line contours are from a total field chart issued by the U.S. Hydrographic Office, 1955 (ref. 3). It will be seen that south of lat. 55° S. the two sets of values diverge considerably. In particular, at lat. 61° S., long. 172° E., where the 1955 data show a maximum of 72,000γ, the value measured in the present instance was only 66,000γ. This discrepancy continues over the area of the Ross Sea shown in Fig. 2. Three isolated values which have been obtained from extrapolated rocket flight measurements[4] are shown in Figs. 1 and 2 with the values that were recorded marked beside them. It will be seen that these values agree with the measurements made in the present survey. Extrapolation of the field-values from the contours show that the maximum of 72,000γ would be located near a position lat. 70° S., long. 150° E.

In Fig. 3 two profiles of the total field which were obtained across an ocean trough are given. This trough is bounded to the north by a line of islands passing through Auckland, Campbell, Bounty and the Antipodes Islands, and the tracks cross it at a latitude of approximately 54° S. To the south the ocean trough is bounded by a portion of the mid-ocean ridge system. This section appears to run from just north of the Balleny Islands to Easter Island and the ship's tracks cross it at a latitude of approximately 68° S. The bathymetric depth over most of the 600 miles of the tracks shown in Fig. 3 was between 2,800 and 3,000 fathoms, and so far as could be ascertained from the depth measurement was flat. These depths were determined from explosive echo soundings made during the transit and from Admiralty Chart 788 and U.S.N. Hydrographic Office Chart No. 6636. The trough runs in a direction N. 74° E.

It will be seen that the peaks of anomaly have amplitudes of between 200 and 500γ, and that at least 25 of these may be correlated between the two

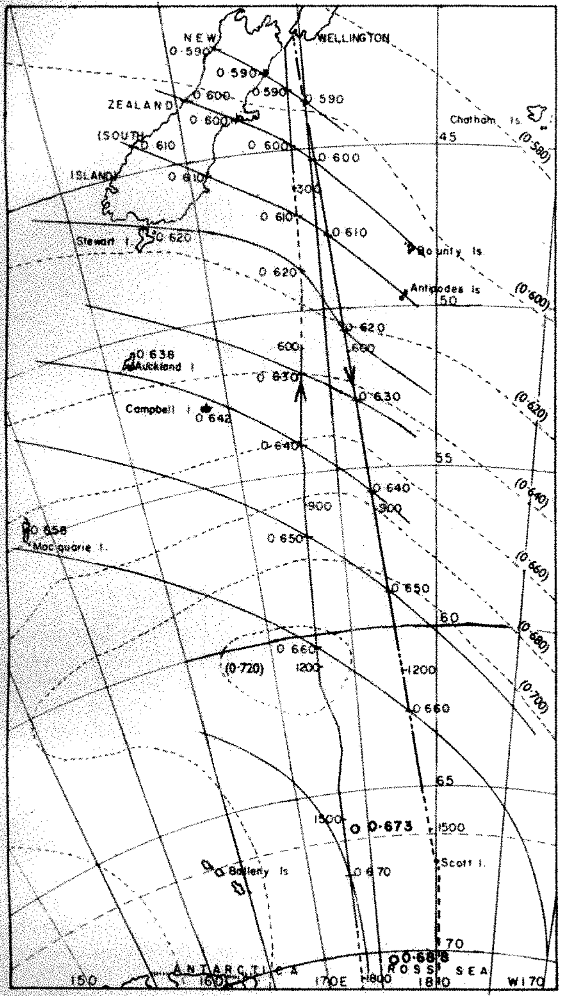

Fig. 1. Smoothed values of total-force magnetic field between Wellington, New Zealand, and Cape Adare, Antarctica
——, Contours extrapolated from values measured on present survey, and from stations in the area. Values in large numerals. – – –, Contours from U.S. Hydrographic Office, 1955 (values in parenthesis)

tracks in the 600 miles shown. The correlated lines are closely parallel to the direction of the bathymetric trough.

The anomalies are generally characterized by steep sides with, in some cases, flattened peaks. On the assumption that these are produced by dykes of magnetic material the thickness of which is comparable with the depth of the ocean, depth and susceptibility calculations have been made using the methods outlined by Henderson and Zietz[5]. It is found that in all cases the depth to the material producing the anomalies lies between 3,000 and 4,000 fathoms below sea-level, that is, only a short distance below the sea bottom. The contrast of susceptibility of the material is between 0·6 and $1·6 \times 10^{-3}$ c.g.s. units and increases uniformly towards the south.

These anomalies bear a remarkable resemblance to those found in the comprehensive surveys conducted off the Californian Coast by the Scripps Institution of Oceanography[6]. In each case, the anomalies have similar amplitude and shape. They are found to occur on essentially flat ocean floors and do not correspond to bathymetric contours. The susceptibilities needed to produce the anomalies are high, indicating that the rocks must be of basic igneous origin.

An explanation advanced by Mason[6] is that these anomalies are produced by comparatively shallow

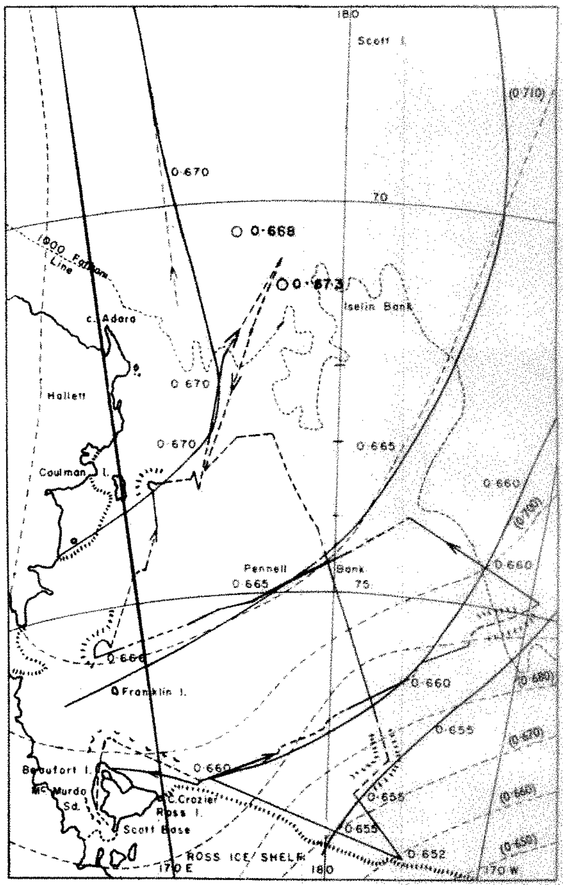

Fig. 2. Smoothed values of total-force magnetic field, Ross Sea, Antarctica
——, Contours plotted from values obtained, January 1959 (measured values shown in large numerals). – – –, Contours on U.S. Hydrographic Office Chart, 1955 (values in parenthesis)

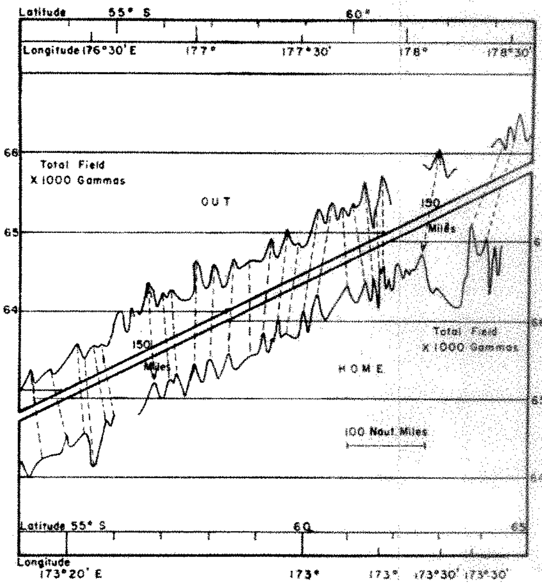

Fig. 3. Correlation, over 600 miles of track, between total field-measurements made on outward and homeward runs

lava flows which have spread out over the floor of the ocean. However, this does not explain why they should flow in parallel streams. It will be noticed that the anomalies off the Californian coast run approximately north and south, that is, parallel to the active primary mountain arc which extends down the west coast of America. In the present case the anomaly lines are parallel to the island arc in the north and the mid-ocean ridge to the south. An alternative explanation might be that, owing to the thrusts from either side, the sub-oceanic basalt layer has been buckled downwards, and fissures normal to the thrust direction have opened up, thus permitting the intrusion of lavas from beneath. This would explain both the nature and direction of the observed anomalies.

This work was part of a scientific programme undertaken by the Naval Research Laboratory, Auckland, New Zealand, and was carried out while I was in the New Zealand Defence Scientific Corps.

[1] Hill, M. N., *Deep-Sea Research*, **5**, 309 (1959).
[2] D.S.I.R. (New Zealand)—Geophysics Division; Total Force Isomagnetic Lines in New Zealand, Epoch 1950.5.
[3] U.S.N. Hydrographic Office Chart No. 1703, *The Total Intensity of the Earth's Magnetic Force for the Year 1955.*
[4] Cahill, L. J., *I.G.Y. Rocket Rep. Ser.* 4, 28 and 38 (1959).
[5] Henderson, R. G., and Zietz, I., *Geophysics*, **13**, 428 (1948).
[6] Mason, R. G., *Geophys. J.*, **1**, 320 (1958).

STAGNATION TEMPERATURE MEASUREMENTS IN A HYPERSONIC WIND-TUNNEL USING THE SODIUM LINE REVERSAL METHOD

By J. L. STOLLERY

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THE sodium line reversal method[1] has recently been used to measure transient temperatures between 1,400° K. and 3,000° K.[2,3]. In the gun tunnel[4], stagnation temperatures in this range can be generated. Measurements were made in the tunnel working section by examining the flow between a blunt body and its bow shock-wave. The gas temperature in this zone is close to the stagnation value.

The experimental arrangement is described fully in ref. 5. The blunt body finally used was a 1¾-in. diameter disk. Originally, a thin layer of common salt was stuck to the front face of the model by moistening the surface. During the test period a 'cloud' of salt particles was 'liberated', interrupted the light beam and gave a false signal of absorption. Since far too much salt was introduced by this first method, the salt was powdered next and rubbed dry on the model surface. This proved to be both simple and effective.

The first tests were made with the background light source switched off. The flow around the model was viewed with the naked eye, as well as being recorded by the photomultiplier tube and oscilloscope. When the stagnation temperature was sufficiently high for useful measurements to be made, a yellow glow within the stand-off distance was clearly visible. It was soon obvious that at the large pressure ratios needed to generate measurable temperatures, the existing piston design was inadequate. Fig 1 shows a trace indicating emission throughout the 30-m.sec. running time but the dural piston body was deformed and the nylon skirt broke and burnt completely, so increasing the test-gas temperature.

During thirty-five runs various piston materials, designs and operating conditions were tried. Finally, an all-dural design generated measurable temperatures and lasted for about ten runs before structural failure. Fig. 2 shows a typical trace with a background source temperature of 1,510° K. Reading left to right at 5 m.sec./cm. it is apparent that 5 m.sec. after the oscilloscope is triggered there is an abrupt emission signal followed immediately by 2 m.sec. of absorption. These initial changes are thought to correspond to piston overshoot followed by piston reversal. The rest of the trace shows absorption, indicating that the gas temperature was less than 1,510° K. Sufficient runs were made to

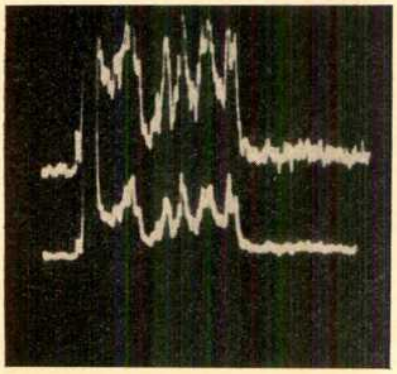

Fig. 1. $P_4 = 2{,}515$ lb./in.^{2} absolute; $P_1 = 5$ lb./in.^{2} absolute; $P_4/P_1 = 503$ (trace 17)

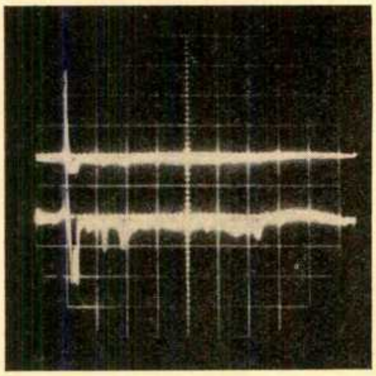

Fig. 2. $P_4 = 3{,}015$ lb./in.^{2} absolute; $P_1 = 7{\cdot}4$ lb./in.^{2} absolute; $P_4/P_1 = 407$; delay, 8·7 m.sec.; source temperature = 1,510° K. (trace 88)

bracket the air stagnation temperatures generated under three operating conditions, namely :

P_4 (lb./in.^{2} absolute	P_1 (lb./in.^{2} absolute)	Damping plate
3,000	7·4	In
3,000	7·4	Out
3,000	10	Out

Though the temperatures measured were accurate to within ± 15° K., there are various corrections to be applied, the most serious of which is due to real gas effects. The sodium line reversal method measures the sodium electronic excitation temperature which, for a diatomic gas, 'follows' the vibrational temperature of the gas extremely closely[3]. If the gas is not in thermal equilibrium, the (vibrational) temperature measured will not be the true stagnation value. Gaydon and Hurle[6] have shown that the vibrational relaxation time for air is comparatively large and increases rapidly as pressure and temperature fall. This means that the nozzle flow will not be in vibrational equilibrium, and that the vibrational temperature will be much higher than the true (translational) flow temperature in the test-jet. On passing through the bow shock-wave, the air is heated again and the situation is reversed. There is insufficient time for the gas vibrational temperature to rise to the true stagnation value though the 'error' is now reasonably small due to the non-equilibrium in the test jet. Preliminary calculations indicate that the temperature measured is about 250° K. below the stagnation equilibrium value. Other corrections allowing for: (1) the effects of longitudinal and transverse temperature gradients ; (2) heat transfer to the disk, are discussed in ref. 5. All the corrections cause the sodium line reversal method to read low. The total correction is estimated to be 350° K.

The results are plotted in Fig. 3 and compared with : (*a*) the idealized estimated values; (*b*) isentropic compression; (*c*) the temperature behind a single reflected shock-wave; (*d*) other experimental data[7] the accuracy of which is ± 100° K. The comparison shows that the gun tunnel is generating temperatures in excess of the isentropic and reflected shock-values, but considerably below the perfect gas idealized estimates. What it does not show is the probable limit on P_4/P_1 of around 500 due solely to the mechanical strength of the piston. It seems likely that to generate temperatures above 2,000° K., wave-heating (zero piston-mass) will be the only solution.

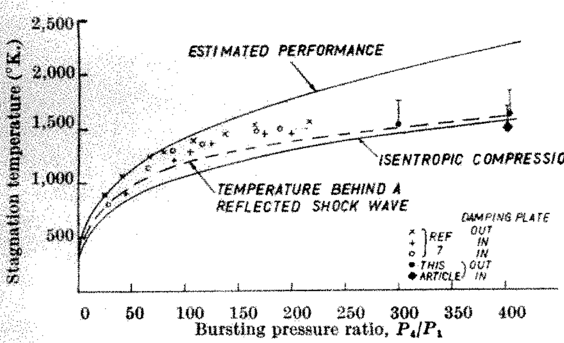

Fig. 3. Gun tunnel temperature measurements

The measured temperatures agree with the trend suggested by the A.R.D.E. experiments[7] and the measured temperature increase that results from removal of the damping plate is similar to that found by A.R.D.E.[7].

Conclusions : (1) Unless a dramatic improvement in piston design is made, enabling a very light, non-inflammable and extremely strong piston to be manufactured, the reservoir temperatures generated in the gun tunnel will be limited to 2,000° K. (2) Removal of the damping plate increases the stagnation temperature for a given bursting-pressure ratio. It also changes the reservoir pressure history to a castellated form, the first step of which has a magnitude which is greater than the bursting pressure, and a duration of nearly 20 m.sec. It would therefore seem sensible to use this period as the effective testing time and operate the tunnel with the damping plate out. (3) Since most traces showed either emission or absorption for the whole of the steady running time of 30 m.sec., and since the type of signal was changed by a 30° increase in source temperature, it is probable that the rate of temperature loss was less than 1,000 deg. K./sec.

This work was done during a visit to the National Research Council, Ottawa, Canada. The co-operative attitude and generous help of W. F. Campbell, R. Meyer and the tunnel technicians is most gratefully acknowledged.

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FORMATION OF HELICAL MICROFIBRILS FROM A STEROID ACID COMPLEX

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THE demonstrated helical molecular structure of many biological polymers has led to the suggestion that super-helices may be formed from the initial coils and may be resolved in the electron microscope[1]. The idea is plausible and important since it provides a bridge between molecular chains and microscopic fibres, but definitive examples of its application are few in number. One example is the observation of a super-helical form in starch[2] and a second is the recent report of helical strands in sodium deoxycholate suspensions[3], which are helical at the molecular level[4]. We report here two additional properties of these helical strands and the results of an extension of observations to six related bile acids. The spontaneous association of deoxycholic acid to form coils at both the 10 Å. and 100 Å. level is of particular interest since no polymerization of the substance takes place[4].

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The morphology of aggregates of seven related bile acids was examined by the electron microscope. Deoxycholic, cholic, apocholic, lithocholic, hyodeoxycholic and glycocholic acids were precipitated by hydrochloric acid from their sodium salts, washed with water, and recrystallized from aqueous ethanol. Taurocholic acid was prepared from its sodium salt with hydrochloric acid, precipitated by ethanol and washed thoroughly with ethanol. All acids except taurocholic were dissolved in ethanol and added dropwise to de-ionized water or an appropriate buffer, until a slightly cloudy suspension was obtained. Taurocholic acid was added directly to water. Preparations without buffer were dried directly on grids covered with a carbon film. Preparations in buffers were dialysed overnight on 'Formvar' films floating on water. After mounting on grids and drying, all specimens were shadowed at an angle of 15° with gold–palladium (60–40).

Of the above bile acids, only deoxycholic formed helical microfibrils when suspended in de-ionized water at pH 5 or in phosphate buffers up to pH 7·4 (Fig. 1). Above pH 7·4, no microfibrils were detectable. When water suspensions of deoxycholic acid were dried on carbon films after zero, two, four or eight hours standing, the form of the helical microfibrils changed progressively to rod-shaped aggregates (Fig. 2). After 8 hr., the super-helices had almost disappeared.

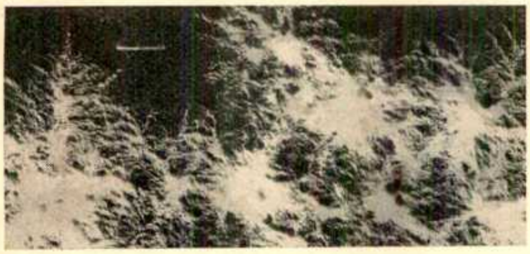

Fig. 1. Helical microfibrils of deoxycholic acid suspended in water and dried immediately

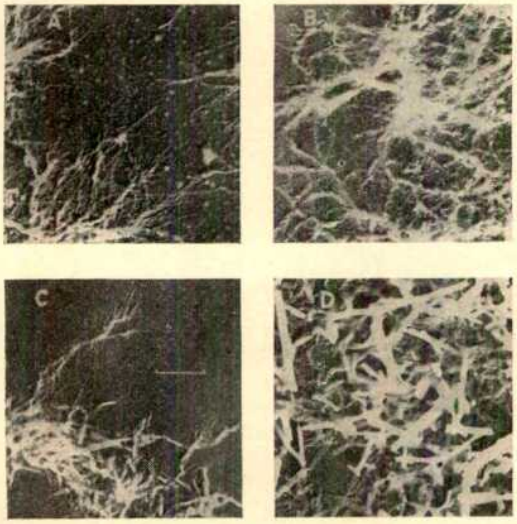

Fig. 2. Microfibrils of deoxycholic acid formed after standing for: *A*, 0 hr.; *B*, 2 hr.; *C*, 4 hr.; *D*, 8 hr.; all of equal magnification

A further property of all preparations of helical microfibrils from deoxycholic acid was a right-handed twist, presumably reflecting a characteristic asymmetry of the form of the molecule (Fig. 1). This asymmetry is not an artefact of the conditions of preparation for comparative preparations of helical microfibrils from potassium palmitate[5] consist of both left- and right-handed helices in approximately equal numbers (Fig. 3).

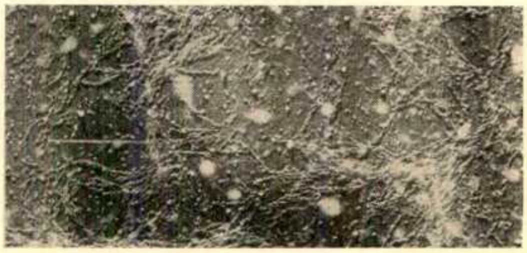

Fig. 3. Microfibrils of potassium palmitate

Deoxycholic acid fibres drawn from a solution buffered with glycylglycine gave an X-ray diffraction pattern identical with that reported previously[4]. When these fibres were dispersed in water on carbon-covered grids, they also showed typical helical microfibrillar substructure.

Of the remaining acids, only lithocholic formed aggregates which showed as bundles of straight microfibrils (Fig. 4).

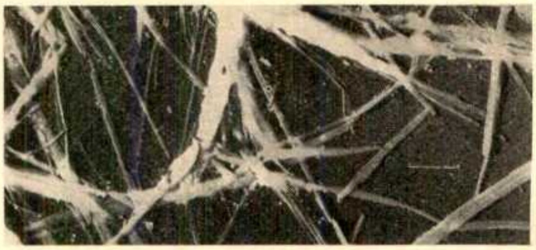

Fig. 4. Lithocholic acid bundles

The present observations, coupled with those of others[3,4], illustrate a particular example of a class of systems analysed by Pauling in qualitative terms, which are of interest in themselves and which may afford a model for some cases of biological fibrogenesis[1]. The asymmetric, un-ionized deoxycholic acid molecules in aqueous solution quickly and spontaneously pack together under the influences of London dispersion forces to form extended linear molecular helices, which are reflected in a high viscosity and the capacity to form brittle, crystalline drawn fibres. Small systematic deviations in the regularity of stacking of the steroid groups cause the molecular helices themselves to have a screw-form, leading to characteristic helical microfibrils or bundles of such microfibrils. Above neutrality, the association of the un-ionized bile-acid molecules is progressively destroyed by Coulomb repulsion of the carboxyl groups. Minor variations of molecular structure (that is, introduction of a double bond in the 8–14 position of deoxycholic acid to give apocholic acid) may eliminate the close packing necessary to give microfibrils or the asymmetry required for a helix (for example, lithocholic acid which is deoxycholic lacking only the hydroxyl group at C_{12}). As might be expected, helical microfibrils are appreciably stronger than straight aggregates since lithocholic acid forms no detectable complexes comparable to those of deoxycholic[4], although it does form straight rods of a comparable size. However, why the packing of the molecular helices in the microfibrils is only metastable with respect to the straight form is not yet clear.

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THE ACTIVE SITE AND MECHANISM OF ACTION OF BOVINE PANCREATIC RIBONUCLEASE

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Department of Biochemistry, University College, London

BOVINE pancreatic ribonuclease (RNase) is the only enzyme the primary structure of which is known[1]. A great deal of work has been done on the secondary and tertiary structure of this protein, but it is not possible to make any definite formulation of the conformation in aqueous solution[2]. In fact, several catalytically active, but physically different conformations can be produced[3]. Richards[4] has shown that subtilisin hydrolyses RNase between amino-acid residues 20 and 21 (numbering from N-terminal) without loss of activity. The peptide of 20 amino-acid residues (S-peptide) remains attached to the protein moiety, from which it may be separated by fractionation with trichloroacetic acid. The enzyme may be reconstituted, with full catalytic activity, by mixing the two fragments in aqueous solution.

RNase has four histidine residues at positions 16, 48, 105 and 119. Weil and Seibles[5], on the basis of photo-oxidation investigations, suggested that one or more of these histidine residues was associated with the active site. Histidine residue 119 reacts with iodoacetate or bromoacetate, with loss of catalytic activity[6], far more readily than the others at *p*H values between 5 and 6. Vithayathil and Richards[7] have shown that histidine 16 of modified S-peptide may be alkylated with iodoacetate with no loss of activity in the reconstituted enzyme. We have accumulated evidence in this laboratory[8] that two histidine residues are probably involved at the catalytic site of RNase and this, together with other data, has been used to formulate a mechanism of action of this enzyme.

Detailed investigation of the *p*H-activity curve of the hydrolysis of cytidine 2′ : 3′-phosphate has shown that the reaction can be interpreted in terms of the following scheme:

$$\begin{tikzcd}
EH_2 \arrow[r, phantom, "\overset{K_b}{\rightleftharpoons}"] & EH \arrow[r, phantom, "\overset{K_a}{\rightleftharpoons}"] \arrow[d, phantom, "\upharpoonleft\downharpoonright"] & E \\
EH_2S \arrow[r, phantom, "\overset{K_{b'}}{\rightleftharpoons}"] & EHS \arrow[r, phantom, "\overset{K_{a'}}{\rightleftharpoons}"] \arrow[d] & ES \\
& \text{products} &
\end{tikzcd}$$

S represents the substrate; EH_2, EH and E represent the enzyme at different stages of ionization; charges are not specified. The constants are defined thus:

$$K_b = \frac{[EH][H]}{[EH_2]} \qquad K_a = \frac{[E][H]}{[EH]}$$

$$K_{b'} = \frac{[EHS][H]}{[EH_2S]} \qquad K_{a'} = \frac{[ES][H]}{[EHS]}$$

At any single *p*H the initial velocity can be fitted accurately to the Michaelis equation:

$$V = \frac{k_3 \cdot [E_0][S]}{K_m + [S]} \tag{1}$$

where E_0 is the total enzyme concentration. The variation of the parameters of equation (1) with *p*H is shown in Fig. 1. The results fit the following expressions:

$$k_3 = \frac{\bar{k}_3}{1 + \frac{[H]}{K_{b'}} + \frac{K_{a'}}{[H]}} \tag{2}$$

$$K_m = \frac{\bar{K}_m\left(1 + \frac{[H]}{K_b} + \frac{K_a}{[H]}\right)}{\left(1 + \frac{[H]}{K_{b'}} + \frac{K_{a'}}{[H]}\right)} \tag{3}$$

where $\bar{k}_3$ and $\bar{K}_m$ are constants independent of *p*H. The values obtained for the constants are shown in Table 1. Serious reservations have to be made in interpreting results such as these[9], though, as a first approximation, it is permissible to identify the acid dissociation constants of equations (2) and (3) with those of the reaction scheme. The values of K_b and K_a suggest that both are dissociations from imidazole groups of histidine residues. One of these is required in the base form and the other in the acid form for catalytic activity. The measured constants are 'macroscopic'[10]; but, because of their relatively large separation, they probably do not differ greatly from the intrinsic *pK* values of the acid and base sites.

Table 1. *pK* VALUES OF THE GROUPS AT THE ACTIVE SITE OF RIBONUCLEASE OBTAINED FROM THE KINETICS OF THE HYDROLYSIS OF CYTIDINE 2′: 3′-PHOSPHATE AT IONIC STRENGTH 0·2 AND 25° C.

Free enzyme	Enzyme-substrate complex
$pK_b = 5{\cdot}22$	$pK_{b'} = 6{\cdot}30$
$pK_a = 6{\cdot}78$	$pK_{a'} = 8{\cdot}10$

If there were two groups on the enzyme, one an acid, the other base, which bind the substrate, the formation of the enzyme-substrate complex would result in lowering the *pK* of the group required in the base form and raising the *pK* of the group required in the acid form[9a]. It can be seen from Table 1 that $pK_{a'}$ is greater than pK_a and $pK_{b'}$ is greater than pK_b; that is, the *pK* values of the groups at the active site are both greater in the enzyme-substrate complex than in the free enzyme: after binding the substrate, both

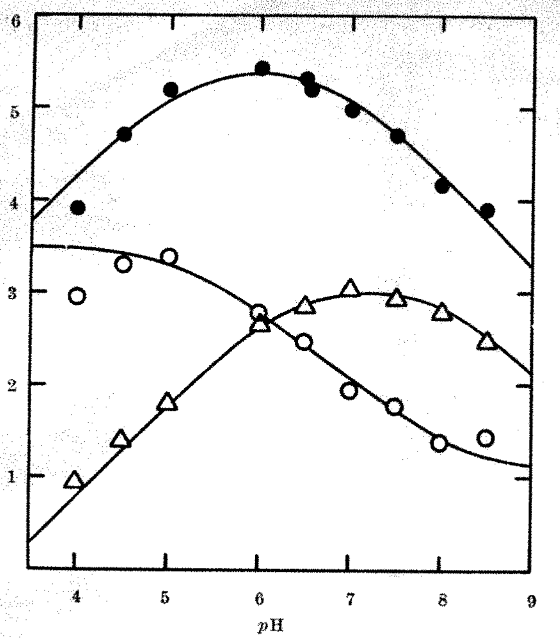


Fig. 1. Variation with pH of the kinetic parameters for the hydrolysis of cytidine 2′ : 3′-phosphate by ribonuclease at 25° C. and ionic strength 0·20. The points shown are experimental and the lines are calculated using the results in Table 1. ●—●—●, log k_3/K_m; △—△—△, log k_3; ○—○—○, pK_m

groups become weaker acids. This is evidence that the base group does not interact with the cyclic phosphate since its pK shifts in the wrong direction on formation of the enzyme-substrate complex. Binding at only the acid site would normally require $K_{a'}$ to be zero, since ES could not exist. A positive value of $K_{a'}$ indicates that, in addition to binding at the acid site, interaction occurs with one or more groups on the protein which do not change their states of ionization over the pH-range used (4–8·5). This interaction might well involve the pyrimidine ring and consequently partially determine the specificity. It is suggested that the base site is required to interact with the other reactant, water or an alcohol, in the hydrolysis, or alcoholysis, of cytidine 2′ : 3′-phosphate. Evidence for the existence of such a 'water-binding' site has already been presented[11], and other data consistent with this interpretation have recently been obtained (Findlay, D., unpublished work).

Investigation of the effects of organic solvents on the pH activity curve shows that the groups with dissociation constants $K_{b'}$ and $K_{a'}$ are cationic acids (Findlay, D., unpublished work). In these experiments the pH activity curve was determined, under conditions in which the substrate did not limit the rate, in water, 50 per cent v/v aqueous dioxane and 50 per cent v/v aqueous formamide. The difficulties of measuring and interpreting the pH of mixed solvents were circumvented by determining the three pH activity curves in buffers of two different types of charge: (a) cationic acids, $HR^+ = H^+ + R$; (b) neutral acids, $HR = H^+ + R^-$. The pK's of acids of type (a) decrease slightly in the presence of organic solvents, whereas those of type (b) increase considerably[12]. The pH activity curve shifts on addition of organic solvents with buffers of type (b) (Fig. 2), but scarcely at all in buffers of type (a) (Fig. 3). Since under the conditions employed the enzyme is saturated with substrate, any changes caused by the organic solvents are probably not due to changes in the binding constant of the substrate, but to changes in the pK's of buffers and enzyme-substrate complex. Both dissociations from the enzyme-substrate complex behave in the same way as buffers of type (a) and very differently from buffers of type (b). This confirms that they are probably both cationic acids. A detailed discussion of this problem will be presented elsewhere.

Further details of the nature of the active site has been obtained by spectrophotometric investigations[13]. Cytidine 2′-phosphate and cytidine 3′-phosphate are both competitive inhibitors of the hydrolysis of cytidine 2′ : 3′-phosphate (ref. 14 and Herries, D. G., and Ross, C. A., unpublished work), and both form complexes with the enzyme which may be

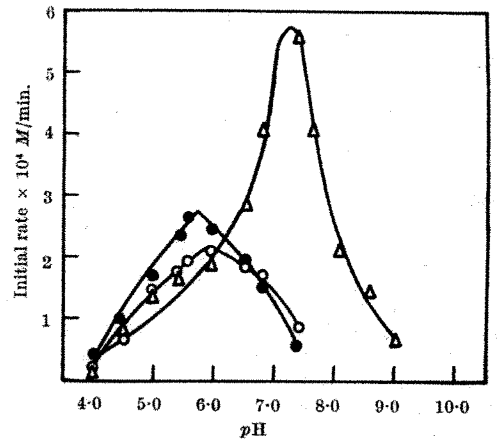


Fig. 2. Effect of solvents on the pH activity curve of the hydrolysis of cytidine 2′ : 3′-phosphate by ribonuclease in 'neutral acid' buffers at 25° C. and ionic strength 0·25. The abscissa is the pH of the appropriate buffer/salt mixture in water. The solvents used were: △—△—△, water; ●—●—●, 50 per cent v/v dioxane; ○—○—○, 50 per cent v/v formamide. The concentrations of enzyme and substrate were, respectively, 0·05 M and 0·0125 mgm./ml. The following buffers were used: pH's 4·00, 4·52, 4·99 and 5·39, acetate; pH's 5·56, 5·96 and 6·53, maleate; pH's 6·82 and 7·40, pentane 3,3-dicarboxylate (pK_2); pH's 7·65, 7·87, 8·08, 8·59 and 9·05, p-phenolsulphonate

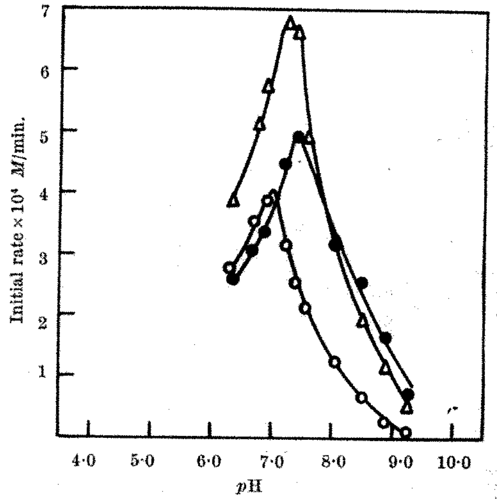


Fig. 3. Effect of solvents on the pH activity curve of the hydrolysis of cytidine 2′ : 3′-phosphate by ribonuclease in 'cationic acid' buffers at 25° C. and ionic strength 0·25. The abscissa is the pH of the appropriate buffer/salt mixture in water. The solvents used were: △—△—△, water; ●—●—●, 50 per cent v/v dioxane; ○—○—○, 50 per cent v/v formamide. The concentrations of enzyme and substrate were, respectively, 0·05 M and 0·0125 mgm./ml. The buffers used were: pH's 6·38, 6·75, 6·91, 7·23 and 7·35, ethylenediamine (pK_1); pH's 7·60, 8·10 and 8·53, N-ethylmorpholine; pH's 8·92 and 9·30, ethylenediamine (pK_2)

detected spectrophotometrically, as shown in Figs. 4 and 5. The computed spectra in these figures were obtained by assuming additivity of the absorbancies of the components, and they differ significantly from the measured spectra. This indicates formation of a complex. Alkylation of histidine residue 119 by bromoacetic acid by the method of Barnard and Stein[6a], to produce enzymically inactive CMRNase, does not alter the enzyme-inhibitor interactions as judged by the spectrophotometric shifts[13b]. Moreover, a clear-cut interaction between the substrate cytidine 2′ : 3′-phosphate and CMRNase can be demonstrated spectrophotometrically as shown in Fig. 6. This suggests that residue 119 does not interact directly with cytidine 2′ : 3′-phosphate but is the water-binding site proposed by Findlay, Mathias and Rabin[11]. Kinetic data suggest that inhibition of RNase by zinc ions[15] is associated with the formation of a ternary complex of RNase, zinc (II) and cytidine 3′-phosphate, the product of the reaction. This ternary complex can be observed spectrophotometrically (Fig. 5), but does not form with CMRNase, zinc (II) and cytidine 3′-phosphate nor with RNase, zinc (II) and cytidine 2′-phosphate. This is consistent with the binding of zinc (II) to residue 119 and provides further support for the view that this residue is not involved in binding cytidine 2′ : 3′-phosphate or cytidine 3′-phosphate. It is important to note that none of the above evidence excludes the possibility that residue 119 is involved in the formation of enzyme – substrate complexes of esters of cytidine 3′-phosphate.

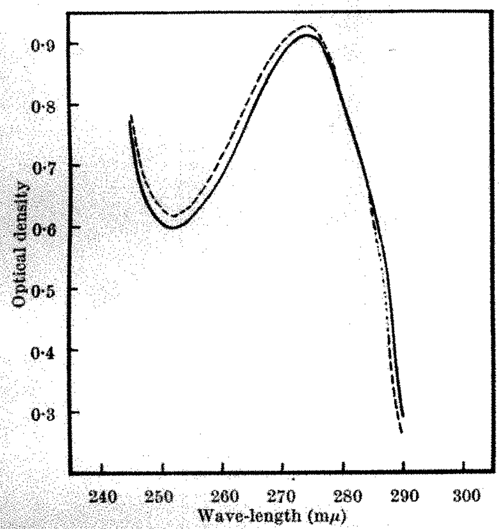


Fig. 4. Absorption spectra of a mixture of cytidine 2′-phosphate and ribonuclease (each 5·44 × 10^{-5} *M*) in N-ethylmorpholine buffer at *p*H 6·90, ionic strength 0·20 and 25° C. ——, measured spectrum; – – –, computed spectrum obtained by adding the absorbancies of the components

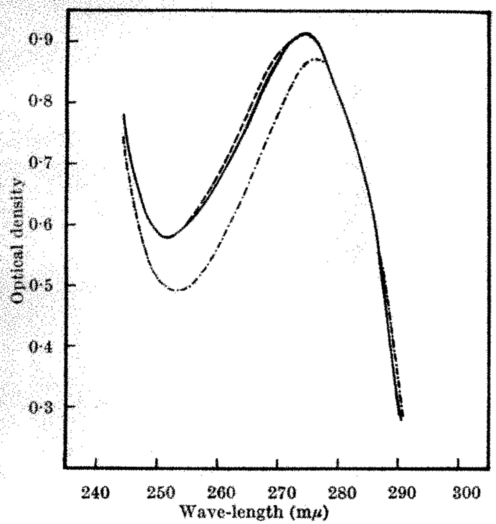


Fig. 5. Absorption spectra of mixtures of cytidine 3′-phosphate and ribonuclease (each 5·44 × 10^{-5} *M*) in N-ethylmorpholine buffer at *p*H 6·90, ionic strength 0·20 and 25° C. ——, measured spectrum; – – –, computed spectrum (cytidine-3′-phosphate + RNAse); –·–·–, in the presence of 2 × 10^{-4} *M* zinc chloride (measured spectrum)

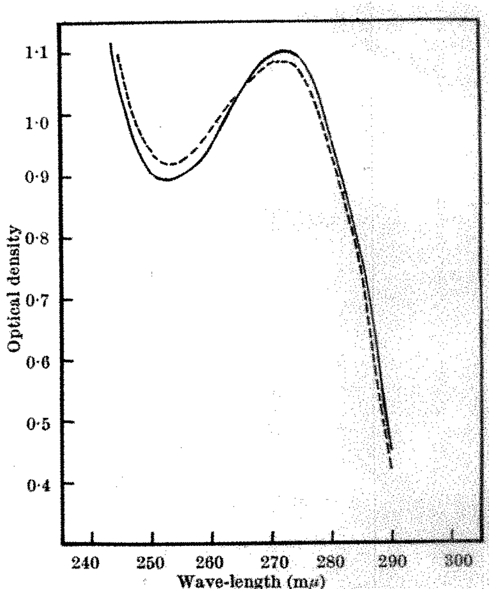
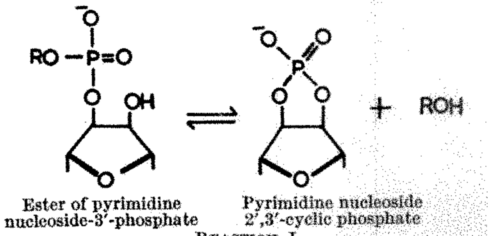


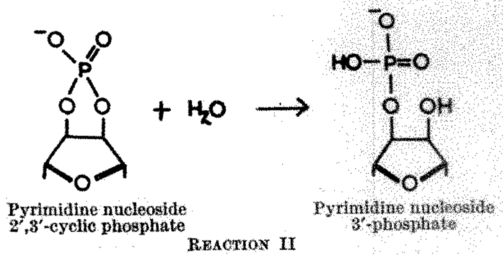
Fig. 6. Absorption spectra of a mixture of cytidine 2′ : 3′-phosphate and CMRNase (ribonuclease with histidine residue at position 119 carboxymethylated) in N-ethylmorpholine buffer at *p*H 6·90, ionic strength 0·20 and 25° C. The concentrations of both components were 5·44 × 10^{-5} *M*. ——, measured spectrum; – – –, computed spectrum obtained by adding the absorbancies of the components

Any mechanism proposed for the action of RNase must explain the finding that cytidine 2′-phosphate is the most powerful inhibitor of the three cytidine


RO
P
O
OH
ROH
Ester of pyrimidine nucleoside-3′-phosphate
Pyrimidine nucleoside 2′,3′-cyclic phosphate

Reaction I

Fig. 7


P
O
H₂O
HO
OH
Pyrimidine nucleoside 2′,3′-cyclic phosphate
Pyrimidine nucleoside 3′-phosphate

Reaction II

Fig. 8

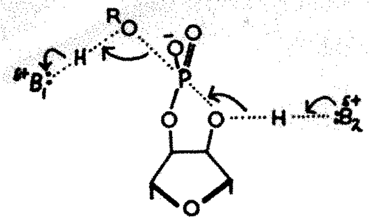


Fig. 9

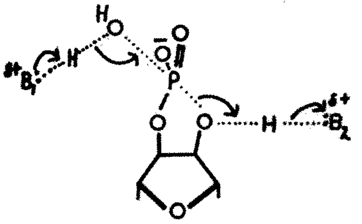


Fig. 10

monophosphates. This would suggest that one of the interactions in the enzyme – substrate or enzyme – inhibitor complex is between the 2′ cytidine oxygen and a positively charged acid-group on the protein. Of the three cytidine monophosphates, cytidine 2′-phosphate has the most basic oxygen in the 2′ position.

RNase catalyses two reactions[16] shown in Figs. 7 and 8. Both these reactions involve nucleophilic displacements about the phosphorus atom. The attacking and departing groups are, respectively: reaction 1 (Fig. 7), 2′ oxygen and OR^-; reaction II (Fig. 8), water oxygen and 2′ oxygen. The attacking reagent would be assisted by co-ordination with a base group and the removal of the departing group would be assisted by co-ordination to an acid group. Thus the acid-base requirements for reaction II are the reverse of those for reaction I in the forward direction and the same as those for reaction I in the reverse direction. We have presented evidence that both these groups are histidine residues and these are represented, in the acid form, by B_1H^+ and B_2H^+. The mechanism we suggest, in which the dotted lines are bonds being formed or broken and curved arrows the direction of electron displacements, is illustrated in Figs. 9 and 10. If ROH replaces HOH in Fig. 10, this represents the reverse of reaction I. Reaction I in the forward direction requires the species B_1H^+ and B_2; reaction II, and reaction I in the reverse direction, require B_1 and B_2H^+. This conforms with the principle of conjugate requirements[17]. B_1 is possibly residue 119 and B_2 either 48 or 105.

We are indebted to the Central Research Fund, University of London, for a grant for the purchase of automatic titration equipment and to the Wellcome Trust for a grant for the purchase of a Cary recording spectrophotometer. The following personal grants are acknowledged: Ontario Research Foundation (to D. F.); Department of Scientific and Industrial Research (to D. G. H.); Medical Research Council (to C. A. R.).

Note added in proof. Recent work from the Rockefeller Institute, New York[18], is in accord with our hypothesis of the structure of the active site of RNase. It was shown that the unique reactivity of histidine 119 towards iodoacetate is probably due to interaction of the carboxyl group of the alkylating reagent with the imidazole group, in the cationic form, of another histidine residue. The separation of the two imidazole groups required for this electrostatic enhancement is of the same order as that expected for the acid/base pair at the active site of RNase.

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VISUAL PIGMENT OF GRAPSOID CRABS

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WITHIN the past few years a number of research workers have isolated visual pigments from a variety of arthropods. Kampa[1] has extracted a pigment from the eyes of a euphausiid crustacean, *Euphausia pacifica*, while Fisher and Goldie[2] have prepared a similar photosensitive substance from the eyes of another member of this order, *Meganyctiphanes norvegica*. Wald and Hubbard[3] have extracted a pigment from the eyes of the decapod crustacean, *Homarus americanus*, and an insect visual pigment has been obtained by Goldsmith[4] from *Apis mellifera*. The first reported arachnoid visual pigment is from *Limulus*[5].

It is apparent that the arthropods offer an excellent opportunity for the study of comparative visual biochemistry[6]. The numerous arthropod species (estimated at 750,000 (ref. 7)), all possess eyes, and, moreover, present tremendous variations in nutrition, ecology, etc.—factors that probably have considerable effects on the visual mechanisms.

This article reports the extraction of visual pigments from the eyes of two species of grapsoid Crustacea. The pigments from the two species are identical, and proved to be rhodopsins of the type previously investigated by other workers from vertebrates and invertebrates[6].

The animals used were adult specimens of *Hemigrapsus edwardsii* and *Leptograpsus variegatus*, collected locally. Experiments were conducted within a few hours of capture, the animals being contained in sea-water.

In experiments to determine the vitamin A content of the eyes, the whole eyestalks were removed and immediately stored in a deep freeze until required. Visual pigments were extracted from freshly removed eyes obtained from animals previously dark-adapted for 3 hr.

Vitamin A was determined by techniques based on those of Wald and Burg[8]. The eye tissues were ground with anhydrous sodium sulphate until dry, and then repeatedly extracted with petroleum ether until the extract was colourless. A further extraction of the tissues was made with acetone to release any protein-bound carotenoids.

The petroleum ether extract was chromatographed on Merck alumina. Vitamin A esters were eluted from the column with 4 per cent acetone in petroleum ether, and free vitamin A alcohol with 40 per cent acetone in petroleum ether. Both these fractions were saponified with 6 per cent potassium hydroxide in methanol for 20 min. at 40° C., and then separately brought into solution in hexane. The absorption spectra of these solutions were determined immediately. The spectra were obtained a second time after 15-min. irradiation with sunlight in the presence of an added 5 μgm. of iodine. The vitamin A of these solutions was brought next into solution in chloroform and a Carr–Price reaction conducted by the addition of saturated antimony trichloride solution in chloroform. From the extinction coefficients of these solutions at 618 mμ the total vitamin A content was calculated.

Visual pigments were isolated from the fresh, dark-adapted eyes by techniques based on those of previous workers[1,3,5]. All stages of preparation were conducted in darkness, or with a dim photographic red light as the only source of illumination.

The eyes were thoroughly ground with a mortar and pestle, and then in a small Potter–Elvehjem glass tissue homogenizer. The ground tissues were repeatedly extracted with petroleum ether to remove light-stable pigments. The resulting tissues were extracted with a small volume of 2 per cent aqueous digitonin, and the extract filtered through sintered glass. The absorption spectrum of this solution was determined immediately, and again after 30 min. in darkness at room temperature. The solution was next exposed to sunlight for 20 min. and the absorption spectrum again determined in the presence of 0·1 *M* hydroxylamine. A difference spectrum was obtained by subtracting the optical density readings of the bleached solution from those of the unbleached solution.

To a portion of the unbleached extract acetone was added and the mixture shaken with ethyl ether. The ether fraction was separated, dried and the residue taken up in chloroform. The absorption spectrum of this extract was determined alone, and again after a Carr–Price reaction.

Extracts of the eyes of both species contained free vitamin A alcohol and vitamin A esters. Astaxanthin and other carotenoids, probably xanthophyll and beta-carotene, were also present. The total vitamin A content of the eye extracts was calculated on the basis that the extinction coefficient, E (1 per cent. 1 cm.), of the vitamin A–antimony trichloride product in chloroform is 4,400 at 618 mμ [9]. The values calculated by taking the means of several determinations are given in Table 1. These levels are comparable with the vitamin A contents reported for other crustacean species[10–14].

Table 1. VITAMIN A IN THE EYES OF GRAPSOID CRABS

	Hemigrapsus edwardsii	*Leptograpsus variegatus*
Total vitamin A (μgm. per eye)	1·3	1·5
Free vitamin A alcohol (per cent of total)	15	11
Esterified vitamin A (per cent of total)	85	89

The addition of iodine and exposure to white light of the solutions containing the extracted vitamin A led to an easily detected increase in extinction around 325 mμ. Moreover, the absorption maximum of the vitamin A in the chromatographed extracts was at 319–321 mμ before this treatment, and at 325–326 mμ afterwards. As the concentration of vitamin A in the extracts was known from the Carr–Price reactions, it was possible to calculate the extinction coefficients (E). The average E (1 per cent, 1 cm.) for all the extracts containing vitamin A (including those derived from the ester forms by saponification) was 1,225, the range of the samples being 1,210–1,255.

These results indicate that the vitamin A from the eyes of these crabs is present largely in the neo-*b* form (the 11-mono-*cis* isomer). This is the only vitamin A isomer to give a large change in extinction on exposure to iodine and sunlight, to have a $\lambda_{\max.}$ at 319–321 mμ, and to have an extinction coefficient at 320 mμ of approximately 1,200 (ref. 8). This deduction was confirmed by measuring the ratio of $E_{\max.}$ of the original extracts at 320 mμ to the $E_{\max.}$ at 325 mμ of the solutions after isomerization. The calculated ratios ranged from 1·44 to 1·50, with a mean of 1·47. The ratio for neo-*b* vitamin A is 1·45 ± 0·05 (ref. 8).

Visual pigments were successfully isolated from the ground eyes of both species by digitonin extraction. The difference spectra of these pigments from both species were identical within experimental errors. The result of a typical determination is given in Fig. 1. The photosensitive pigment has an absorption maximum at 513 ± 2 mμ. No change in spectrum was detected after the pigment solutions had been kept at room temperature for 30 min.

Examination of the spectrum of the products produced by the bleaching of the crab rhodopsin revealed the presence of a second pigment with $\lambda_{\max.}$ 495 mμ. This pigment appears to be in many ways analogous to the metarhodopsin obtained by Wald and Hubbard[3] from the lobster. On standing in the dark, the crab metarhodopsin is gradually converted to a mixture of retinene and opsin. This change is demonstrated by the slow decline in intensity of absorption at 495 mμ, and by the increased quantity of retinene extractable from the solution. The conversion of crab metarhodopsin to retinene and opsin is complete within 2 hr.

Three compounds were identified in the ether extract of the acetone-treated, unbleached solution of crab rhodopsin. A narrow absorption due to vitamin A was detected at 325 mμ, a second absorp-

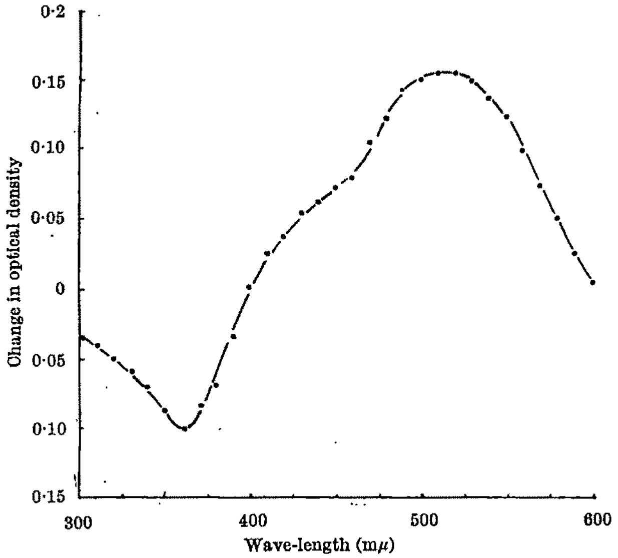

Fig. 1. Difference spectrum of rhodopsin from grapsoid crabs. Since retinene oxime has no absorption above 450 mμ, the difference spectrum is equivalent to the true absorption spectrum of the rhodopsin above this wave-length. The minimum around 362 mμ corresponds to the absorption of retinene oxime

tion at 366 mμ indicated the presence of retinene, while a broad-band absorption in the blue-green at around 470 mμ was assumed to be due to astaxanthin. The presence of vitamin A and retinene in the extract was confirmed by a Carr–Price reaction which yielded an absorption spectrum with maxima at 618 mμ (vitamin A) and at 666 mμ (retinene).

These results indicate that the digitonin extract contains a variety of compounds other than crab rhodopsin. The vitamin A detected is presumably a contaminant due to the incomplete extraction of the tissues with petroleum ether. It is unlikely to be present as a protein complex. The astaxanthin also may be a contaminant of a similar nature, but the amounts detected favour the view that the digitonin extracts an astaxanthin–protein complex from which free astaxanthin is liberated by the acetone treatment.

Considered as a whole, the above results demonstrate that crab rhodopsin is a protein with a retinene chromophore. The preponderance of neo-*b* vitamin A in the crab eyes is strong evidence that the retinene chromophore is also in the hindered 11-mono-*cis* configuration, and is thus analogous to previously isolated rhodopsins[6].

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CONTRACTION OF SMOOTH MUSCLES IN NON-IONIC SOLUTIONS

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IT is generally assumed that muscular contraction and electrical activity depend on the presence of an extra-cellular fluid containing the ions of Ringer's solution or one of its variants. However, I. Singh *et al.*[1–3] have repeatedly reported that smooth muscles from the stomach of the frog continue to contract and to conduct action potentials when soaked in sucrose solutions. These experiments are often disregarded. Only recently, Bozler[4] confirmed the report that smooth muscles soaked in sucrose containing calcium chloride retain the ability to contract. We have repeated some of Singh's other experiments and are also able to duplicate his results.

In most of our experiments the smooth muscle of the stomach of the frog, *Rana pipiens*, was used. Strips of the stomach muscle were fastened with one end tied to a strain gauge for recording isometric tension and suspended in a simple bath. The observations were made at room temperatures. The muscles were immersed in a 0·12 *M* sucrose solution, which has about half of the tonicity of normal frog Ringer's. The sucrose was dissolved in distilled water which had been passed afterwards through a demineralizing column. The first response by the muscle to the sucrose medium is a series of large contractions. The spontaneous contractions gradually die out while the muscle undergoes a slow increase in tone. After about 2 hr. in sucrose, the muscle rarely contracts spontaneously, but contractions can be readily elicited by stimulation with $2{\cdot}9 \times 10^{-4}$ *M* epinephrine, as shown in Fig. 1*A* or with $2{\cdot}2 \times 10^{-4}$ *M* acetylcholine. The muscles continue to respond to stimulation for as long as 24 hr. after immersion in 0·12 *M* sucrose.

Muscles soaked for hours in sucrose also retain excitability to electrical stimulation. The responses fall into two general catagories. With some preparations, there is a rapid increase in the tension exerted by the muscle as soon as a d.c. stimulating current (0·05–0·6 m. amp.) begins to flow through the muscle bath; when the stimulating current is turned off, the muscle relaxes to the pre-stimulus tension. With other muscles, under the same conditions, there is little change in the tension exerted by the muscle as long as the stimulating current is flowing. But once the stimulus is turned off, there is a brief latent period and then the muscle contracts, reaches a maximum tension, and relaxes. The longer the stimulus, the shorter the latent period and the greater the contraction. The variables determining whether the muscle contracts along with, or after, electrical stimulation have not yet been resolved; perhaps the most important factor is the portion of the stomach selected as the source of the muscle strip.

In a smaller series of experiments, it was found that short lengths of the taenia coli of the guinea pig soaked for hours in 0·16 *M* sucrose remain responsive to chemical and electrical stimulation.

Singh and Acharya also reported that sucrose-soaked frog stomach muscle continues to conduct action potentials, judging from an extra-cellular recording. We have taken strips of frog stomach muscle soaked in 0·12 *M* sucrose and simultaneously recorded tension, with a strain gauge, and electrical activity between a pair of fine platinum electrodes pushed into the muscle. As shown in Fig. 1*B*, the contractions of the muscle were accompanied by a marked increase in the amount of electrical activity recorded from the muscle.

We have also begun to study conduction in isolated ureters from the guinea pig, which are ideally suited for measurements of conduction velocities. At 33° C. in Tyrode's solution the conduction velocity is about 2·5 cm./sec. After 220 min. in 0·32 *M* sucrose, the conduction velocity falls to 0·026 cm./sec., probably because the resistance of the extra-cellular fluid has markedly increased.

In a final series of experiments, fine capillary electrodes filled with 3 *M* potassium chloride were thrust into strips of frog stomach muscle which had been soaked in 0·12 *M* sucrose for 3 or more hours. The records are poor technically, owing to the high resistance of the sugar solution and of the micro-electrodes. It is striking, however, how similar the records from sucrose-soaked muscles are to those from muscles in Ringer's solution. Estimates of the 'resting' potentials are uncertain because high tip potentials are common and uncertain with such fine electrodes. The recorded values ranged between 20 and 70 mV., inside the cell negative. A section of a record is shown as Fig. 2. In only one case was a spike recorded which seemed to overshoot the zero potential baseline. Therefore, it cannot be definitely decided whether or not an action potential can overshoot when the external medium is a sucrose solution. Preliminary results show that in sucrose-soaked muscles treated with epinephrine there is an increase in the rate of spiking and a decrease in the 'resting' potential. Spiking was reversibly eliminated by chilling the sucrose solution bathing the muscle.

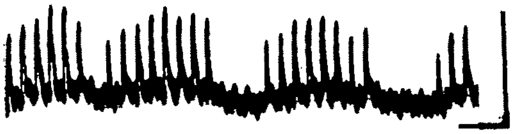

Fig. 2. A recording made with a micropipette inserted into a strip of frog stomach muscle soaked for 3 hr. in 0·12 *M* sucrose. The base line is marred by a high noise-level. Calibrations: time, 0·1 sec.; voltage, 50 mV.

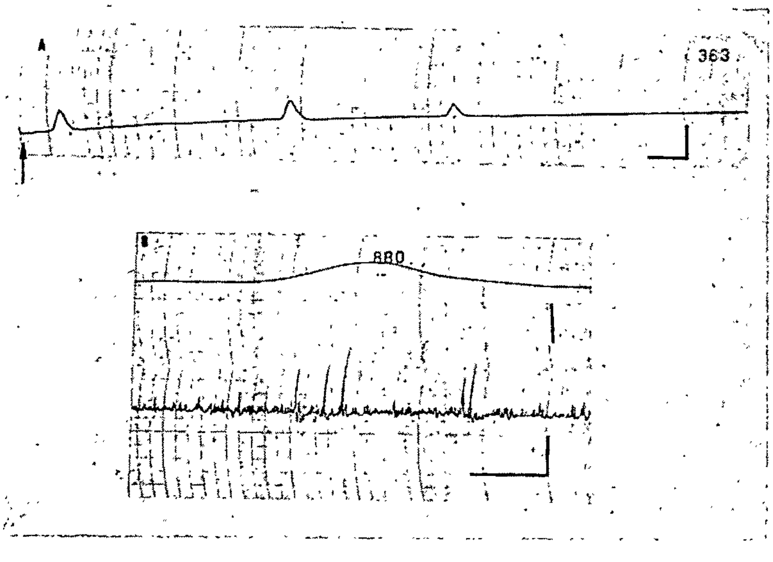

Fig. 1. *A*, response of a strip of frog stomach muscle, soaked for 3½ hr. in 0·12 *M* sucrose, to epinephrine hydrochloride (added at the arrow). Note the increase in tone and the three rhythmic contractions. Calibrations: time, 30 sec.; tension, 0·5 gm. *B*, simultaneous electrical and mechanical recordings from a strip of frog stomach muscle soaked for 3 hr. in 0·12 *M* sucrose. The record shows a spontaneous contraction and the simultaneous electrical activity. Calibrations: time, 30 sec.; tension, 0·5 gm.; voltage, 50 µV.

The principal conclusion is that the mechanical and electrical behaviour of these smooth muscles are changed relatively little when the tissue is transferred from an environment of Ringer's to a sucrose solution which is about half isotonic. This is true in spite of the change in the extra-cellular fluid and a drastic change in the inorganic composition of the tissue. In sucrose-soaked muscles, ashed and analysed by flame photometry, the concentration of sodium is about 4·5 m*M*/kgm. of muscle water and the concentration of potassium is about 45·7 m*M*/kgm. The values for the Ringer's-soaked muscles are: sodium, 66 m*M*/kgm.; potassium, 129 m*M*/kgm. (ref. 5).

Obviously these results challenge some of the present ideas about the electrical activity and the contraction of smooth muscles. Since the sodium concentration of the muscles falls during the soaking in sucrose to about 10 per cent of the initial value, there is little possibility that the extra-cellular fluid is protected by a diffusion barrier from exchanging with the external medium. Therefore, whatever ions are present in the extra-cellular fluid of a muscle soaked in sucrose must come from the impurities in the reagent grade sucrose, from the distilled and demineralized water, or by leaching from the muscle cells. Although we cannot as yet place a precise upper limit on the concentration of ions in the extra-cellular fluid of the sucrose-soaked muscles, it must be extremely low compared with the normal environment of the cells. Holman[6] showed that the electrical activity of smooth muscles does not depend on the sodium concentration of the external medium in the way that is familiar with nerve and with skeletal muscle. However, it is still surprising to see electrical signals which look very similar to the normal action potentials occurring when the extra-cellular fluid can contain only a fraction of a millimole of sodium.

The continued contraction by sucrose-soaked smooth muscles is equally surprising. Axelsson and

Bülbring[7] showed that the removal of calcium from Ringer's solution led to the loss of tension by the taenia coli, and Robertson[8] showed that the mechanical response of smooth muscles (depolarized in potassium sulphate-Ringer's and stimulated with acetylcholine) declined markedly when calcium was withdrawn from the medium. These experiments were interpreted as showing that extra-cellular calcium is needed for contraction. The concentration of calcium in the extra-cellular fluid is unlikely to be markedly greater in a muscle soaked for many hours in 0·12 *M* sucrose than in a muscle soaked for a shorter time in a calcium-free Ringer's. Nevertheless, the sucrose-soaked muscles can contract. It appears that ionic solutions which do not contain calcium may stop smooth muscles from contracting—perhaps by displacing a critical fraction of the calcium from the cell. However, from the evidence now at hand it cannot be concluded that the contraction of smooth muscles depends on the presence of calcium in the extra-cellular fluid.

It is also remarkable to find continued electrical and mechanical activity, supported by the metabolism of the cells, coming from a tissue the intracellular ionic composition of which must be changed so drastically.

In summary, we have confirmed the observations by Singh *et al.* that smooth muscles soaked in half-isotonic sucrose solutions retain electrical activity and contractility. The behaviour of 'slow' skeletal muscle fibres in sucrose is different from that of smooth muscles; acetylcholine, which normally produces depolarization and contraction, causes sucrose-soaked frog recti to become hyperpolarized and to relax[9].

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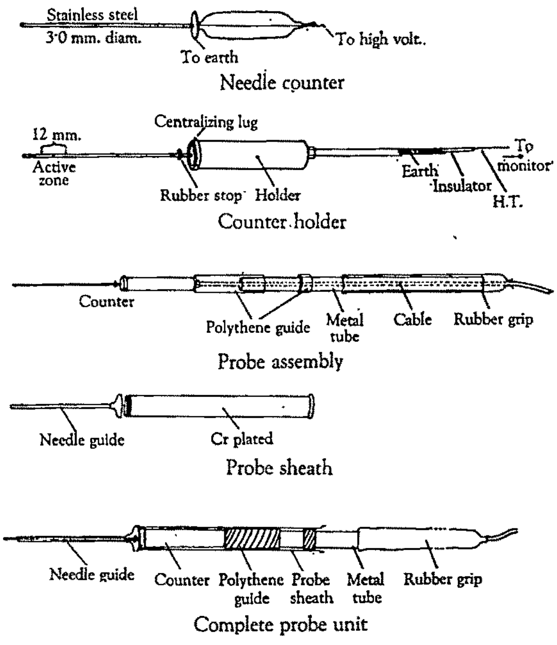
MOVEMENT OF THE OVUM IN THE REPRODUCTIVE TRACT

A Needle Probe Geiger-Müller Counter for the Study of the Passage of the Ovum in the Reproductive Tract

In an endeavour to inquire into the reasons for the failure of non-surgical transfers of ova into recipient cows[1], small radioactive spheres[2], the passage of which in the reproductive tract could be traced with a newly designed probe unit, were used to simulate cow ova. The 'artificial ova', consisting of an ion exchange resin ('Permutit'), impregnated with radioactive gold, gold-198, of density 1·2 and 120μ in diameter, were transferred to the cow uterus via the cervix. They were suspended in a small quantity of cow serum delivered from a syringe attached to a narrow metal tube extending into the uterine horn. The needle probe apparatus was then used to search the internal walls of the uterine horns, cervix and vagina for the presence of the radioactive artificial ova at varying intervals of time after transfer. A report of the results of this investigation forms the subject of the next communication. The gold-198 spheres are also being used in some other work on the tubal transport of ova in the rabbit[2] and in the ewe[3].

The essential part of the needle probe apparatus (Fig. 1) is the stainless steel *NIb* needle counter (20th Century Electronics), contained in a chromium plated counter holder. The needle counter itself is 150 mm. long and the active length of the Geiger–Müller counter is 12 mm., situated 7 mm. from the tip of the needle. The probe assembly consists of the counter holder bolted on to a hollow polythene guide of length 58 mm. and outside diameter 34 mm., utilizing the earthing nut at the rear of the holder. Into this guide is inserted a thin-walled aluminium metal tube of 25 mm. outside diameter. A further polythene guide 15 mm. long is situated 5 cm. behind the first. The rear part of the aluminium tube is covered with a rubber sheath handle, through which the high-tension cable passes out to a rate-meter which measures any radioactivity detected. The whole of the probe assembly as shown in Fig. 1 is inserted into a chromium plated probe sheath 34 cm. long with an outside diameter of 3·8 cm. Part of the probe sheath consists of a detachable blunt-ended needle guide, 12·5 cm. long and 4·0 mm. outside diameter, screwed on to the end. The needle of the counter is directed into the needle guide by means of three centralizing lugs on the counter holder, and by the polythene guides on the probe assembly.



Needle counter

Counter holder

Probe assembly

Probe sheath

Complete probe unit

Fig. 1. Needle probe equipment

In order to search the cow reproductive tract for any radioactivity emitted from the gold-198 spheres, the complete probe unit (Fig. 1) is grasped in one hand by means of the rubber handle of the probe assembly. The other hand is inserted into the rectum of the cow to help guide the needle through the cervix and into the uterine horns. A little 'Vaseline' on the outside of the probe sheath is sometimes needed to ease the probe sheath up the vaginal tract. With the needle retracted into the needle guide, the complete probe unit is inserted and the needle guide passed through the cervix into the uterus of the cow. The counter is then eased forward up the probe sheath by applying forward pressure on the rubber grip with the free hand. The needle slides out from the guide, exposing the active counting zone to any radiation present in the near vicinity. The intensity of radiation present is measured on the rate-meter. It was found possible to estimate very rapidly the approximate number of gold-198 spheres in the uterus or the vagina merely by judging the noise emitted by the loudspeaker in the rate-meter. The vagina was always searched for radioactivity before investigating the cervix and uterus. This ensured that any radioactivity detected in the vagina was not due to gold-198 spheres which might be dislodged from the uterus during manipulation of the needle guide through the cervix.

In withdrawing the probe unit, the needle is retracted into the needle guide by pulling on the rubber grip, two marks on the aluminium tube indicating full extension and full retraction of the needle. The probe sheath is then drawn slowly back to the exterior. By repeating the procedure described, the counter may be used to search the walls of the uterus, cervix and vagina and the external organs of reproduction. If a sharp-pointed needle guide is attached to the probe sheath in place of the blunt-ended one, then such a point can be used to pierce the wall of the vagina, and search for activity in the Fallopian tubes or ovaries or in the abdominal cavity.

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A Possible Explanation for the Failure of Non-Surgical Ovum Transfers in the Cow

SURGICAL transfer of fertilized ova by puncture of the uterine wall has been successful in several mammalian species including the cow[1], but the non-surgical approach through the cervix has so far been successful only in the mouse[2]. In the rabbit, transfer of the ovum through the cervix has also been successful, but it involved a surgical incision in the vagina[3]. In the cow many attempts have been made to obtain pregnancies by the non-surgical transfer of ova through the cervix, but all have been unsuccessful[4]. Work on cows, however, has shown that stimulation of the cervix causes the release of oxytocin, thus increasing uterine contractions, and that an injection of oxytocin induces uterine contractions at all stages of the œstrous cycle[5].

The experiments reported here were designed to throw some light on the problems involved in the non-surgical transfer of ova to the cow. It was felt that such a transfer, which is carried out in the early luteal phase of the cycle owing to the necessity of synchronizing the development of the ovum with that of the uterine endometrium, might stimulate the cervix, release oxytocin, and produce uterine contractions, as a result of which the ova would be either expelled into the vagina or driven towards the utero-tubal junction. The technique employed depended on the use of 'artificial ova', consisting of resin spheres impregnated with radioactive gold[6]. Between 10 and 30 such spheres were transferred, in 0·75–1·0 c.c. of sterile homologous blood serum, non-surgically through the cervix of recipient heifers about three days after œstrus, using a stainless steel catheter of external diameter 1·5 mm. and length 40 cm. connected to a 5-c.c. syringe by a 15-cm. length of polythene tubing (external diameter 2·25 mm.). It was hoped that by using such a fine catheter for the transfer, stimulation of the cervix would be reduced to a minimum. At varying times after the insertion of the spheres the uterus, cervix and vagina were checked for radioactivity with a needle-probe Geiger counter, which is described in the previous communication.

It was found (Table 1) that, 1¼–1½ hr. after transfer, the spheres started to move through the cervix, and that after 1½ hr. a large proportion of the spheres had been expelled from the uterus altogether. In exp. 7 (Table 1) 11 out of 25 spheres were recovered from the vagina 4¼ hr. after insertion. In two animals examined (exp. 8 and 9), 20½ hr. after insertion, some spheres had already been voided from the vagina and consequently little or no radioactivity could be demonstrated in the vagina, whereas some other spheres were still retained by the uterus. In another animal (exp. 10) the spheres were transferred surgically, and these, too, were found in the vagina 4¾ hr. later, probably as a result of the traction applied to the uterus during the laparotomy, which may have been quite sufficient to cause the release of oxytocin.

In another group of four experiments (Table 2), cow ova were transferred non-surgically to recipient heifers, and at varying intervals after the transfer the animals were slaughtered, and the reproductive tracts searched for the ova. Of the 37 ova transferred,

Table 1. FATE OF RADIOACTIVE SPHERES TRANSFERRED NON-SURGICALLY TO THE UTERUS OF HEIFERS 3–3¼ DAYS AFTER ŒSTRUS

Exp. No.	Time from insertion of spheres to checking for activity (hr.)	Portions of the reproductive tract checked				
		Vagina		Cervix	Uterus	
		Posterior	Anterior		Near cervix	Cornua†
1	¼	–	–	N.C.	N.C.	N.C.
2	1¼	–	–	+	N.C.	N.C.
3	1⅓	–	+ + +	–	–	–
4	1¾	–	+ + +	–	+ + +	–
5	1½	–	+ + +	–	+ + +	+ +
6	3¼	+ + +	+ +	–	–	–
7*	4¼	+ +	+	–	+ + +	–
8	20½	–	–	–	+ +	–
9	20½	–	+	–	+ + +	–
Surgical transfers						
10	4¾	+ + +	–	–	–	–

* Eleven out of twenty-five radioactive spheres transferred were recovered from the vagina.

† Site of deposition was about half-way up the uterine horn.

Scale of radioactivity: N.C., not checked; + + +, very active; + +, active; +, slightly active; –, not active.

Table 2. Fate of Cow Ova Transferred Non-surgically into the Uterus of Recipient Heifers

Exp. No.	Days from œstrus to transfer	Ova transferred (No.)	Time from insertion to autopsy (hr.)	Ova recovered in:			Recipient's own ovum recovered from the Fallopian tube
				vagina	cervix	uterus	
11	3	7	1¼	1	0	2	+
12	3	8	2	1	0	0	+
13	3	11	3	4	0	3	+
14	9	11	2½	0	8	0	−
	Total	37	Total	6	8	5 = 19 (51·4 per cent)	

only 19 (51·4 per cent) were recovered. Some of the ova were expelled into the vagina just as rapidly as the radioactive spheres, but it is possible that at the peak of the luteal phase (exp. 14) expulsion is slower than during its early stage (exp. 1–13); during œstrus, expulsion occurs even more rapidly. Altogether, our results show that the radioactive 'artificial ova' do in fact behave in a similar manner to normal ova.

Preliminary attempts to block the ejection mechanism by the injection of adrenaline have been so far unsuccessful. This does not necessarily mean that the factor causing the ejection is not oxytocin, since the action of adrenaline is known to be a transient one, only blocking uterine activity for 4–8 min.[5d]. Spinal anæsthetics sufficient to paralyse the whole hind quarters of the cows were also tried, but again the spheres were ejected as in the untreated cows.

From the observations reported in this communication it is concluded that one of the reasons for the failure of non-surgical ovum transfers in the cow is the ejection of the ova about 1½ hr. after insertion. This may be due to stimulation of the cervix at the time of the transfer, which causes the release of oxytocin from the pituitary gland, and consequently uterine contractions.

We are grateful to Dr. T. R. R. Mann for discussing this manuscript. We acknowledge generous financial assistance from the Milk Marketing Board, and one of us (M. J. K. H.) acknowledges the receipt of an Agricultural Research Council Postgraduate Studentship.

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COPRECIPITATION OF MITOCHONDRIA AND CHLOROPLASTS

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To obtain critical evidence for the presence of an enzyme or multi-enzyme system in a particular cell organelle, it is necessary to prepare this organelle free from all other types of sub-cellular particle. In the case of isolated chloroplasts, differential centrifugation is the method generally employed in attempts to free chloroplasts from smaller particles. Since 1956, many workers have followed the procedure of Arnon *et al.*[1] in which the chloroplasts are sedimented from the leaf homogenates at 1,000*g* after removal of debris and whole cells. Resuspension of the chloroplast pellet, followed by centrifuging at 1,000*g*, has been commonly employed with the view of removing residual amounts of smaller particles[1–3].

However, the absence of biochemically active mitochondria from these preparations has usually been assumed rather than demonstrated. With the advent of a technique for separating chloroplasts from contaminating mitochondria by centrifugation in a density gradient[4], it has been shown that *Vicia faba* chloroplasts, prepared by the usual method[1] and twice washed, are still accompanied by numerous mitochondria. The aim of the present work was to determine how far the presence of mitochondria in chloroplast preparations can account for one of the features attributed to the plastids, namely, the presence of glutamic–oxaloacetic transaminase.

Aspartate is one of the compounds labelled when isolated spinach chloroplasts fix $^{14}CO_2$ in the light[5,6], while the addition of phosphoenolpyruvate and $^{14}CO_2$ to a water extract of spinach chloroplasts leads to the formation of labelled oxaloacetate and labelled aspartate[7]. Although mitochondria from leaves have not been extensively studied in this respect, glutamic–oxaloacetic transaminase has been shown to be present in mitochondria from a number of higher plant sources[8–11]. In view of these findings, the assumption that the formation of aspartate is an aspect of chloroplast metabolism[7] has been further investigated.

Chloroplasts and mitochondria were isolated from leaves just fully expanded of *Vicia faba* plants 2–3

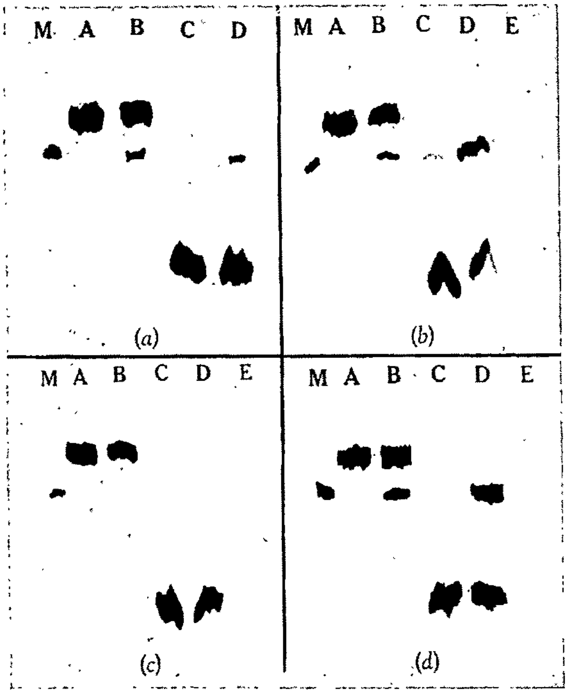

Fig. 1. Assay of transaminases by paper chromatography. Pellets of unpurified chloroplasts (*a*), mitochondria (*b*), purified chloroplasts (*c*), and a mixture of mitochondria and purified chloroplasts spun at 1,000*g* for 12 min. (*d*), were washed once in sucrose–phosphate medium and taken up in 6 ml. distilled water. Samples (2 ml.) were incubated with α-ketoglutarate (75 μmoles) and either DL-aspartate (150 μmoles) or L-alanine (50 μmoles) in a total volume of 3 ml. phosphate buffer (0·067 *M*, *p*H 7·3) for 4 hr. at 30°. The ethanol-soluble material was chromatogrammed in phenol. *M*, marker glutamate; *A*, aspartate + α-ketoglutarate, zero time; *B*, aspartate + α-ketoglutarate, 4 hr.; *C*, alanine + α-ketoglutarate, zero time; *D*, alanine + α-ketoglutarate, 4 hr.; *E*, α-ketoglutarate, 4 hr.

weeks old. The procedure of James and Das was employed[4], except that the leaves were ground by hand in sucrose (0·3 *M*) containing phosphate buffer (0·067 *M*, *p*H 7·3). Mitochondrial and crude chloroplast preparations were obtained from the same homogenate. The crude chloroplast preparations were then purified by centrifugation in a sucrose–glycerol density gradient. Purified chloroplasts were disintegrated at 2° C. by ultrasonic vibration and the green fragments ('grana') separated from the straw-coloured supernatant solution ('stroma') by centrifuging at 105,000*g* for 30 min. Water extracts were prepared by adding small volumes (5–6 ml.) of ice-cold distilled water or dilute phosphate buffer to the mitochondrial and chloroplast pellets; after 30 min. the particulate material was centrifuged down and the clear solution decanted. Glutamic–oxaloacetic transaminase and glutamic–pyruvic transaminase were detected qualitatively by the chromatographic separation of amino-acids present in reaction mixtures; glutamic–oxaloacetic transaminase was assayed quantitatively in water extracts by the spectrophotometric measurement of oxaloacetate at 280 mμ[12].

Crude chloroplast preparations were found by chromatography to contain the two transaminases (Fig. 1*a*); the glutamic–oxaloacetic transaminase was also detected in water extracts of crude chloroplast preparations, confirming previous results[7]. However, mitochondrial preparations obtained from the same homogenates as were used for the isolation of chloroplasts also contained both transaminases (Fig. 1*b*). The purified chloroplasts were next examined; on four occasions, no transaminase activity could be demonstrated by the chromatographic method in either intact chloroplasts (Fig. 1c) or the 'grana' or 'stroma' fractions prepared from these chloroplasts. In addition, glutamic–oxaloacetic transaminase could not be detected by the spectrophotometric method either in the 'stroma' or in water extracts of whole chloroplasts. Chloroplast suspensions containing as much as 2·5 mgm. chlorophyll or the 'stroma' obtained from chloroplasts equivalent to 4 mgm. chlorophyll were used in the reaction mixtures (final vol., 3 ml.). The addition of pyridoxal 5-phosphate ($0{\cdot}75 \times 10^{-4}$ *M*) had no effect.

From these results it seemed possible that the transaminase activity of the crude chloroplast preparations could be attributed to the presence of mitochondria. An experiment was therefore designed to test the possibility that mitochondria are co-precipitated with chloroplasts. Mitochondria and purified chloroplasts were prepared from bean leaves (fresh weight 325 gm.) and suspended in sucrose–phosphate medium. Increasing volumes of the mitochondrial suspension were added to a constant volume of the chloroplast suspension, and the mixtures centrifuged at 1,000*g* for 12 min. The pellets were washed once in 10 ml. of medium and resuspended in 5 ml. phosphate buffer (0·005 *M*, *p*H 7·3). After 30 min. at 2° C., the suspensions were centrifuged at 17,000*g* for 45 min., and the clear supernatant solutions assayed for glutamic–oxaloacetic transaminase by the spectrophotometric method. The results (Table 1) show that a linear relationship exists between the amount of transaminase and the volume of mitochondrial suspension added to the purified chloroplasts. The contamination of the chloroplast pellets by mitochondria as shown by the presence of the transaminases was confirmed by the chromatographic method (Fig. 1*d*). Mitochondria were observed microscopically in washed pellets from tubes *B*–*G*, but were absent from tube *A* (Table 1).

In view of the assumption that washing removes the mitochondria, the effect of washing a mixture of purified chloroplasts and mitochondria was studied. Fig. 2 shows that the first wash removes a considerable fraction of the transaminase activity, but that additional washing has little further effect.

These experiments clearly demonstrate that mitochondria sediment with chloroplasts when suspensions containing both types of particle are spun at 'chloroplast' speed (1,000*g*), and that these mitochondria are not completely removed by two washings. The possibility must therefore be considered that mitochondrial contamination is responsible for some of the properties of chloroplasts isolated by procedures involving only differential centrifugation. It is

Table 1. COPRECIPITATION OF MITOCHONDRIA AND CHLOROPLASTS

Contents of centrifuge tubes	Units of Transaminase
A. 5 ml. Ch. + 5 ml. medium	0·0
B. 5 ml. Ch. + 0·5 ml. M. + 4·5 ml. medium	0·53
C. 5 ml. Ch. + 1·0 ml. M. + 4 ml. medium	1·87
D. 5 ml. Ch. + 2·0 ml. M. + 3 ml. medium	3·25
E. 5 ml. Ch. + 3·0 ml. M. + 2 ml. medium	4·44
F. 5 ml. Ch. + 5·0 ml. M.	8·7
G. 1 ml. M. + 9·0 ml. medium	3·06

M., mitochondrial suspension; Ch., purified chloroplast suspension. Medium: sucrose (0·3 *M*) containing phosphate buffer (0·067 *M*, *p*H 7·3).

Tubes *A*–*F* were centrifuged at 1,000*g* for 12 min.; tube *G* was centrifuged at 17,000*g* for 20 min. The pellets were washed once with sucrose–phosphate medium before water extracts were made. The extracts were assayed at 20° for glutamic–oxaloacetic transaminase (ref. 12). Units as defined by Ellis and Davies (ref. 12).

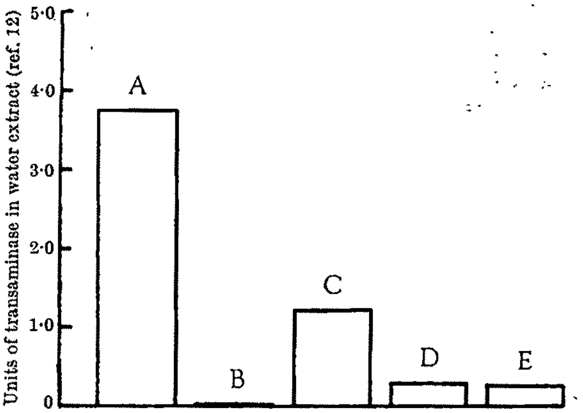

Fig. 2. Effect of washing on mitochondrial contamination. *A*, 5 ml. M. + 5 ml. medium, centrifuged at 17,000*g* for 20 min.; *B*, 5 ml. Ch. + 5 ml. medium, centrifuged at 1,000*g* for 12 min.; *C*, 5 ml. Ch. + 5 ml. M., centrifuged at 1,000*g* for 12 min.; *D*, as *C*, but washed once in 10 ml. medium; *E*, as *C*, but washed twice in 10 ml. medium.
Water extracts were made from all the final pellets, and assayed for glutamic-oxaloacetic transaminase (ref. 12).
M., mitochondrial suspension; Ch., purified chloroplast suspension; both in sucrose–phosphate medium

suggested that the amount of glutamic–oxaloacetic transaminase could be used as an index of the degree of contamination of chloroplast preparations by mitochondria. As we have found that the purified chloroplasts, as well as the mitochondria, contain highly active malic dehydrogenase[13], the sensitivity of the transaminase assay could be increased considerably by linking the production of oxaloacetate with the oxidation of reduced diphosphopyridine nucleotide[14].

The possibility cannot be ruled out that purification by the glycerol technique completely removes transaminases from the chloroplasts. Zelitch and Barber have found that high concentrations of glycerol reduce, but do not eliminate, the oxidative activity of spinach mitochondria[15]. The glutamic–oxaloacetic transaminase activity of water extracts of bean leaf mitochondria is also reduced (by about 50 per cent) by pretreatment of the mitochondria with 60 per cent glycerol[13]. In neither case does the glycerol treatment result in a complete disappearance of enzymic activity. The chloroplasts purified by the glycerol technique are not devoid of enzymes; they are able to carry out the complete process of photophosphorylation, and contain triphosphopyridine nucleotide-triose phosphate dehydrogenase and malic dehydrogenase[16]. In addition, chloroplasts freed from mitochondria by an alternative technique (centrifuging in a sucrose–diodone density gradient[4]) do not contain any glutamic–oxaloacetic transaminase but still exhibit triphosphopyridine nucleotide-triose phosphate dehydrogenase and malic dehydrogenase activity. The available evidence, therefore, suggests that the absence of transaminases from the purified chloroplasts is not a result of the purification procedure. In any event, the great advantage of working with chloroplasts free from mitochondria must be weighed against any possible disadvantage associated with the purification procedure.

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INDUCTION OF HERITABLE RESPIRATORY DEFICIENCY IN *SACCHAROMYCES* BY PANTOTHENATE STARVATION

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CYTOPLASMICALLY inherited respiratory deficiency in *Saccharomyces* is characterized by the permanent loss or depression of the activities of a number of enzymes structurally associated with the mitochondrion[1]. Though a variety of chemical and physical treatments have been described which induce this condition[2–5], the particular metabolic disturbances which comprise the inducing event are not yet defined. Bearing on this question, we have reported that anaerobically grown yeast cells are more responsive to inducers of respiratory deficiency than cells grown aerobically[6]. Since the terminal electron transport system is inoperative in anaerobically grown cells, it is possible that at least one of the metabolic processes which sustain inducing damage is partially debilitated simply by a limitation of intracellular hydrogen acceptors. The severe reduction of overall lipid synthesis which occurs characteristically when yeasts are grown anaerobically[7] suggested to us that the oxidation of pyruvate to acetate might represent such a process. If this were so, then limiting the ability of cells to generate acetate during aerobic growth on glucose should increase their susceptibilities to induction of respiratory deficiency. To test this possibility we have attempted to prepare acetate-deficient yeasts by both aerobic and anaerobic propagations on a pantothenate-free medium. Growth under these conditions is known to depress cellular coenzyme *A*-levels and result in a consequent depression of both acetate formation and utilization[8]. The present article describes our observations that cells are transformed directly into respiration-deficient mutants when grown aerobically in the absence of pantothenate and that such transformation can be prevented by the provision of exogenous acetate or certain higher fatty acids.

The test organism was a tetraploid strain of *Saccharomyces* possessing absolute and partial growth requirements for adenine and pantothenate respectively. Respiration-deficient mutants of spontaneous origin occur within this strain in a frequency of about 0·3 per cent. The basal pantothenate-free growth medium used was of the following composition : glucose, 40 gm. ; ammonium tartrate, 2 gm. ; potassium dihydrogen phosphate, 1·5 gm.; dipotassium hydrogen phosphate, 0·3 gm. ; crystalline magnesium sulphate, 0·5 gm. ; calcium chloride, 0·33 gm. ; crystalline ferrous sulphate, 3 mgm. ; crystalline zinc sulphate, 3 mgm. ; crystalline copper sulphate, 0·5 mgm. ; crystalline manganous sulphate, 0·4 mgm.; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0·15 mgm.; $Na_2B_4O_7.10H_2O$, 0·9 mgm.; inositol, 10 mgm.; thiamine hydrochloride, 1 mgm.; *para*aminobenzoic acid, 0·6 mgm.; pyridoxine, 0·6 mgm.; nicotinic acid, 0·6 mgm.; biotin, 0·02 mgm.; adenine sulphate, 150 mgm.; water, 1,000 ml. To prepare pantothenate-supplemented medium, 1 mgm. of calcium pantothenate was added per litre. This as well as other additions to the medium as indicated in the text were made prior to autoclaving at 10 lb. pressure for 10 min. For aerobic growth, the medium was dispensed in 50-ml. quantities into 250-ml. Erlenmeyer flasks; following inoculation the flasks were incubated at 28° C. on a reciprocal shaker operating at 100 2-in. strokes per minute. For anaerobic growth, screw-cap test-tubes 20 cm. × 2 cm. were filled to a depth of about 17 cm. with 50 ml. of medium, inoculated and incubated statically at 28° C. Though these static growth conditions are properly described as micro-aerobic, they are effectively anaerobic since cells so cultivated fail to achieve the enzymatic adaptation to oxygen utilization which occurs under aerobiosis[9]. Cells for experimental inocula were harvested from the log phase of pantothenate-supplemented aerobic cultures, washed four times in *M*/15 potassium dihydrogen phosphate and introduced to a density of about $3{\cdot}0 \times 10^4$ cells per ml. into the experimental flasks or tubes. At intervals during subsequent incubation 1-ml. samples of the cultures were plated to determine increases in cell numbers and the frequencies of respiratory mutants appearing in the populations. The composition of the plating medium and the technique for scoring respiration-deficient mutants have been described previously[10].

Curves presented in Fig. 1 illustrate the increases in number of cells and the frequencies of respiratory variants scored in cultures prepared as indicated above. It is seen that cells express a partial growth requirement for pantothenate under both aerobic and anaerobic conditions. It may also be noted that in pantothenate-free medium both aerobically and anaerobically cultivated cells show initially rapid, though declining, rates of increase which are stabilized into logarithmic rates of growth approximately 12 hr. after inoculation. Undoubtedly, this anomalous growth behaviour is due to a carry-over of stored pantothenate in the cells of the inoculum. Through the early and middle portions of the logarithmic periods of growth, the frequencies of respiratory variants are maintained at the spontaneous levels in all cultures. However, during the period of transition from the logarithmic into the stationary phase of growth, cells cultivated aerobically in the absence of pantothenate are converted *en masse* into respiration-deficient mutants; no such transformation occurs in either the pantothenate-supplemented culture or in anaerobic cultures with or without pantothenate.

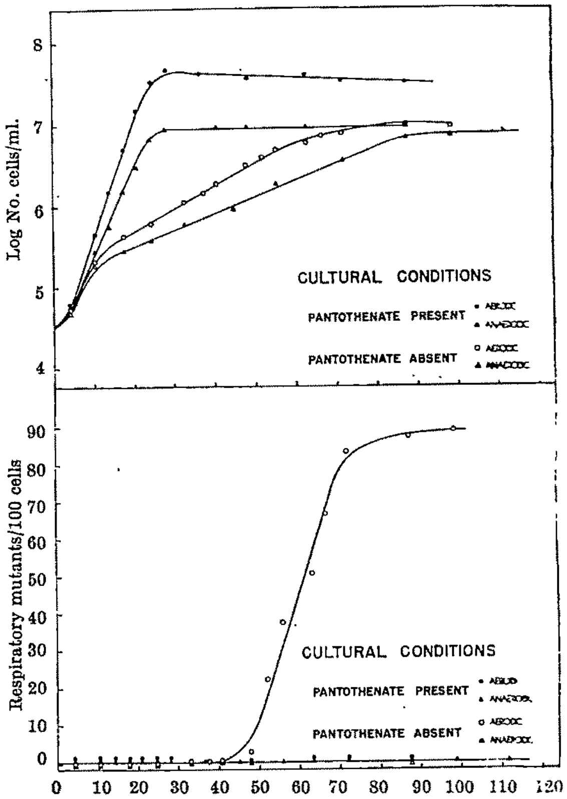

Fig. 1. Growth and mutation curves of cells cultivated aerobically or anaerobically in the presence or absence of pantothenate

To determine what bearing lipid metabolism might have on this mutation, acetate as well as representative sterols and higher saturated and unsaturated fatty acids were tested individually for possible influences on the compositions of early stationary-phase populations attained in pantothenate-free aerobic cultures. The results summarized in Table 1 show that, with the exception of cholesterol, each of the lipids examined increases the total cell yield over that obtained in the absence of lipid. This is consistent with a previous report that pantothenate starvation causes multiple lipid deficiencies in *Saccharomyces*[11]. On the other hand, only acetate and the mono-unsaturated oleic acid appear highly effective in preventing the occurrence of respiratory

Table 1. EFFECTS OF ACETATE, HIGHER FATTY ACID AND STEROL SUPPLEMENTS ON THE TOTAL NUMBER OF CELLS AND THE FREQUENCIES OF RESPIRATION-DEFICIENT MUTANTS PRODUCED IN PANTOTHENATE-FREE CULTURES AFTER 72 HR. INCUBATION. CULTURES WERE INOCULATED TO AN INITIAL DENSITY OF $2{\cdot}9 \times 10^4$ CELLS/ML.

	Cell No./ml.	Mutants/100 cells
Supplement*		
None	$7{\cdot}6 \times 10^6$	86·6
Potassium acetate	$2{\cdot}4 \times 10^7$	1·7
Unsaturated fatty acids		
Linoleic acid	$1{\cdot}1 \times 10^7$	81·3
Oleic acid	$2{\cdot}1 \times 10^7$	1·2
Saturated fatty acids		
Palmitic acid	$1{\cdot}7 \times 10^7$	37·8
Stearic acid	$1{\cdot}9 \times 10^7$	12·9
Sterols		
Cholesterol	$6{\cdot}9 \times 10^6$	88·1
Ergosterol	$2{\cdot}5 \times 10^7$	93·0

* Potassium acetate was added directly to the medium to a concentration of 4·5 mgm./ml.; other fatty acids and the sterols were added to the medium from ethanolic solutions to concentrations of 50 μgm./ml. The ethanol content of each experimental flask, including the lipid-free control, was adjusted to a concentration of 0·2 per cent (v/v) prior to autoclaving.

mutation; the fully saturated stearic and palmitic acids are considerably less effective in this regard while the di-unsaturated linoleic acid and the sterols, ergosterol and cholesterol, are without effect.

Evidently yeast cells require a supply of certain fatty acids in order to ensure the genetic stability of their aerobic respiratory apparatus. Their need for oleate is particularly critical. Under conditions of pantothenate deficiency, cells are incapable of providing these materials for themselves when approaching the stationary phase of growth in aerobic culture. The fact that exogenous acetate is as effective as oleic acid in satisfying this requirement establishes that the pantothenate-starved cells possess sufficient quantities of coenzyme *A* to permit an adequate rate of fatty acid production from acetate and indicates that their lipid need results from their limited ability to generate acetate during growth on glucose. It is known that yeast cells grown aerobically incorporate acetate into lipids at equivalent rates during the logarithmic and stationary phases of growth; their ability to oxidize acetate, however, does not develop appreciably until the termination of the logarithmic phase[12]. Since the oxidation of acetate by yeast is an aerobic process, it can be concluded that pantothenate-starved cells conduct no significant acetate oxidation either during logarithmic aerobic growth or at any state of anaerobic growth. The fact that they also do not mutate under these conditions indicates that their production of acetate is at least sufficient for the biosynthesis of the fatty acids which stabilize respiration. Accordingly, the extensive mutation which pantothenate-starved cells undergo at the inception of the stationary phase of aerobic growth can be attributed to their oxidative depletion of that limited supply of endogenous acetate which is essential for biosynthetic purposes. Thus acetate metabolism does appear to be of basic importance in the genetic maintenance of cellular respiratory competence.

The respiratory activity of the normal mitochondrion depends uniquely on the structural orientation of its constituent enzymes[13]. Lipids and ribonucleic acid along with the enzymic proteins themselves contribute to the preservation of this organization. While it may be assumed that the biosyntheses of mitochondrial enzymes, like those of other enzymes, are genically directed, the fact that mitochondria do not arise *de novo* shows that their fabrication is contingent on the structural organization of pre-existent mitochondria. Thus treatments which upset normal mitochondrial organization might be expected to give rise to lines of mutant mitochondria exhibiting depressed respiratory activities. The heritable loss of respiratory competence induced in cells by pantothenate starvation can most simply be ascribed, at present, to such a disorganization resulting from a cellular deficiency for particular fatty acids.

This work was aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Wichita, *NR* 103-459, and a grant from the Kansas Division of the American Cancer Society.

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RADIOSENSITIZATION OF *E. COLI* BY PURINE AND PYRIMIDINE ANALOGUES INCORPORATED IN DEOXYRIBONUCLEIC ACID

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THE radiosensitivity of a given species and strain of bacteria is characteristic of that species and strain, but is subject to modification by a variety of physiological and environmental factors[1]. Among the factors which modify radiation sensitivity are oxygen[2], nitric oxide[3], and the sulphydryl-amines, such as cysteine[4], cysteamine[5] and AET[6].

A number of considerations have led investigators to postulate that the lethal effects of radiation might be mediated by damage induced in the molecular structure of the nucleic acids, in particular deoxyribonucleic acid (DNA). The discovery in recent years that certain purine and pyrimidine analogues are incorporated into bacterial DNA[7-12] makes it of interest to study the effect of the resulting alteration of molecular structure of DNA on radiosensitivity.

The work described here was initiated about a year ago[13], and is focused primarily on the X-ray sensitivity of various strains of *E. coli*, though some experiments with ultra-violet have also been performed.

Greer[14] has recently reported a striking increase of ultra-violet sensitivity in *E. coli*, strain $15T^-$, a thymine-deficient mutant, after incorporation of the thymine analogue, 5-bromouracil (BU). Djordjevic and Szybalski[15] have reported a similar response to ultra-violet in mammalian cells cultivated *in vitro* in the presence of 5-bromouracil deoxyriboside (BUDR), as well as the corresponding iodinated compound (IUDR). These authors also reported limited experiments with X-rays, indicating increased lethality in mammalian cells the DNA of which was labelled with these halogenated deoxyribosides.

We have used the wild-type *E. coli* strains *B* (*W*3292, radiosensitive) and *B*/*r* (radioresistant[16]); a thymine-deficient mutant of strain *B* (*W*4516); and a purine- and thiamin-deficient mutant of strain *K*12 (*W*3687), all obtained through the kindness of Dr.

Esther Lederberg of the Department of Genetics, Stanford University.

All incubations were carried out under aerated conditions at 37° C. The mutant strains were grown from slants in a fortified medium[17] to which thymine (strain *W*4516) or purines (strain *W*3687) were added. Primary cultures of strains *B* and *B*/*r* were grown on mineral medium[18]. Secondary cultures employed mineral medium to which 2 per cent sulphanilamide was added to block *de novo* purine and pyrimidine synthesis. The sulphanilamide medium required supplementation with several other bases, vitamins and minerals. The purine and pyrimidine analogues under investigation were usually added at a level of 100 μgm./ml. Analogues studied to date include BU, IU, 5-fluorouracil (FU), thioguanine (TG), 2-aminopurine (AP), and 2,6-diaminopurine (DAP).

X-irradiation was carried out at 250 kV., 15 m.amp., 30 cm. distance, 0·25 mm. copper + 1·0 mm. aluminium filter, 1·10 mm. copper half-value layer, dose-rate 360–390 r./min. Cultures were collected by centrifugation, washed, suspended and diluted in phosphate buffer to concentrations of about 1×10^7 organisms/ml., and irradiated in 'Teflon' cups fitted in a 'Lucite' block, as described by Gunter and Kohn[19]. Ultra-violet irradiations were performed with an unfiltered low-pressure mercury lamp with a measured output of 16·6 ergs/sq. mm./sec. at 43 cm. distance. Samples were withdrawn at successive time-intervals from an open Petri dish buffer suspension. After irradiation, samples were appropriately diluted and plated on nutrient agar (fortified with thymine or adenine for the mutant strains) and incubated at 37° C. Colony counts at each radiation dose-level are expressed as a fraction of the viable counts of non-irradiated samples. Radiosensitivity is expressed in terms of the experimental/control slope ratios of dose – log survival curves.

DNA was isolated from aliquots of control and experimental cultures by differential chemical extraction; mixed nucleic acids by lysis with 2 per cent sodium lauryl sulphate. When necessary, RNA was removed by alkaline hydrolysis. BU incorporation into DNA was established by three different methods: cæsium chloride density gradient determination[20]; incorporation of radioactive ^{82}Br–BU (synthesized by Dr. Joseph P. Kriss[21]); and base hydrolysis and chromatography. TG incorporation was determined with the analogue labelled with sulphur-35. Detection of DAP incorporation into DNA required the use of carbon-14-labelled analogue.

The incorporation of ^{82}Br–BU into DNA was linear with time until the end of the phase of exponential growth, by which time there was 35–50 per cent replacement of thymine by BU. Stationary phase cultures showed further incorporation with a plateau at about 70 per cent replacement of thymine by BU. Base hydrolysis and chromatographic analysis of DNA from stationary phase cultures indicated 68 per cent replacement. The flotation density of the isolated DNA was 1·763 (control 1·712), corresponding to 70 per cent replacement of thymine by BU. There was little or no decrease of viability of organisms that incorporated BU through the log phase; but a rapid decrease in viability with further BU incorporation during stationary phase was noted. For this reason most of the radiation experiments were carried out on organisms collected during the log phase.

Strains *W*4516 and *B*/*r* labelled with BU exhibited a striking enhancement of sensitivity to ultra-violet, the curves being essentially identical to those reported by Greer[14]. The thymineless mutant, strain *W*4516, showed a multi-hit type of X-ray dose-survival curve, with an extrapolation number which fluctuated somewhat but was usually in the range 1·5–2·0. When this organism was grown on BU, the shoulder of the X-ray survival curve narrowed appreciably, and in some instances vanished, while the slope of the linear portion of the curve exceeded that of the control curve by a factor of 1·8–2·2 in various experiments (Fig. 1). For the wild-type strains, the control curves of which were of the single-hit type, radiosensitization by BU yielded slope ratios of approximately 1·6–1·8 for strain *B*/*r* and 1·4–1·5 for strain *B* under log-phase conditions. Organisms grown on reciprocal 9 : 1 mixtures of thymine and BU exhibited responses intermediate between the extremes of 100 per cent thymine- or 100 per cent BU-grown cultures. Radiosensitization was a function of incubation time in the presence of BU, being undetectable after 2 hr. (8 per cent thymine replacement), barely detectable at 4 hr. (about 20 per cent replacement), and maximal by 8 hr. (approximately 40 per cent replacement). The possibility that some threshold-level of BU incorporation is required merits further study in the time-interval between 2 and 4 hr. When the thymineless mutant strain *W*4516 was grown through log phase in BU, then diluted ten-fold and re-incubated in thymine long enough to permit one replication of DNA, the resulting organisms, containing DNA which was presumably labelled in only one strand with BU, exhibited a radiosensitivity intermediate between that of controls and fully BU-labelled cells. A somewhat greater loss of radiosensitivity has been reported in BUDR-labelled mammalian cells after one replication in the presence of thymidine[15].

In one experiment, IU-grown strain *W*4516 cells exhibited conversion to a single-hit type of curve, but the slope was essentially identical to that of the controls. In the same experiment, cells grown on BUDR showed slightly greater radiosensitization than BU-grown cells, with slope ratios of 1·80 versus 1·35 relative to thymine-grown controls, respectively. The mere presence of the analogues in the culture medium, under conditions in which incorporation into DNA could not proceed (for example, in the absence of sulphanilamide) yielded radiation dose-survival curves identical with those of controls. In

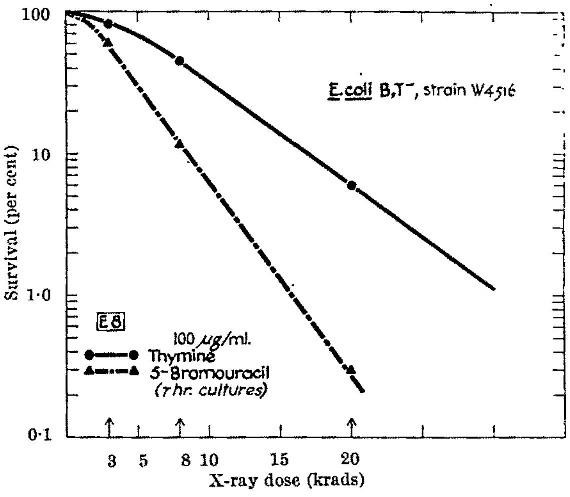

Fig. 1

limited trials to date, pre-incubation of strain *B/r* with 5-FU has revealed no radiosensitization. This analogue, unlike IU and BU, is incorporated into RNA, but not into DNA (ref. 22).

Thioguanine is incorporated into log-phase strain *B/r* to the extent of about 0·6 per cent, calculated on the basis of replacement of DNA guanine. Although the extent of incorporation is considerably less than that of BU at corresponding time-intervals, the degree of radiosensitization to both ultra-violet and X-rays was approximately the same (Fig. 2). Consistent with this observation is the finding (to be reported in detail in a separate paper) that aqueous solutions of TG were appreciably more sensitive than any of the natural bases to spectral degradation by ultra-violet, whereas BU was only slightly more sensitive than thymine. The spectral degradation of TG by X-rays was markedly more sensitive than that of guanine and essentially identical with the responses of BU and thymine. Since purines and pyrimidines are presumably incorporated into DNA more or less independently, the effect of combined BU and TG was studied in sulphanilamide-grown strain *B/r*. In the presence of TG, BU incorporation was markedly reduced, reaching a level of only 12 per cent thymine replacement, as compared with 70 per cent in the absence of TG. None the less, organisms which had incorporated both analogues into their DNA exhibited a further increase in X-ray sensitivity (slope ratio 2·95 versus 2·2 for TG or BU alone, Fig. 3).

Although most of the radioactivity of DAP labelled with carbon-14 incorporated into DNA of purine-deficient strain *W*3687 cells was in guanine and adenine, the incorporation of unchanged DAP to the extent of about 1 per cent was demonstrable by careful chromatographic separation from guanine in the presence of carrier DAP. Replicate experiments with this strain indicated a slight increase in radiosensitivity, with slope ratios in the range 1·15–1·25. There was a departure from linearity at higher dose-levels, indicating the presence of a small proportion of cells which had failed to incorporate the analogue. In one experiment with AP in strain *W*3687, neither radiosensitization nor incorporation into DNA was demonstrable.

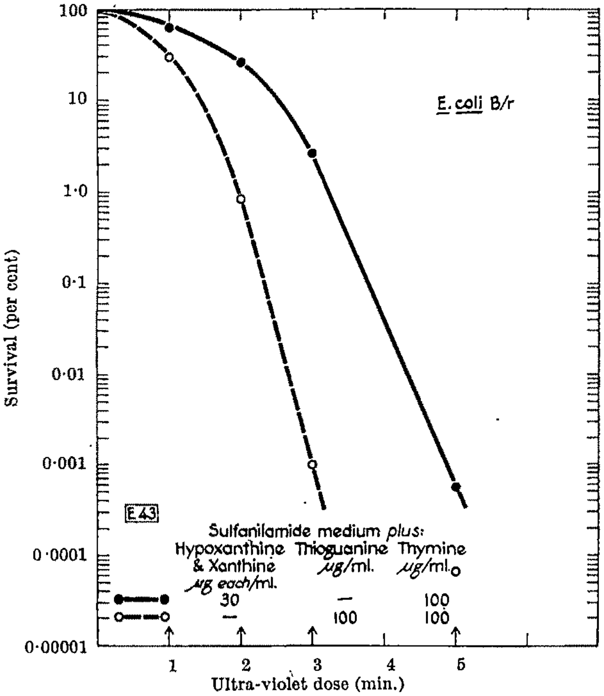

Fig. 2

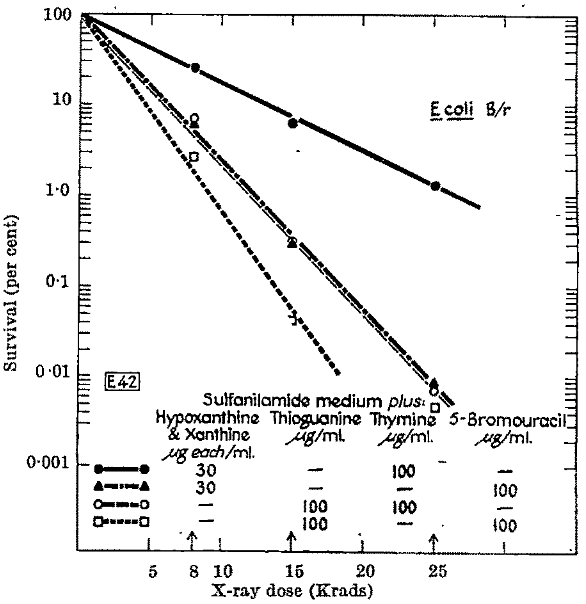

Fig. 3

In summary, it appears that both ultra-violet and X-ray sensitivity of *E. coli* are enhanced when appreciable proportions of the natural bases of DNA are substituted by any of several purine and pyrimidine analogues. Analogues which fail to enter bacterial DNA (FU, AP) have to date failed to elicit this response. Based on relative extent of incorporation, TG is the most active radiosensitizing agent thus far reported, with BUDR, BU, IU, and DAP following in order of decreasing activity.

These investigations were aided by grants *C*-3352 and *C*-2896 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service. Analogues were obtained from the California Corporation for Biochemical Research, Los Angeles, California; ^{35}S-TG from Isotopes Specialties Company, Burbank, California.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Solar Flare Effects in the *F* Region of the Ionosphere

ELEVEN years ago, Dieminger reported that the large solar flare of November 19, 1949, had a marked effect on the *F* region over Lindau, Germany[1]. A similar *F*-region effect was observed at Okinawa and probably at Singapore at the time of the outstanding flare of February 23, 1956[2,3]. In addition to being very large, these flares were notable in that they were two of the five flares that had occurred up to that time during which increases in sea-level cosmic ray had been observed. Yet, because the *F*-region effect had been detected at so few of the sunlit ionospheric sounding stations, some doubt remained as to the correct interpretation of these observations.

The purpose of this communication is to report two additional cases of *F*-region effects which were recently observed accompanying the two large solar flares that occurred at 1323 U.T. on November 12, 1960, and at 0207 U.T. on November 15, 1960. Because these flares were also followed by cosmic ray increases recorded at sea-level (flares number eight and nine in the series now numbering ten, the tenth having been observed on November 20, 1960), the connexion between the relatively few flares that are associated with the emission of these very high energy particles ($\gtrsim 2$ BeV.) and apparent *F*-region effects is considerably strengthened. It is likely that the *F*-region effects are not caused by the cosmic-ray particles themselves but rather that flares associated with the emission of these very high energy particles are also unusual in their photon radiation. However, it should be noted that, in the course of this work, several apparently similar, but considerably smaller, *F*-region effects have been found in association with solar flares that were not observed to be followed by sea-level cosmic ray increases or polar cap absorption events.

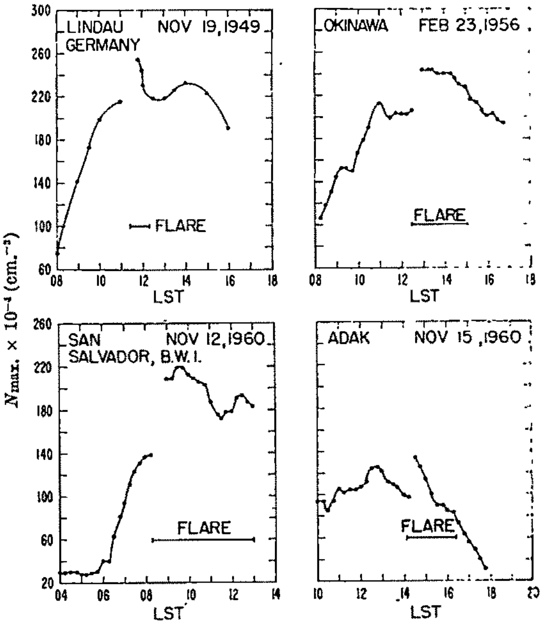

Fig. 1. Variations in *F*-layer maximum electron density during four cosmic ray-associated solar flares

Fig. 1 shows the variation of *F*-region maximum electron density, as determined from observations of the vertical-incidence penetration frequency, around the time of the two November 1960 flares and the two earlier flares discussed above. It can be seen that increases of about 20–50 per cent in maximum electron density occurred during the course of each flare. Examination of the soundings data taken on November 12, 1960, at other sounding stations along the east coast of the United States and in the Caribbean suggests that the *F*-region effect may have extended over the entire area, but at no other station was the effect as definite as at San Salvador, British West Indies, possibly due to the fact that the rapid diurnal increase in electron density was occurring at most of the stations at the time of the flare. Fortunately, however, an independent observation of the *F*-region effect associated with the flare of November 12, employing a different technique, is also available. Since January 1960, an experiment has been going on at the National Bureau of Standards that involves the study of oblique incidence propagation characteristics by means of recordings of frequency changes on high-frequency transmissions[4]. At the time of the November 12 event, recordings were being made in Boulder, Colorado, of the precise frequency of the *WWV*-20 Mc./s. transmission from Washington, 2,370 km. away. The effect shown (Fig. 2), which begins within about a minute of the time of commencement of the flare, can be interpreted as a 22 ± 5 km. lowering of the reflexion height at the midpoint of the path. (Note that no other effect, comparable in magnitude, has been observed in the 14 months since recording began.) While it was not possible to obtain meaningful electron density profiles from the November 12 San Salvador ionograms, due to large *D*-region absorption effects, it was possible to deduce profiles from the soundings taken at Adak during the November 15 event. Examination of the Adak profile data, shown in Fig. 3, reveals that the height of the plasma frequency-level of 6 Mc./s., which approximates to the

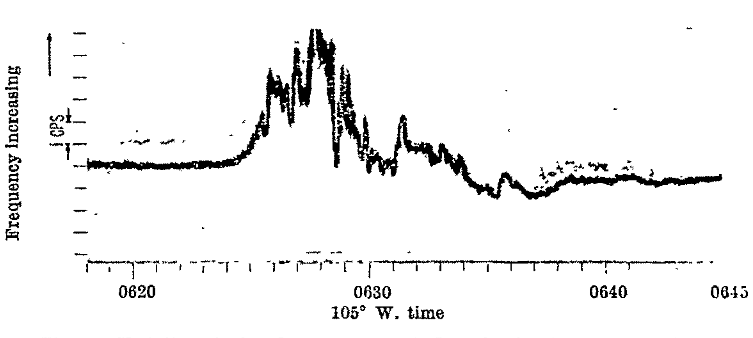

Fig. 2. Variations in the frequency of *WWV*-20 Mc./s. signal as recorded at Boulder, Colorado, November 12, 1960. The flare began at 0623 and the increase in sea-level cosmic ray at about 0640 (Deep River, Ont.)

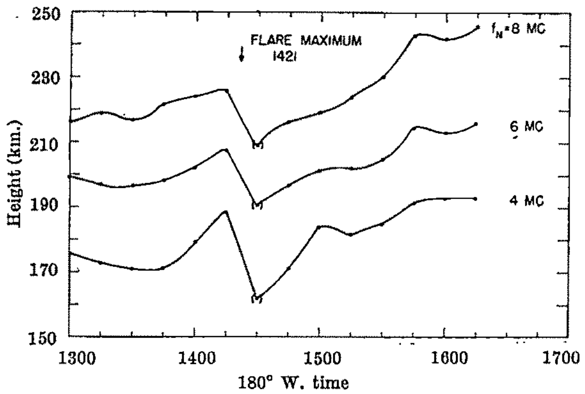

Fig. 3. Height variation of several fixed plasma frequencies during the solar flare of November 15, 1960; Adak, Alaska

equivalent vertical incidence frequency for 20 Mc./s. propagation over a 2,370-km. oblique path, decreased about 18 km. during this flare event, an effect of the same magnitude as that observed on the records of change in frequency during the November 12 event.

As mentioned above, less well-marked *F*-region effects that may be flare-associated were observed at several other locations during the November 1960 events. However, many sunlit hemisphere stations, with good *F*-region observations throughout most of the duration of the flares, show no change in *F*-region maximum electron density. A more complete examination of vertical soundings data obtained at sunlit locations during flares having sea-level cosmic ray increases is now under way.

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PHYSICS

Effects of High Pressure on Glass : a Possible Piezometer for the 100-Kilobar Region

THE measurement of pressures of the order of 10,000–200,000 bars and upward on uniaxial pressure transmitting devices is a formidable problem. At present, inter-laboratory comparison of results obtained at ultra-high pressures is not satisfactory. The availability of a convenient pressure calibration substance that is useful over a wide temperature-range, and the agreement on a satisfactory scale of pressure based on this calibration substance, are imperative for future work.

To date, the only convenient high-pressure calibration substances for extreme pressure devices depend on the temperature-dependent phase transitions which occur in some metals. This is unsatisfactory, since it allows only point-wise calibration over a limited range of pressures and is limited to the room-temperature 'fixed' points.

Bridgman and Simon[1] have reported that silica glass could, above a threshold pressure of about 100,000 atmospheres, be made permanently more dense, and at pressures of about 200,000 atmospheres this increase in density amounted to about 6–7 per cent. In an attempt to check pressure apparatus in this laboratory against Bridgman's data, some very surprising findings were encountered.

The apparatus used has been described by Dachille and Roy[2] and is similar to that employed by Bridgman and Simon[1]. Automatically regulated pressure was applied to two opposed anvils of the Bridgman type by means of a hydraulic press. The pressure was calculated on the basis of the total force developed by the ram of the press and the area of the anvil face after removal of pressure. The sample was heated and its temperature automatically regulated by means of a split external furnace. A basic difference in procedure used by us was the use of powdered glass instead of a continuous disk.

The effect of pressure on the density and refractive index of silica glass was found to be much greater than that reported by Bridgman and Simon[1]. Further, the change in density was found to be a continuous function of pressure above 20,000 atmospheres at room temperature. No threshold effect was observed at 600° C. The density of silica glass, which has a normal refractive index of 1·458, could be increased by a pressure of 150,000 atmospheres at room temperature to a form with a refractive index as high as α-quartz, namely, 1·54 (see Fig. 1). The changes achieved by Bridgman and Simon[1] at a reported pressure of 200,000 atmospheres could be achieved at pressures as low as 55,000 atmospheres at room temperature.

The effect of temperature is also very important. Silica glass which has been subjected to 40,000 atmospheres at 25° C. has an index of refraction, after returning to ambient conditions, of 1·48, whereas the index of the glass subjected to 40,000 atmospheres pressure at 500° C. has an index of refraction of 1·50 under ambient conditions.

For each pressure and temperature there is a pseudo-equilibrium volume for the silica glass. This volume is attained in less than 15 sec. and does not alter after several days at pressures and at temperatures (less than 600° C.) less than those initially applied.

The compressed silica glass is quite stable under ambient conditions. Samples of the compacted silica glass have been kept undesiccated for 1½ years without any noticeable change in index of refraction. Further, the glass can be kept for a week at 600° C. at atmospheric pressure without undergoing any noticeable relaxation. Glasses compacted by the uniaxial device show a spread of about ±0·005 index of refraction unit. This is probably a result, as Bridgman

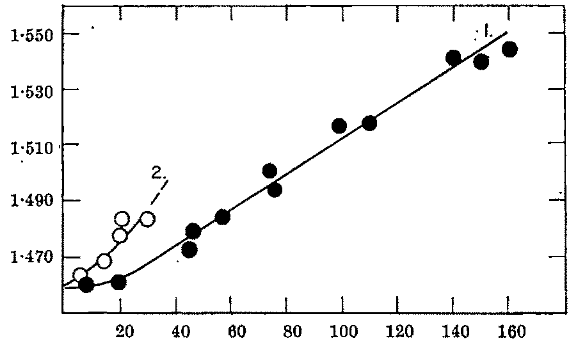

Fig. 1. Index of refraction of the quenched phase of silica glass as a function of the pressure of the run: (1) at 25° C.; (2) at 600° C.

and Simon suggest, of the uniaxial nature of the pressure-transmitting device. Glasses subjected to 10,000 atmospheres argon pressure (hydrostatic pressure) in a bomb appear homogeneous when examined by the standard immersion-oil technique (that is, within ± 0·001 unit), with indices falling on the same curve.

The continuous compaction of glass as a function of pressure is not unique to silica glass. Similar results of somewhat lesser magnitude have been observed in germanium oxide, boron oxide, as well as silver phosphate and 'window' glass. A detailed report on the effects of pressure and temperature on these glasses will be given elsewhere[3].

In view of the observed continuous increase of density of silica glass as a function of both temperature and pressure, it is proposed that the refractive index of silica glass be used as a piezometer. Silica is particularly inert and could be mixed with many samples to provide a check on the actual pressure and/or temperature of a run. It is suggested that it would be quite valuable if several laboratories interested in high-pressure measurements determined independently the effect of pressure and temperature on the refractive index of silica glass. Perhaps a satisfactory pressure-scale could then be agreed upon.

This investigation has been supported by a grant to the University for fundamental studies in defect solids by the Bausch and Lomb Optical Company. We are much indebted to our colleague, Dr. F. Dachille, for help with equipment and ideas.

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Estimation of Dynamic Modulus and Dynamic Yield Stress for 'Perspex'

IT has been found that a steel ball dropped on to a thick flat plate of 'Perspex' produces an electrostatic charge over the contact area. This effect has been investigated with the view of developing a simple method for estimating the dynamic modulus and dynamic yield stress.

The technique for determining modulus was as follows: A cylindrical 'Perspex' rod 2·54 cm. diameter and 30 cm. long with polished flat ends was supported in a horizontal position. One end was placed against a fixed steel plate and a 1-cm. thick 'Perspex' end-piece was wrung on the other end with 'Vaseline'. A clean steel ball of diameter 3·2 cm. was suspended as a simple pendulum, so that on release from a given height, h cm., impact took place on the end-piece. A fine wire connected the ball to an oscilloscope amplifier which had a high resistance across the input. The voltage–time record showed that as the ball approached the polymer surface, it became charged by induction due to the surface electrostatic charge which is readily built up on 'Perspex'. In close proximity to the polymer the ball began to discharge, but on contact, the rate of induction decreased rapidly so that the potential of the ball fell below a minimum discharge potential resulting in a marked decrease in rate of discharge. However, once good contact had been established between ball and polymer, the rate of discharging increased until contact was broken when the process ceased. The duration of contact could thus be obtained from the oscilloscope record. For an impact velocity of 77 cm. sec.$^{-1}$ the measured duration of contact, t, was $37{\cdot}8 \times 10^{-5}$ sec. According to the Hertz[1] theory:

$$t = \frac{2{\cdot}94}{v^{1/5}}\left(\frac{5}{4}\,\frac{m}{n}\right)^{2/5}$$

where m is the mass of the ball, v the ball velocity on impact and n is given by:

$$n = \frac{4\pi\sqrt{R}E_1^2E_2^2}{3[(1-V_1^2)E_2 + (1-V_2^2)E_1]^2}$$

The quantities E_1, E_2, V_1 and V_2 are the Young's moduli and Poisson's ratios of steel and 'Perspex' respectively. The radius of the ball is denoted by R. Putting the above-measured value of t in the equations with values of E_1 and V_1 given by Davies[2] and V_2 given by Davies and Hunter[3], resulted in a dynamic Young's modulus (E_2) of $5{\cdot}3 \times 10^{10}$ dynes cm.$^{-2}$ for 'Perspex'. A different form of ball impact experiment by Tillet[4] using a 0·63-cm. diameter ball at a velocity of 90 cm. sec.$^{-1}$ gave with the Hertz calculation a modulus of $5{\cdot}4 \times 10^{10}$ dynes cm.$^{-2}$. These dynamic values compare favourably with the 'static' value of $1{\cdot}8 \times 10^{10}$ dynes cm.$^{-2}$ computed from Kolsky's[5] 'static' stress–strain curve and the dynamic moduli of $6{\cdot}8 \times 10^{10}$ (ref. 5) and $6{\cdot}0 \times 10^{10}$ dynes cm.$^{-2}$ (ref. 3), obtained at very high rates of stressing by pressure-bar methods.

Attempts have also been made to measure the diameter of the maximum indentation. A specimen was impacted and held over a tray of lycopodium powder and gradually lowered towards it until a quite distinct circular patch of powder had been attracted to the impact area. It was then assumed that the diameter of the powder patch d_p was equal to the diameter of maximum indentation d_e given by Hertz as:

$$d_e^3 = 6PR\left(\frac{1-V_1^2}{E_1} + \frac{1-V_2^2}{E_2}\right)$$

where P is given by:

$$P = n\left(\frac{5mv^2}{4n}\right)^{3/5}$$

There was a range of values of d_p for a given height of impact, but it seemed reasonable to suppose that it was the maximum value of d_p which was required. The maximum values of d_p obtained using an impactor of diameter 3·8 cm. at impact velocities in the range 58–310 cm. sec.$^{-1}$ gave calculated moduli in the range 8×10^{10}–$6{\cdot}3 \times 10^{10}$ dynes cm.$^{-2}$. As will be seen from the previous paragraph, these values of modulus are impossibly large; they should not be greater than moduli obtained by pressure-bar methods. Thus it must be concluded that $d_p < d_e$.

The above conclusion and the fact that the stresses vary over the contact area are used to estimate the dynamic yield stress. It is assumed that during impact the ball discharges most efficiently where really good contact is made. Thus powder is primarily attracted where plastic yielding has occurred. Hence maximum values of d_p may correspond to a yield stress of the material. There may be other effects involved, but the above is assumed to predominate.

To calculate the yield stress corresponding to a maximum of the ratio d_p/d_e for a given h, where d_e was calculated using the modulus $5{\cdot}3 \times 10^{10}$ dynes cm.$^{-2}$, the Guest–Mohr hypothesis of elastic failure was taken in conjunction with Huber's[6] equations,

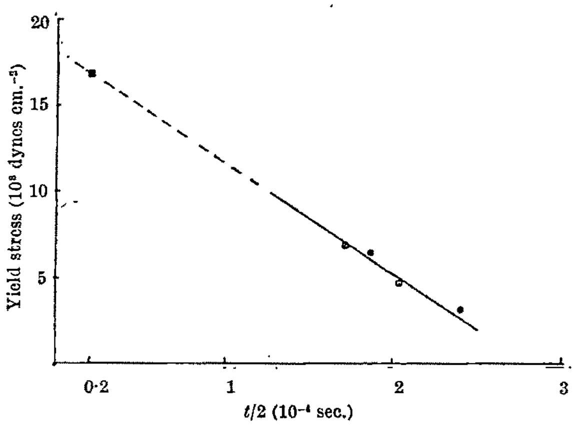

Fig. 1. Estimated yield stresses

which were then solved using a value of 0·35 for Poisson's ratio to give solutions of a graphical form described by Davies[2]. For values of h ranging from 48 cm. to 2 cm. the values of dynamic yield stress obtained were $6\cdot9 \times 10^8$–$3\cdot1 \times 10^8$ dynes cm.$^{-2}$. A straight-line plot was obtained for dynamic yield stress against half the duration of contact, $t/2$, given by the Hertz calculation with the modulus $E_3 = 5\cdot3 \times 10^{10}$ dynes cm.$^{-2}$. A typical set of results is shown in Fig. 1. Extrapolating the line to $t/2 = 0\cdot2 \times 10^{-4}$ sec., which is approximately the time to reach maximum stress in the pressure-bar experiments[3,5], gave a yield stress of $16\cdot9 \times 10^8$ dyne cm.$^{-2}$. Thus, assuming 'Perspex' behaves as a Hookean solid at pressure-bar rates of stressing, a yield stress of about $16\cdot9 \times 10^8$ dynes cm.$^{-2}$ should be observed. The dynamic stress–strain curve of Kolsky[5] does not show this, but the more recent results of Davies and Hunter[3] show a linear stress–strain curve up to a yield stress of approximately $16\cdot6 \times 10^8$ dynes cm.$^{-2}$. Thus the present simple technique may be of value in predicting high stress-rate behaviour.

A more detailed account of the above experiments for 'Perspex' and additional results for a number of other polymers will be published elsewhere.

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Packing of Spheres

In connexion with Bernal's theory of liquids, Scott[1] has determined the packing density of equal spheres by experiments with steel balls. In this laboratory we were interested in the dense random packing of beads of ion-exchange resins. Although particles of ion-exchange resins may vary in diameter from 1·3 mm. to 0·03 mm. or less, if the ratio of the diameters of the larger to the smaller beads in any batch exceeds 3 or 4, segregation may occur when the beads are backwashed or allowed to settle under water. If the ratio exceeded 6·5, it would be possible for the smallest spheres to fit into spaces between close-packed spheres of the largest diameter and so increase the packing density; but, if the problem is limited to the smaller ratios which occur in practice, only holes and not spaces can be filled by the smaller beads and the packing density should be 0·64–0·74.

The beads were sieved and packed under water, and to avoid peripheral errors at least 50,000 beads were taken for each determination. Narrow fractions with few irregular particles had packing densities close to 0·64. The value obtained for the most regular narrow fraction (irregular particles <4 per cent) was 0·63(8), in good agreement with Scott's extrapolated value of 0·63(7) for equal spheres. In this fraction the mean deviation from the mean diameter was 10·5 per cent. Wider fractions had increased packing densities, and a value of 0·66(7) was obtained for a fraction with mean deviation from the mean diameter of 23 per cent. Even higher values were obtained by mixing in various proportions beads of mean diameter 0·09 mm. with beads of mean diameter 0·31 mm. The smaller and larger beads alone each had packing densities of 0·66, but the packing density of the mixture reached a maximum of 0·70(5) when the proportion of the smaller beads was about 30 per cent of the mixture. Most batches of ion-exchange resin contain irregular fragments produced by the shattering of some of the beads, and these fragments probably affect the packing more than do rounded particles that are not truly spherical. The packing density of crushed resin was found to be 0·54 for particles of similar size. When various proportions of irregular particles were mixed with spheres of the same average size, the packing density remained at 0·64 until 10 per cent by intrinsic volume of irregular particles had been added. Thereafter the packing density decreased in proportion to the percentage of irregular particles in excess of 10 per cent.

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GEOLOGY

Potassium–Argon Ages of the Dartmoor and Shap Granites using the Total Volume and Isotopic Dilution Techniques of Argon Measurement

Early in 1960, J. L. Kulp and others reported potassium–argon and rubidium–strontium ages for a number of granites from Great Britain and Eire[1]. The argon measurements were made at the Lamont Geological Observatory by the isotopic dilution technique, and the rubidium–strontium determinations were made at Oxford and Harwell. Some of the rocks examined, including the Shap and Dartmoor granites, had been measured by Mayne *et al.*[2], using the total volume technique for measuring the argon. There was a serious discrepancy between the potassium–argon measurements reported in these two communications, the results obtained by Kulp *et al.* being in agreement with the rubidium–strontium data (Table 2).

In this communication the results of new potassium–argon measurements on the Shap and Dartmoor granites, using both total volume and isotopic

Table 1. NEW POTASSIUM-ARGON AGES

Mineral	Sample No.	K (per cent)	Argon technique	Radiogenic argon (std. c.c./gm. ×10⁴)	Atmospheric argon (per cent)	δV (per cent)	δt	Age (million years)
Dartmoor granite biotite		6·78	Total volume (Cambridge)	0·762	5·4	1·4	2·45	263
				0·766	2·4	0·6	2·47	264
			,,	0·758	2·8	0·5	2·44	262
,, ,,	20503 (I)	7·03	Isotopic dilution (Oxford)	0·835	18·5			276 ± 15
,, ,,	20503 (IV)	6·65		0·756	36			265 ± 12
,, ,,	20503 (II)	6·61	Total volume (Oxford)	0·734	13·5			260
,, ,,	20503 (III)	5·89		0·667	14·0			265
Shap granite biotite	20512	6·47	Total volume (Cambridge)	1·154	2·6	0·3	3·62	401
				1·176	3·6	0·3	3·67	408
			,, ,,	1·152	6·3	0·4	3·61	401
			,, ,,	1·150	3·6	0·1	3·65	400
,, ,,	,,	6·42	Isotopic dilution (Oxford)	1·055	12·2			374 ± 20
				1·147	12·5			404 ± 20
			,, ,,	1·091	12·7			386 ± 13
			,, ,,	1·099	8·8			387 ± 13
			,, ,,	1·083	15·0			381 ± 12
,, ,,	7680	6·28	Total volume (Oxford)	1·072	13·5			405
,, ,,	7682	6·46		1·177	12·5			410

Decay constants: $\lambda_\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$. $\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$. Isotopic abundance of ^{40}K = 0·0119 atom per cent.
δV = percentage standard deviation in volume of radiogenic argon due to uncertainty in isotopic ratio of the specimen.
δt = error due to a 1 per cent error in proportion of K_2O or volume of radiogenic argon.

dilution techniques, are reported. The total volume measurements were carried out at Cambridge on newly developed apparatus, and at Oxford on the apparatus employed previously by Mayne *et al.* The isotopic dilution measurements were made at Oxford.

The total volume apparatus used at Oxford is similar to that described by Thomson and Mayne[3]. The apparatus used for the isotopic dilution measurements is a bakeable glass system which will be described elsewhere. Aliquots of argon-38 were prepared by a method similar to that of Wasserburg and Hayden[4], and calibrated by mixing with known amounts of spectroscopically pure argon. The accuracy of this system of argon measurement was checked from time to time by measuring the argon content of the reference biotite *B*.3203 supplied by Dr. P. M. Hurley of the Massachusetts Institute of Technology. The results of three measurements differed by 0·2–1·5 per cent from the mean of the American measurements. Duplicate determinations on younger materials suggest, however, that the accuracy is ± 3 per cent for the more recent measurements quoted in this communication, and rather more for the earlier ones.

The Cambridge apparatus used in the extraction and purification of argon is built mainly of stainless steel. It is small in size, and is connected directly to the mass spectrometer, which makes it easy to operate and reduces the likelihood of loss of gas by absorption, or the presence of small leaks which would add to the volume of gas finally measured. For successful total volume work both these troubles must be avoided, and also the McLeod gauge on which the volumes are measured must be accurately calibrated. This has been done in three ways:

(1) By the measurement of accurately known volumes of argon of different sizes, made up from spectroscopically pure argon using a gas pipette.

(2) By introducing samples of air from containers of known volume filled at a measured temperature and pressure.

(3) By measuring argon liberated by fusing the reference sample, *B*.3203.

Results from these experiments were consistent to about 1 per cent, which besides giving a reliable calibration of the McLeod gauge, indicated that the loss of argon by absorption was negligible, and that the method of purification of the gas was adequate.

For the work in both Oxford and Cambridge the potassium determinations were made by flame photometric methods. Each determination at Cambridge is the average of six analyses, and at Oxford the average of four. The accuracy of the Cambridge measurements is estimated to be ± 1 per cent, and of the Oxford ones ± 2 per cent.

The results of the new determinations are given in Table 1. Their mean values, weighing all determinations equally, are 265 ± 5 million yr. for the Dartmoor granite and 397 ± 8 million yr. for the Shap. In Table 2 these are compared with the corresponding results from the earlier reports. The new figures are in good agreement with both the potassium–argon and the rubidium–strontium ages of Kulp *et al.* (except that the difference between the new age for the Shap granite and the rubidium–strontium age slightly exceeds the uncertainties given). They confirm that the results of Mayne *et al.* are too high, particularly for the Shap granite. The reason for this is not known.

The differences in the levels of contamination by atmospheric argon found in the two laboratories (Table 1) arise mainly from the use at Oxford of graphite crucibles, which have a large surface area for absorption of gas, while molybdenum crucibles are used at Cambridge.

Table 2. COMPARISON WITH EARLIER RESULTS

Reference	Method	Age of Dartmoor granite (million yr.)	Age of Shap granite (million yr.)
Mayne *et al.* (ref. 2)	K/Ar, total volume	298*	465–499*
Kulp *et al.* (ref. 1)	K/Ar, isotopic dilution	271 ± 5	391 ± 7
Kulp *et al.* (ref. 1)	Rb/Sr	277 ± 5	381 ± 7
This work	K/Ar, both techniques	265 ± 5†	397 ± 8†

* Recalculated values.
† Error is twice standard deviation of mean.

In the figures obtained for the argon content of the Shap sample 20512 there is some evidence for a small systematic difference between the results by the two techniques. However, this is not large enough to be investigated easily at separate laboratories. The Cambridge apparatus is being modified to permit both techniques to be used on the same sample of gas.

It may be concluded from these results that the total volume technique can give good results on materials of Palæozoic age. The chief limit to its accuracy is the error in reading the McLeod gauge. The possibility of incomplete purification, however, must always be borne in mind, since the amount and composition of the contaminating gases which are evolved when a sample is fused are both variable. These limitations become less important for older rocks, and in our experience there seems to be little to choose in most respects between the two methods for work on Palæozoic and earlier materials. In general, however, it is considered that the isotopic dilution technique is preferable, since its accuracy can be maintained for much younger samples.

We wish to thank the Department of Scientific and Industrial Research for its financial support for these projects, and Prof. L. R. Wager and Sir Edward Bullard for their interest and encouragement.

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Karroo Igneous Activity and Tectonics in South-East Southern Rhodesia

During the period 1959–60 the work of the Research Institute of African Geology on Karroo igneous activity in Southern Rhodesia was extended to include the suite of Karroo igneous rocks which lie between the Lundi and Sabi Rivers some eighty to ninety miles to the north-east of those described by Cox *et al.*[1] (Fig. 1*a*). It is hoped to publish the detailed results of the work on this area soon.

Between the Sabi and Lundi Rivers the geological sequence is identical with that to the south-west. Basement gneisses, schists and quartzites of the east-north-east trending Limpopo Orogenic Belt[4] are unconformably overlain by Karroo sediments[6], and those in their turn underlie a considerable thickness of Karroo volcanic rocks.

The Karroo lavas in this region are estimated to be about 13,000 ft. thick and have a uniform dip of 7–10° to the south-east. They may be divided into a lower group of olivine-rich basalts and an upper group of more tholeiitic basalts in which olivine is noticeably scarce or absent. The ignimbrites, which characterize the uppermost part of the volcanic succession in the Nuanetsi District[1], are absent or overlain by later formations.

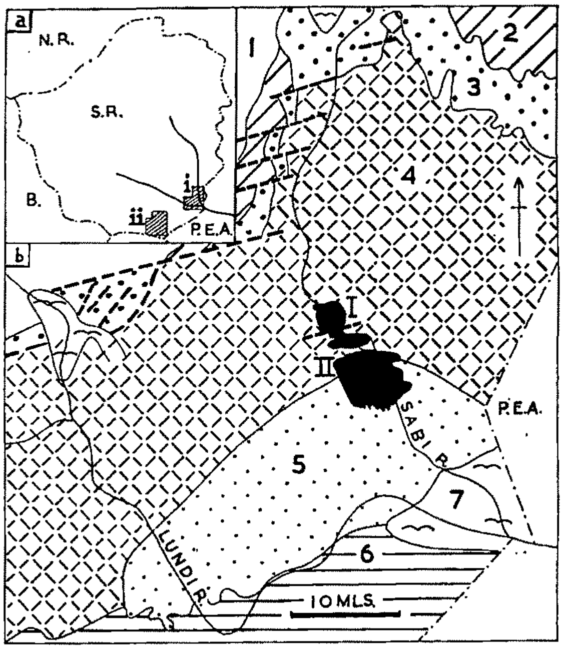

Fig. 1. (*a*) *N.R.*, Northern Rhodesia; *B*, Bechuanaland; *P.E.A.*, Portuguese East Africa; i, area shown in (*b*); ii, area described by Cox *et al.* (ref. 1). (*b*) 1, Basement Complex; 2, Umkondo System; 3, Karroo sediments; 4, Karroo basalts; 5, Main Granophyre; 6, Cretaceous; 7, Alluvium; I, Mutandawhe Complex; II, Chiwonje Complex

The extrusion of this thick sequence of lavas in the Sabi-Lundi area was followed by the intrusion of a series of rocks mainly of granitic affinities. Apart from a small horizontal tonalite body situated to the south-east of Mutandawhe, the earliest of these intrusions was a large sheet of porphyritic granophyre, the intrusion of which was localized at, or near, the upper limit of the basalts. This granophyre has an areal extent of 500 sq. miles and crops out from beyond the Lundi River in the south-west to beyond the Sabi River in the north-east (Fig. 1*b*); it can be correlated with the Main Granophyre of the Nuanetsi Area[1].

Subsequent phases of Karroo igneous activity are represented by two intrusive complexes in the Lower Sabi Valley. Mutandawhe, the more northerly of the complexes, is composed of granites and syenites and is characterized by a series of ring intrusions, but the Chiwonje complex to the south is composed entirely of granites which are intruded in part concordantly and in part discordantly. The rocks of these complexes have been responsible for copper, lead and tungsten mineralization in the surrounding basalts.

The final phase of igneous activity in the area is represented by a series of minor acid and basic intrusions.

In the southern part of the area the Main Granophyre and lavas are overlain by the conglomeratic basal member of a sedimentary series presumed to be Cretaceous in age. This latter group is in outcrop continuity with the post-Karroo sediments of the Nuanetsi area.

Structurally, the rocks as a whole overlie the east-north-east trending Limpopo Orogenic Belt[3,4], and the Chiwonje and Mutandawhe Complexes lie at the intersection of this belt with the southward projection of the Sabi Valley Fault[5]. The influence of the Limpopo Orogenic Belt during post Pre-Cambrian times has been noted by Dixey[2] and is further suggested by the many faults which affect the Karroo rocks and which strike parallel to the orogenic belt.

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CHEMISTRY

Sorption of Copper by Cellulose

Belford, Preston and co-workers[1,2] have made a study of the sorption of metal ions, particularly the cupric ion, by native cellulose. They suggest that the ions are adsorbed to form a regular two-dimensional lattice on the surfaces of microfibrils by complex formation with hydroxyl groups of the cellulose molecules. Should this be so, copper sorption might give information relating to the surface area of the crystalline fraction in cellulose, and, with such a possibility in view, it was considered worth while to make a further study of the interaction of cellulose with the cupric ion.

Preliminary experiments confirmed that beech α-cellulose[3] and bleached cotton thread do in fact take up copper from copper sulphate solution, but the extent of sorption by the α-cellulose (namely, 0·9 m.mole copper per 100 gm.) was found to be lower than that reported by Belford, Myers and Preston[1] for similar material by a factor of five. In seeking an explanation for this discrepancy, the possibility was considered that acidic groups within the cellulose molecule might be responsible for the uptake of copper, variation in acid content resulting in varying sorption of copper: as a result of further investigation, it now seems reasonably certain that this is the case.

Experiments were carried out on de-waxed, scoured Egyptian cotton, two samples of periodate–chlorite oxycellulose[4], and two samples of nitrogen dioxide oxycellulose[5] prepared from cotton; also studied were 'Tenasco Super 70', 'Viscose Rayon' and 'Fortisan *H*'. Specimens (about 0·5 gm.) were immersed for at least 15 min. in filtered 0·1 *M* copper sulphate solution (100 ml.). The *p*H of the medium was determined before and after immersion of the cellulose samples; in every case immersion was followed by a fall in *p*H of 0·01–0·7 unit (increasing with uptake of copper). Bound copper was removed from the thoroughly washed material in three extractions with 0·1 *N* hydrochloric acid and determined by the standard iodimetric method, using 0·005 *N* sodium thiosulphate. The cellulose specimens were then washed until free from acid and the carboxyl content determined by the alkali back-titration method of Neale and Stringfellow[4,6] using 0·01 *N* hydrochloric acid. Duplicate determinations were made in every case.

Fig. 1 shows that uptake of copper is proportional to the carboxyl content. This would appear convincing evidence that sorption of copper in these experiments is due solely to the presence of carboxylic acid groups in the cellulose molecule: sulphonic acid groups, which might occur in α-cellulose isolated from wood, would presumably act in the same way.

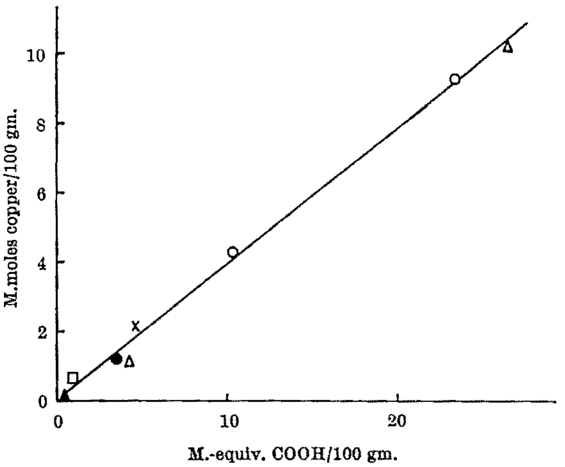

Fig. 1. ▲, Cotton; ○, periodate-chlorite oxycellulose; △, NO_2-oxycellulose; ●, 'Tenasco'; ×, 'Viscose'; □, 'Fortisan'

Further evidence that uptake of copper by cellulose is not due to the acidic action of hydroxyl groups was obtained by dissolving 5 gm. of α-methyl-D-glucopyranoside in 100 ml. of 0·1 *M* copper sulphate solution. The *p*H remained unchanged, establishing the absence of any reaction liberating hydrogen ions comparable with that occurring when cellulose takes up copper.

In attempting to interpret the results quantitatively, two apparently conflicting observations must be taken into account: (1) uptake of copper by a beech α-cellulose from 0·1 *M* copper acetate was approximately double that from 0·1 *M* copper sulphate solution[1]; (2) sorption from copper sulphate is governed by a Langmuir isotherm, maximum uptake being virtually attained with 0·1 *M* solution[1]. This would normally be taken to imply that at such concentration all the active sites are occupied.

A possible explanation for the apparent anomaly is forthcoming from a consideration of the equilibria involved, with the simplifying assumption that the system can be treated as one phase. Thus, if K_a is the acid dissociation constant of the carboxyl groups, K_s the dissociation constant of the copper salt ($=[RCOO^-][Cu^{2+}]/[RCOOCu^+]$) and C the total carboxyl content ($=[RCOOH] + [RCOO^-] + [RCOOCu^+]$), it can be shown that:

$$\frac{C}{[RCOOCu^+]} = \frac{K_s}{[Cu^{2+}]} + \frac{K_s[H^+]}{K_a[Cu^{2+}]} + 1 \qquad (1)$$

This would lead to a linear relation between reciprocal copper uptake and reciprocal copper concentration, with a limiting uptake at high copper concentration equal to half the theoretical maximum (that is, equal to half the carboxyl content) if the term $K_s[H^+]/K_a\,[Cu^{2+}]$ were constant and equal to unity. In fact, *p*H measurements on solutions of copper sulphate in the concentration-range 0·005–0·1 *M* have shown that the hydrogen ion activity is approximately proportional to the copper concentration, particularly in the region of higher concentration where more accurate sorption determinations are possible. If the constants K_s and K_a are given respectively the values of approximately 10^{-2} and 10^{-5} (as would appear not inconsistent with published results[6,7]), equation (1) predicts the observed sorption isotherm and also predicts about a two-fold increase in uptake of copper if the hydrogen ion concentration is decreased by a factor of 10 or more, as is the case in passing from 0·1 *M* copper sulphate to 0·1 *M* copper acetate (namely, sulphate *p*H 4·2, acetate *p*H 5·4). The system is obviously more complicated than depicted here, but it seems reasonable to suppose that the foregoing interpretation of the facts is essentially correct.

To sum up, therefore, the evidence now available makes it appear most probable that sorption of copper from solution by cellulose is due entirely to the action of acidic groups in, or associated with, the cellulose molecules. From this it follows that measurements of sorption of copper cannot be expected to yield any information necessarily related to the physical structure of the cellulose.

I am indebted to Dr. T. P. Nevell, of the Shirley Institute, for providing the specimens of oxycellulose used in this investigation. The work forms part of the programme of fundamental research undertaken by the Council of the British Rayon Research Association.

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I am not completely certain that Dr. Michie is discussing quite the same problem as that investigated by my co-workers and me. Although we used the term 'copper-cellulose complex' in some of our papers, we were consciously using the term 'cellulose' as defined by two of us in another connexion[1] as a family of polysaccharides built into microfibrils of a substance which, by virtue of the extraction procedure by which it is obtained and of certain physical properties, is normally called cellulose; we were therefore not discussing those rare products—which we term 'eucellulose'[1]—which yield glucose only on hydrolysis.

In collaboration with Mr. Dennis, I am following up our earlier work on what should be called a 'copper-microfibril' complex. Our results and conclusions will eventually be published *in extenso* elsewhere; at the moment we can say only that the bulk of the uptake of copper is not by the 'pure' cellulose component but by pentosans so firmly bound to the microfibril that they withstand the chemical procedures normally used for the extraction of α-cellulose. It is complexes with these, moreover, which give the sharp electron diffraction diagrams. We are ourselves not certain that copper adsorbed on 'pure' cellulose yields such a diagram; certainly none has been obtained with *Valonia* cellulose in spite of a measurable uptake of copper.

On the point of adsorption in 'pure' cellulose, Dr. Michie may well be correct for the materials and conditions he uses, though his interpretation rests on the straight-line relationship between uptake of copper and the so-called $-COO^-$ content of the cellulose as determined by a method which itself depends on cation adsorption. The difference between the cotton used by him and by us was that, whereas our cotton had been treated with strong alkali in the cold, his had been scoured (that is, boiled in 1 per cent sodium hydroxide). We have reason to believe that the effect of scouring is complex, but we cannot report on this at the moment.

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The Borohydride Ion in the Lithium Borohydride Lattice

In a recent communication in *Nature*, Dr. C. J. H. Schutte[1] has suggested that the anomalous band occurring at about 1,250 cm.⁻¹ in the paraffin-oil mull spectrum of lithium borohydride at room temperature[2] may be assigned to the doubly degenerate frequency, $\bar{\nu}_2(E)$, of the tetrahedral BH_4^- ion. This frequency is normally inactive in the infra-red, but it is suggested that under the space-group V_h of crystalline, orthorhombic lithium borohydride, the BH_4^- ions occupy sites of symmetry C_s; the degeneracy is thereby removed so that all the fundamentals are now allowed.

I suggest that confirmation of the above assignment is provided by a previous calculation of some of the vibration frequencies of the tetrahedral hydride ions[3]. The calculation was based on a theoretical discussion of the dynamics of binary hydrides[4], in which it was shown that, with the assumption of a spherical and rigid electron distribution in the molecule, the doubly degenerate frequency, $\bar{\nu}_2(E)$, of a tetrahedral XH_4 molecule (or ion) could be determined to within 5 per cent by the formula:

$$4\pi^2\bar{\nu}_2^{\,2} = 9(6)^{1/2}e^2/32\ mr_0^{\,3}$$

where e and m are the charge and mass of the proton and r_0 is the equilibrium XH distance. With the value of the B-H distance obtained from proton magnetic resonance measurements on the BH_4^- ion[5], namely 1·25 Å., the above formula gave a calculated frequency of 1,171 cm.⁻¹ (±59 cm.⁻¹) for $\bar{\nu}_2$. This value, therefore, gives good support to the above assignment of Schutte and is also in good agreement with the value of 1,210 cm.⁻¹ obtained[6] from measurements of the Raman spectra of solutions of the borohydride ion in liquid ammonia.

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ALTHOUGH the comments of Dr. Brown on my communication are very encouraging for further theoretical investigations, it should be pointed out that the equation used by Brown to calculate the doubly degenerate $\nu_2(E)$ vibration of the BH_4^- ion was derived for a tetrahedral ion or molecule which has a spherical and rigid electron distribution. This assumption is certainly not valid in the case of the BH_4^- ion in the $LiBH_4$ lattice. In this lattice the tetrahedral ion is considerably deformed, having the site symmetry C_s. In addition, it must be pointed out that the crystalline field in which any ion is embedded has a profound influence on the frequencies of vibrational modes (see, for example, refs. 1 and 2). The frequency of ν_2, calculated according to the formula derived by Brown, is that of the free tetrahedral BH_4^- ion and can only be compared, for example, with the Raman frequencies of BH_4^- in liquid ammonia.

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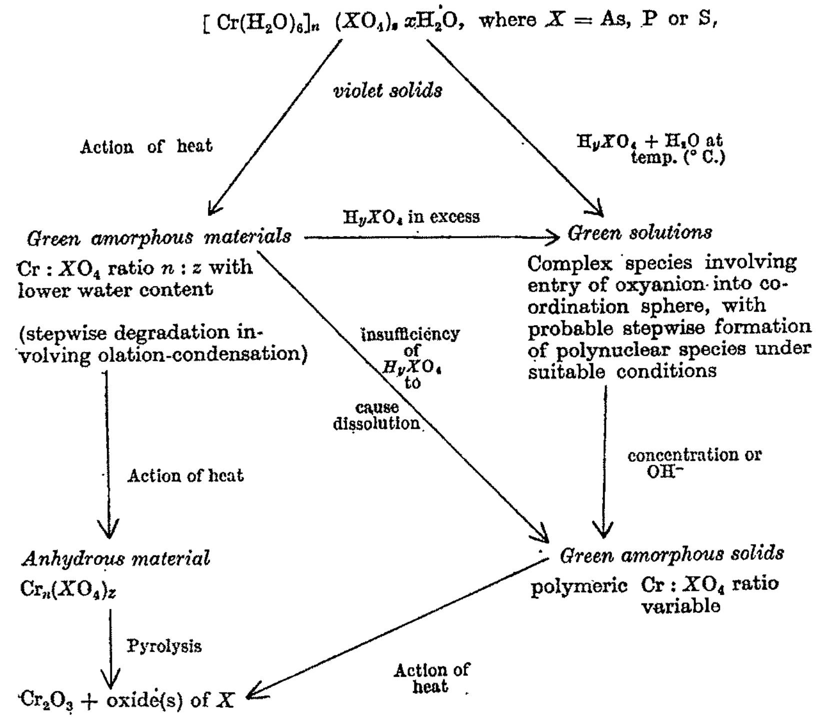
Chromium(III) – Oxyanion Systems

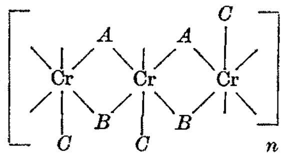
CERTAIN hexaquochromium(III) oxyanion compounds (in particular, the phosphate, arsenate and sulphate) have shown marked similarities in their behaviour on thermal degradation and in solutions with their corresponding oxyacids. This behaviour can best be explained on the basis of olation-condensation reactions which are believed to occur both in the solid state and in solution.

The behaviour may be represented schematically :


$[Cr(H_2O)_6]_n\ (XO_4)_z\ xH_2O$, where X = As, P or S,
violet solids
Action of heat
$H_yXO_4 + H_2O$ at temp. (° C.)
H_yXO_4 in excess
Green amorphous materials
Cr : XO_4 ratio $n : z$ with lower water content
(stepwise degradation involving olation-condensation)
Green solutions
Complex species involving entry of oxyanion into co-ordination sphere, with probable stepwise formation of polynuclear species under suitable conditions
insufficiency of H_yXO_4 to cause dissolution
Action of heat
concentration or OH^-
Anhydrous material
$Cr_n(XO_4)_z$
Green amorphous solids
polymeric Cr : XO_4 ratio variable
Pyrolysis
Action of heat
Cr_2O_3 + oxide(s) of X

Work on the solution and solid phases of the systems: Cr_2O_3–P_2O_5–H_2O at 0°, 40°[1], and 70°[2], Cr_2O_3–As_2O_5–H_2O at 25°[3] has already been reported. The system Cr_2O_3–SO_3–H_2O is being studied at 25° and 70°.

Thermogravimetric studies of hexaquochromium(III) phosphate, arsenate and sulphate indicate that the dehydration process is complex. Inflexions in curves of the loss in weight against temperature are interpreted as stages in a process of olation-condensation occurring in the solid phase, which is initiated by the entry of the oxyanion into the chromium(III) co-ordination sphere. Such a process is expected to result in the formation of cross-linked polymers incorporating groupings of the type :



Where $A = HXO_4^{a-}$, $B = OH^-$ or HXO_4^{a-} or no linkage, and $C = HXO_4^{a-}$, $H_2XO^{(a-1)-}$, or H_2O.

This type of structure accounts for the ion-exchange properties encountered in these systems[2,3]. X-ray studies have shown that the products of dehydration of these hexaquochromium(III) salts are amorphous and possess no long-range order. This might be accounted for by a random arrangement of the bridging groups. However, recrystallization occurs in these materials prior to complete dehydration to the phase $Cr_n(XO_4)_z$.

The kinetics of thermal decomposition have been studied by the method developed by Freeman and Carroll[4]. The results are presented in Tables 1–3, together with supporting evidence obtained from preliminary differential thermal analysis.

Infra-red studies of the products of progressive dehydration show that the characteristic absorption bands associated with water in the complex are initially extremely broad due to the effect of hydrogen bonding. In the course of the dehydration this band (3,750–2,100 cm.$^{-1}$) becomes modified in length and intensity, shortening occurring in the 3,750–3,100 cm.$^{-1}$ region. Finally the band disappears on complete dehydration (together with that due to the bending vibrations (~1,600 cm.$^{-1}$)). The characteristic bands of the oxyanion undergo broadening and splitting in the course of dehydration—implying a lowering of T_d symmetry, which might be expected if the formation of HXO_4-groups were involved. Magnetic studies on the arsenate have shown that the compound obeys the Curie–Weiss law down to liquid-nitrogen temperatures and has a very small value of the Weiss constant θ. Progressive dehydration of this material

Table 1. Hexaquochromium(III) Sulphate—$[Cr(H_2O)_6]_2 (SO_4)_3.16H_2O$

Temp. (° C.)	Loss	Stage (hydration)	Colour	Activation energy (*E*) kcal.	Reaction order (*n*)	Differential thermal analysis observations
25		16-H_2O	violet	—	—	
60 100	1-H_2O (lattice water)		violet	5	1	Two broad and consecutive endothermic effects
		15-H_2O				
120						
	5-H_2O quickly		deep green	30	1	
170		10-H_2O (inflexion)				
	5-H_2O quickly		green	≃ 50	2	
390		5-H_2O				
	~ 3·5-H_2O		lime green	6·5	1·2	Small endothermic effect
450		1·5-H_2O	yellow green			
	0·5-H_2O		light brown	very small	—	Exothermic effect
530		1-H_2O				
	1-H_2O lost		deep brown	very small		
635		Anhydrous				
	loss of SO_3		green	11·5	0·6	Endothermic effect
780		Cr_2O_3 formed	deep green			

Table 2. Hexaquochromium(III) Arsenate—$[Cr(H_2O)_6] AsO_4$

Temp. (° C.)	Loss	Stage (hydration)	Colour	Activation energy (*E*) kcal.	Reaction order (*n*)	Differential thermal analysis observations
		(slow decomposition)	grey-violet	—	—	—
25		6-H_2O				
						30°
	2-H_2O lost		light green	5	0·6	
60		4-H_2O				
	2-H_2O lost		green	17	2	Broad endothermic effect
110		2-H_2O				
	1-H_2O lost					
280		1-H_2O	grey	—	—	260°
	½-H_2O lost					
400		½-H_2O	light brown	22	1	450°
	very gradual water loss					600° Exothermic effect
		≏ anhydrous	brown	—	—	
900						
1,200	loss of As_2O_5		deep green	39	2	~ 1,200° Endothermic effect
1,400		Cr_2O_3 formed				

Table 3. Hexaquochromium(III) Phosphate $[Cr(H_2O)_6].PO_4$

Temp. (° C.)	Loss	Stage (hydration)	Colour	Activation energy (*E*) kcal.	Reaction order (*n*)	Differential thermal analysis observations*
25		6-H_2O	violet	—	—	
100	2-H_2O lost		violet	10·5	1	
250		4-H_2O (inflexion)	bright green			Endothermic effect observed between 150–300°
	2-H_2O lost		light brown	7	1·8	
300		2-H_2O				
	1-H_2O lost		dark brown	5·5	1	
400		1-H_2O				
650	½-H_2O lost slowly		black	very small	—	
1,000		~ ½-H_2O	black			
1,300		~ Anhydrous	light green	—	—	Exothermic effect at around 1,100° C.
1,400		Anhydrous $CrPO_4$ stable				
> 1,400			light green	—	—	

* Due to Sullivan and McMurdie (ref. 6)

does not show any departure from the linear law, although a progressive increase in θ is observed, a value of ~30° K. being obtained for $CrAsO_4\frac{1}{2}H_2O$. Such behaviour would seem to indicate the absence of —O—links in the polymeric compounds[5].

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Assurance of Thermostatic Control during Electrophoretic Experiments

Precise thermostatic control is of paramount importance if absolute electrophoretic mobilities are to be measured. Moreover, recently proposed supporting media in the form of gels (starch gel and cyanogum gel) introduce an additional need for constant temperature since the diameters of the gel pores (a factor largely determining migration in those media) depends on thermal agitation of the pore-forming bridges[1].

Generally, electrophoresis in a supporting medium is conducted without positive control of temperature. At best, the medium is placed in a container maintained at constant temperature (for example, an ice chest) and the temperature gradient existing between the room and the medium is kept within reasonable limits by using small field strengths and low ionicities. Another method is that of using a cooling fluid circulating in rather close contact with the electrophoretic medium. Yet in all those methods it is the temperature of the cooling medium that is directly controlled and not that of the electrophoretic medium itself.

This could, nevertheless, easily be achieved if use were made of the following property: since the medium used for electrophoresis has nearly always electrolytic conductivity, it possesses a rather large (negative) temperature coefficient. Thus the resistance of the medium could be used as a temperature-sensing element. Resistance variations can easily be converted into voltage variations if the electric field is established from a power supply regulating for constant intensity. Indeed, if the temperature of the electrophoretic medium rises, its resistance decreases, and to maintain a constant current intensity, the voltage at the electrodes will automatically be reduced. This decrease in voltage can be made to

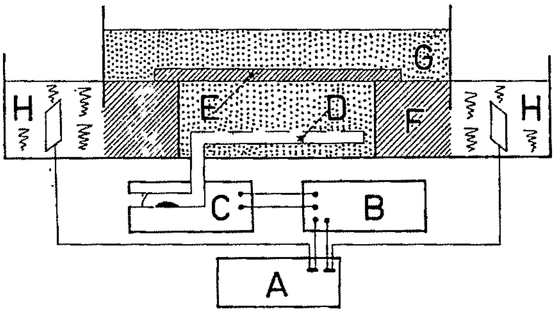

Fig. 1. Diagram of the apparatus for conducting high-voltage agar-gel electrophoresis at constant temperature. *A*, Electric power supply regulating for constant intensity; *B*, amplifier that closes a relay if the voltage at *A* decreases; *C*, air pump switched on by relay in *B*; *D*, tube provided with small holes for blowing air in *G*; *E*, electrophoresis plate; *F*, agar gel; *G*, petroleum ether; *H*, electrode vessels

close a relay, switching on some cooling device. The temperature will go down again and the voltage will rise to its initial value. At that moment active cooling will be cut out. In fact, electrophoresis is then conducted at (nearly) constant intensity and voltage.

Very effective cooling, giving the additional advantage of sealing the electrophoretic medium against evaporation, is obtained by bathing that medium in a neutral, volatile fluid : if its evaporation is activated, the heat generated in the electrolyte is absorbed. Evaporation of the volatile fluid is easily controlled by blowing finely dispersed air through it ; this makes at the same time for effective stirring.

These principles[2] have been applied to a technique of agar-gel electrophoresis described some time ago[3]. The experimental apparatus is illustrated in Fig. 1. The electrophoresis plate is bathed in petroleum ether of low boiling point (25–50° C.). A small air pump blows air through a tube provided at its distal end with small holes. If the temperature of the electrophoresis plate tends to rise, the pump starts blowing air until the temperature in the plate has reverted to its initial value. In an actual experiment effected at a field-strength of 30 V./cm. (agar 1 per cent in barbital buffer of *p*H 8·4 and ionicity 0·05) and conducted at 12° C., the temperature variations in the plate were found to be less than 1° C., with the pump acting in short cycles at a frequency of 8/min. With this method any temperature between +4° C. and 25° C. can be set by activating the evaporation of the petroleum ether prior to the experiment, until the fluid is at the desired temperature. The plate is then left for a few minutes to reach temperature equilibrium. The electric field is then applied and the temperature in the plate will automatically be maintained at a constant value.

Separations, already of outstanding quality when agar gel is used, are even better if the separation is effected at low temperature (+4° C.), since diffusion is then strongly checked while the decrease in mobility can be compensated for by increasing the electric field strength. Application of the principles here exposed to other types of electrophoresis, for example to the column type, could be fruitful.

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BIOCHEMISTRY

Quinine Oxidase in Liver and Blood Plasma of Rabbits poisoned with Carbon Tetrachloride

RABBIT liver has been shown to be a rich source of quinine oxidase[1,2]. The enzyme was also demonstrated in other organs of the rabbit but in much smaller amount (spleen, lungs) ; none was found in the pancreas, heart, kidney and skeletal muscles[2]. In normal human blood, quinine oxidase is present in negligible amounts. However, in cases of parenchymatous lesions of the liver, increased amounts could be detected in the blood. Therefore, the determination of this enzyme has been recommended by Baier[3] and by Roman and Dulmanis[4] as a test for liver damage.

Since carbon tetrachloride is regarded as a strong hepatotoxic agent, I decided to investigate its effect on quinine oxidase of the liver and blood plasma.

Quinine oxidase was determined using a method previously worked out and based on the reduction of the dye triphenyltetrazolium chloride in evacuated Thunberg tubes, the substrate being N′-methylnicotinamide[5]. The formazan produced after the incubation of the reaction mixture for 15 min. at 37° C. was extracted with petroleum ether and the colour measured in a Beckman quartz spectrophotometer at 480 mμ. N′-methylnicotinamide (7 × 10^{-3} *M*) was used instead of quinine hydrochloride since Knox[1] and also Lang and Keuer[1] demonstrated that this compound is the natural substrate for the enzyme. Blood plasma quinine oxidase was also measured by the increase in the absorption at 366 mμ of the carbostiryl oxidation product of quinine, using Baier's method[3,6]. Heparinized plasma was used throughout.

The enzyme could not be detected in the blood plasma of normal rabbits by the formazan reagent or by Baier's methods. It was, however, soon verified that a few hours after the injection of carbon tetrachloride the enzyme decreases in liver and appears in the blood. Table 1 summarizes this result.

Table 1. EFFECT OF INJECTED CARBON TETRACHLORIDE ON THE LIVER AND BLOOD PLASMA OF THE RABBIT

Conditions	No. of rabbits	Averages and standard deviations. Liver (μgm. formazan/gm. wet weight)	
			Blood plasma (Baier units)
Normal	10	21·5 ± 3·4	0
Carbon tetrachloride	10	9·2 ± 5·7	138 ± 42

Rabbits were dosed with carbon tetrachloride, 0·05 ml./100 gm. body-weight intraperitoneally and killed after 12 hr. Baier unit – increase of 0·001 in optical density at 366 mμ.

Some experiments were also performed to find the time of appearance and the level of this enzyme in blood plasma during the course of poisoning. A group of four rabbits weighing 600–920 gm. were injected intraperitoneally with 0·05 ml. of carbon tetrachloride/100 gm. body-weight and the blood withdrawn by heart puncture and analysed 3, 5 and 10 hr. after the injection. Four other animals were bled after 15 and 20 hr. Fig. 1 shows the average increase up to 20 hr.

Blood plasma of poisoned rabbits was saturated with ammonium sulphate solution (v/v) to find which fraction carries the enzyme. The protein precipitate was separated, dissolved in distilled water made slightly alkaline (*p*H 7·2) and dialysed in 'Cellophane' bags against distilled water at 4° C. The enzyme

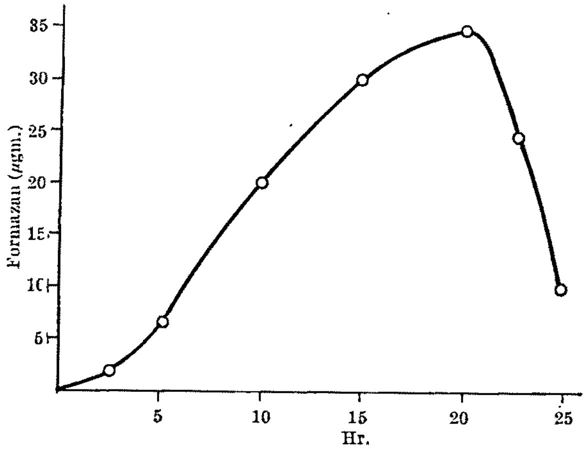

Fig. 1. Increase of plasma quinine oxidase in rabbits treated with carbon tetrachloride. Values expressed in μgm. formazan/1 ml. of plasma

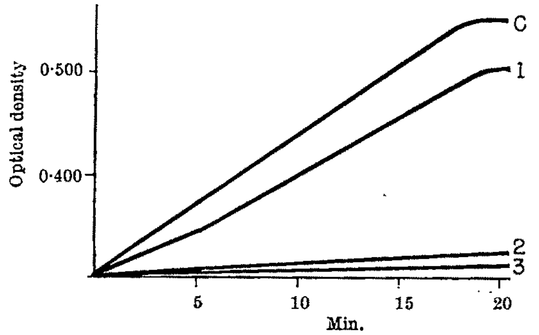

Fig. 2. Kinetics of the oxidation of quinine chloride. *C*, controls. 1, *p*-chloromercuribenzoate, 1×10^{-3} *M*. 2, crotonaldehyde, 7×10^{-3} *M*. 3, 0·1 ml. menadione, 0·4 *M*

activity was found only in the globulin fraction. A typical experiment is reproduced in Table 2.

Neither normal rats nor rats treated with carbon tetrachloride showed any quinine oxidase activity in the blood plasma. This suggests that in the rat the enzyme is not easily removed from the liver cells as in the rabbit. It appears, therefore, that this enzyme behaves differently from xanthine oxidase, which increases rapidly in the liver and blood of rats treated with carbon tetrachloride as previously reported by us[7]. It must be remembered that the liver of the rabbit is poor in xanthine oxidase and the blood is devoid of it. The localization in the liver cells of the rat and the association of this enzyme with lipids[8] may be responsible for the differences observed between their behaviour in carbon tetrachloride poisoning.

Liver quinine oxidase was inhibited by crotonaldehyde, *o*-iodobenzoic acid and 2-methyl-1,4-naphthoquinone(menadione), slightly inhibited by *p*-chloromercuribenzoate and not affected by 2-amino-4-hydroxy-6-pteridine aldehyde (Table 3). The kinetics of the oxidation of quinine chloride were followed in plasma by the increase in optical density at 366 mμ in the presence of *p*-chloromercuribenzoate, crotonaldehyde and menadione.

Table 2. Quinine Oxidase in Blood Fractions

	Activity*
Control plasma	150
Globulin fraction (corrected to the initial volume)	147
Albumin fraction	0

* Activity in μgm. formazan/100 ml. blood plasma.

Table 3. Effect of Inhibitors on the Quinine Oxidase of Cell-free Liver Homogenate

Inhibitors	Formazan produced (μgm.)	Inhibition (per cent)
Controls	30·8	—
6-Pteridyl aldehyde (30 μgm.)	30·0	0·9
p-Chloromercuribenzoate, 1×10^{-3} *M*	26·0	16
o-Iodosobenzoic acid, 2×10^{-3} *M*	13·0	58
Crotonaldehyde, 1×10^{-3} *M*	12·1	61
Menadione, 2×10^{-2} *M*	0	100

Liver homogenate 1:1 in phosphate buffer *p*H 7·4. Centrifuged 30 min. at 3,500 r.p.m. Total volume of the reaction mixture in the Thunberg tube, 2 ml. (ref. 5).

These results indicate that quinine oxidase is partly dependent to SH— groups for full activity. Work is now in progress to investigate the effect of other inhibitors.

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Isolation of the Toxic Principle in *Acacia georginae*

Serious losses in livestock, apparently due to poisoning by natural fodder, have occurred in the Georgina River watershed since early settlement. These occur over an area of approximately 14,000 square miles of north-western Queensland and over an equally large area of the adjoining Northern Territory. *Acacia georginae* is widely distributed throughout the affected areas, and its pods, seeds and leaves have proved toxic to sheep and cattle[1,2].

The isolation and identification of the toxic principle in the seeds of *A. georginae* is now reported. Previous investigations[3] showed seeds to contain a complex mixture of carbohydrates, amino-acids and pinitol, but failed to characterize the toxic factor. This has now been identified as the fluoracetate ion.

Seeds were extracted with ethanol and standard procedures used to separate into acidic, basic and neutral fractions. The acid extract was purified by repetitive re-extraction from ether with sodium bicarbonate and finally by fractional distillation of the volatile acids so obtained. The toxic factor was identified as the fluoracetate ion by conversion to the butyl ester and the use of gas chromatography aided by an authentic specimen of butyl fluoracetate. Infra-red absorption spectra confirmed the identification.

Toxicity tests on laboratory animals and on sheep with potassium fluoracetate in quantities comparable with those found in the seeds of *A. georginae* produced identical symptoms.

The occurrence of fluoracetate has been reported in *Dichapetalum cymosum* (*Chailletia cymosa*) in South

Africa[4], this plant being responsible for serious stock losses.

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Origin of β-Amino*iso*butyric Acid in Iris

Asen *et al.*[1] have recently added β-amino*iso*butyric acid (BAIB) to the growing list of amino-acids found in higher plants by isolating this compound from the roots of *Iris tingitana* var. Wedgewood. Fink *et al.*[2,3] have shown that BAIB can arise metabolically from thymine via dihydrothymine and β-ureido*iso*butyric acid in the rat. This discovery, taken in conjunction with our recent observation[4] that the higher plant *Brassica napus* (rape) forms radioactive dihydrothymine, β-ureido*iso*butyric acid and carbon dioxide from 2-^{14}C-thymine, strongly suggested to us that, in iris, thymine might well be the source of BAIB. This possibility was tested by supplying totally tritiated thymine to tissue sections of *Iris tingitana* var. Wedgewood.

Small disks (5 mm. diam., 1 mm. thick) were cut from the interior of a freshly dug iris root which had just finished blossoming. The disks (0·3 gm.) were incubated in air, at 28° C., in 1·0 ml. 0·03 *M* phosphate buffer, *p*H 5·0, which contained 5×10^{-3} μmoles of ^{3}H-thymine ($2{\cdot}4 \times 10^{7}$ c.p.m. counted as a weightless sample with a windowless Geiger counter). Following incubation for periods of 2, 4, 6 and 20 hr. the incubation mixtures were homogenized in a Potter–Elvehjem homogenizer with sufficient 75 per cent ethanol to yield a final volume of 5·0 ml. and filtered. The filtrates were taken to dryness in a stream of nitrogen, freed of lipoidal materials by extraction with petroleum ether and dissolved in a small quantity of water. The extracts were chromatographed on Whatman No. 1 filter paper employing solvent No. 5 of Fink *et al.*[3]. The radioactive regions on the developed chromatograms were located with a recording windowless scanning device (Forro radioscanner). In all cases the scanning records revealed the presence of a radioactive area corresponding precisely with authentic BAIB, the presence of which was revealed with ninhydrin. To establish further the identity of the putative BAIB, the radioactive product in question was eluted and portions thereof re-chromatographed on Whatman No. 1 paper, with three additional solvents: solvent No. 1 and No. 2 of Fink *et al.*[3] and *n*-butanol/acetic acid/water, 6 : 1 : 2 (v/v/v), of Asen *et al.*[1]. Correspondence between the radioactive area and the position of authentic unlabelled BAIB was satisfactory.

Still other portions of the eluate were converted to the N-dinitrophenyl derivative by treatment with fluoro-2,4-dinitrobenzene[5]. Since the quantities of the suspected BAIB were too small to yield sufficient dinitrophenyl derivative to be visibly yellow when applied to the paper chromatogram a small quantity of authentic unlabelled BAIB (100 μgm.) was added to the dinitrophenylation reaction mixture. The product was isolated and chromatographed on paper which had been previously treated with phthalate buffer[6]. Development was carried out with water-saturated butanol. On scanning the chromatogram for radioactivity, the dinitrophenyl derivative of the unknown was found to coincide with the yellow dinitrophenyl-BAIB. BAIB was detected in all the incubation experiments.

It is, therefore, reasonable to assume that the breakdown of thymine can account for the presence of BAIB which Asen *et al.* discovered in iris. ^{3}H-thymidine also yielded BAIB under similar conditions although it was a much poorer source than thymine. The thymidine was contaminated with a trace of thymine too slight to account for the BAIB formed. Presumably the free base is liberated from thymidine by a phosphorylase or hydrolase.

It is quite likely that the route to BAIB formation is via the Fink pathway[3], that is, through dihydrothymine and β-ureido*iso*butyric acid, since these two substances were found to be radioactive in the extracts obtained in the above experiments with thymine. These compounds were identified as described elsewhere[4] using slight modifications of the procedures of Fink *et al.*[3]. Still further evidence for the participation of this pathway comes from the observation that when the radioactive thymine was diluted 5,000-fold with unlabelled dihydrothymine (25 μmoles), no radioactive BAIB could be detected after 20 hr. incubation.

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A Supplement to the Kober Reaction for Urinary Œstrogens

Ittrich[1] has shown that the pink colour given by natural œstrogens in the Kober reaction with sulphuric acid can be extracted by chloroform when the acid is diluted to 20–30 per cent. The colour fades, but can be stabilized somewhat by using a 2 per cent solution of *p*-nitrophenol for extraction. Other workers[2] have preferred tetrachloroethane to chloroform.

The Kober colour could be extracted more readily if dilution were performed with aqueous trichloroacetic acid instead of water, and was then more resistant to fading. Under these conditions, *p*-nitrophenol was not needed. Because of the favourable partition ratio, the colour can be concentrated in a small volume of organic phase, and much of the 'background colour' remains in the diluted sulphuric acid. The extracted Kober colour absorbs maximally at a longer wave-length than in sulphuric acid.

Colorimetric measurement of œstrogen in male and post-menopausal urine is frequently near the limits of sensitivity of the method. Concentration of feeble colours by extraction into chloroform–trichloroacetic acid should enable low levels of œstrogen to be measured spectrophotometrically with greater confidence, and the spectral shift is a check on the authenticity of the Kober colour. It is suggested that the following type of procedure may be valuable as a supplement to the conventional analytical procedures for œstrogen utilizing the Kober reaction[3,4]. For œstrone or its methyl ether: dilute a 3-ml. aliquot of the Kober colour (after reading) with 1·8 ml. of 2 *N* trichloroacetic acid in a pointed centrifuge tube, stopper, mix, cool under the tap, add 1·2 ml. of chloroform, shake for 20 sec , centrifuge briefly, suck off top layer and discard, read the bottom layer in a 1 ml. cuvette at 502, 532, 562 m*M* (508, 538, 568 m*M* for the ether) in a spectrophotometer. This gives an Allen corrected value about three times as great as that derived from measurements on the original Kober colour. The chloroform extract fades about 3 per cent in ½ hr. Tetrachloroethane is objectionably toxic, though an excellent solvent.

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Controlled Use of Adsorbents in Enzyme Purification

Published methods for the purification of many enzymes specify the use of *C*γ-aluminium hydroxide gel[1] or calcium phosphate gel[2] as adsorbents, and Colowick[3] has reviewed the use of these methods.

The adsorbents may be used (*a*) preferentially to adsorb contaminants from solution or (*b*) preferentially to adsorb the enzyme from solution. These may be regarded as the two gross extremes of (*c*) a graded fractional adsorption, and Ochoa[4] has used all three procedures for the purification of the 'malic' enzyme from different sources.

However, the use of fractional procedures is reported only rarely in the literature, and most authors seem to make use of a technique which superficially resembles (*b*). This procedure is carried out in such a way as to afford little chance for investigating, and thus utilizing, the possibly fine resolving power of the adsorbents. The gel suspension is usually added in one or two large batches to adsorb all the enzyme and, after sedimentation, the gel is washed and eluted.

In this communication the behaviour is reported of two different enzyme preparations on different gels, and evidence is presented of differential adsorption of protein during the procedure. The results emphasize the importance of fractional adsorption in enzyme purification with gels, and indicate that this technique can lead to greater purification than is possible using the popular technique above.

The method requires titration of the enzyme remaining in solution after addition of small batches of adsorbent, and a rapid method for the assay of enzymatic activity is desirable for convenient working.

(1) *Preparation of glucose-6-phosphate dehydrogenase.* This enzyme can be conveniently prepared from dried baker's yeast, but is usually contaminated with hexokinase. In a method used in this laboratory (unpublished), the hexokinase activity is destroyed by mild-heat treatment of a yeast autolysate and nucleic acids are precipitated by protamine sulphate. Some impurities are removed by iso-electric precipitation and the crude enzyme is fractionated with ammonium sulphate. Treatment with *C*γ alumina results in further purification.

Table 1 shows the results obtained in this step by the popular procedure and the fractional method.

Table 1. FRACTIONATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE WITH ALUMINA GEL

Method 1:

Enzyme adsorbed from 0·05 *M* acetate buffer *p*H 5·5 with 2·5 ml. of *C*γ alumina (11 mgm. solids/ml.). Gel precipitate collected by centrifugation, washed with 0·05 *M* acetate *p*H 5·5, eluted three times with 0·1 *M* phosphate buffer *p*H 7·4.

Volume of gel added (ml.)	Total units* of enzyme in solution	Total protein† (mgm.)	Specific activity (units/mgm. protein)
0	260	20	13
2·5	31	—	—
Alumina eluate	230	9·7	24

Method 2:

Enzyme solution in 0·05 *M* acetate buffer *p*H 5·5, 29·0 ml. of *C*γ alumina suspension added. Gel precipitate discarded, 30 ml. of *C*γ alumina added to enzyme solution. Gel collected by centrifugation washed and eluted as above.

Volume of gel added (ml.)	Total units* of enzyme in solution	Total protein† (mgm.)	Specific activity (units/mgm. protein)
0	2,430	200	12
29	2,300	—	—
30	36	—	—
Alumina eluate	2,010	34	59

* Determined by the rate of reduction of triphosphopyridine nucleotide ($0{\cdot}2 \times 10^{-3}$ *M*) in the presence of *tris* (hydroxymethyl)-aminomethane buffer (0·03 *M*, *p*H 7·4), magnesium chloride (3×10^{-3} *M*) and glucose-6-phosphate (10^{-3} *M*) in a Beckman *DU* spectrophotometer at 30° C. The unit of dehydrogenase activity is defined as an optical density change of 0·100 per min. at 340 mμ and 30° C.

† Determined by the biuret method (ref. 5).

These results indicate that the fractional procedure gives a superior purification factor in this case. In method 1, the specific activity of the enzyme increases by a factor of about 1·9, while in the second method the increase in the factor is about 5.

(2) *Preparation of uridinediphosphoglucose* (*UDPG*) *dehydrogenase.* This enzyme is prepared from fresh calf-liver by a modification of a method previously applied to acetone powders of liver[6].

Fresh calf-liver is treated in a Waring blender with 0·15 *M* potassium chloride and centrifuged. The supernatant solution is fractionated with ammonium sulphate, heat treated and fractionated again with ammonium sulphate. Calcium phosphate gel is then used in the fractional adsorption procedure.

In this step, the differential adsorption occurs of some other system (of unknown type) which interferes strongly with the assay method for UDPG dehydrogenase. This is demonstrated by the data in Table 2. As more gel suspension is added, the apparent activity of the dehydrogenase in solution rises to a peak value, and then begins to fall. At the peak value

Table 2. FRACTIONAL ADSORPTION OF UDPG-DEHYDROGENASE WITH CALCIUM PHOSPHATE GEL

Adsorption of enzyme from solution in 0·01 *M* phosphate *p*H 6·8

Total volume of gel added (ml.)	Total units* of enzyme in solution
0	12·4
9·0	36·3
18·0	49·5
30·0†	60·2
3·0	50·5
6·0	46·5
21·0	31·5
51·0	0

* Measured by the rate of reduction of diphosphopyridine nucleotide (7×10^{-4} *M*) in presence of glycine buffer (0·15 *M*, *p*H 8·7) and UDPG ($1·5 \times 10^{-4}$ *M*). Micro-cells with total volumes of 0·7 ml. were used in a Beckman *DU* spectrophotometer at 30° C. The unit of enzyme activity is defined as a change in optical density at 340 mμ of 0·100 per min. at 30° C.

† At this point all the added gel was discarded ; 23 per cent of the total protein had disappeared from solution.

in a typical experiment, 23 per cent of the total protein had been adsorbed to the gel.

It can be seen that the fractional procedure brings about removal of an interfering system as previously mentioned, together with a substantial amount of protein. During the latter part of the step, the enzyme is adsorbed to the gel. The gel is washed with 0·01 *M* phosphate *p*H 6·8 and the enzyme eluted with 0·1 *M* phosphate buffer *p*H 7·4. Quantitative recovery of the enzymes (peak values) have been obtained in these experiments.

The results indicate that in procedures for the purification of other enzymes or proteins which involve the use of adsorption-elution techniques, the fractional methods described here may be of considerable value. Such procedures are relatively easy to apply provided that rapid assay methods are available.

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The Pasteur Effect in a Respiration-deficient Mutant of Baker's Yeast

THE respiration-deficient mutant of baker's yeast, *Saccharomyces cerevisiae R XII A*, induced by high-temperature cultivation, was used for examining the point of action of oxygen in the Pasteur effect. The mutant was identical with the *petite colonie* mutant described by Ephrussi[1] and Raut[2]. The argument was that, if oxygen acted directly on some enzyme involved in glucose degradation (for example, glyceraldehyde phosphate dehydrogenase), it would decrease the rate of glucose catabolism even in the mutant lacking essential components of the cytochrome system.

It was found that in the presence of glucose there was no demonstrable uptake of oxygen by the suspension and that the Q_{CO_2} (=100–130) was not affected by the introduction of oxygen into the suspension. The uptake of glucose, as measured by quantitative analysis as well as by distribution of radioactivity following application of universally labelled ^{14}C-glucose, revealed no differences between aerobic and anaerobic conditions. As another criterion of the Pasteur effect, the change of inorganic phosphate-level was examined. Here again no change due to oxygen could be observed to take place in the steady-state level in the presence of glucose (Table 1).

Table 1. INORGANIC PHOSPHATE AND LABILE PHOSPHATE LEVELS IN THE RESPIRATION-DEFICIENT MUTANT (μGM. PHOSPHORUS/MGM. DRY WEIGHT ± *S.E.*)

Incubation conditions	Inorganic phosphate	Labile phosphate
No substrate, anaerobically	4·24 ± 0·24	5·84 ± 0·31
No substrate, 10 min. aeration	3·63 ± 0·26	6·39 ± 0·67
5 per cent glucose, anaerobically	2·37 ± 0·23	7·15 ± 0·71
5 per cent glucose, 10 min. aeration	2·40 ± 0·21	7·26 ± 0·75

If these results can be generalized they may serve as a weighty support for the theory that oxygen brings about the Pasteur effect not by affecting directly any enzyme involved in the anaerobic breakdown of glucose but rather by making it possible for other catabolic pathways to function (much in line with Lynen's views[3,4]).

It was observed, however, that under conditions when no substrate was present in the medium, oxygen was effective in producing changes in the amounts or, at any rate, in the distribution of some metabolically active components of the mutant cell. Some of these changes (for example, in the penetration of free glucose into the cell) are dealt with elsewhere[5]. It was found here that oxygen decreased the level of inorganic phosphate in the absence of glucose, with simultaneous production of labile phosphate bonds (Table 1), the process being completely blocked by 10^{-4} *M* potassium cyanide but only partly inhibited (at most by 20 per cent of the steady-state level change) by 5×10^{-4} *M* 2,4-dinitrophenol. The generation of high-energy phosphate bonds was accompanied by only a minute oxygen consumption ($Q_{O_2} = 0·25–0·32$; $Q_{CO_2} = 1·09–1·48$) so that the apparent phosphorus : oxygen ratio reached values far exceeding those commonly reported for whole cells and even for purified enzyme systems. Thus endogenous phosphorylation in the mutant (and very likely in ordinary baker's yeast, ref. 6) appears to be qualitatively different from phosphorylation proceeding with glucose, and produces high-energy bonds at the expense of other energy sources (physicochemical state of proteins, nucleic acids ?) than those involved in oxidative phosphorylation known heretofore.

Detailed results will be published in *Folia microbiologica*.

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Is *Mycobacterium phlei* Glutamotransferase endowed with Glutamine Synthetase Activity?

IN a previous paper[1] we reported the finding of glutamotransferase (an enzyme catalysing the transfer of the γ-glutamyl residue of glutamine to hydroxylamine)[2] in cell-free extracts of *M. phlei*. The enzyme required the presence of manganese ions, which could not be substituted by magnesium ions. No glutamine synthetase (an enzyme catalysing the synthesis of glutamine from glutamic acid and ammonia) activity was found in the *M. phlei* preparations, when tested in the presence of magnesium ions under conditions optimal for this enzyme in preparations of bacterial[2], plant[3] and animal[4] origin. The *M. phlei* extracts possessed only traces, if any, of glutaminase activity and did not hydrolyse glutamohydroxamic acid at all. On the other hand, these preparations showed very high asparaginase activity, lacking any aspartotransferase and asparagine synthetase activities. Aspartohydroxamic acid was very strongly hydrolysed by the *M. phlei* extracts; however, this activity was dissociated from asparaginase activity. We have, therefore, concluded that in *M. phlei* the reactions of transfer, synthesis and hydrolysis are due to the action of different enzymes.

IT was reported recently[5] that glutamine synthetase and glutamotransferase of sheep brain were activated by either manganese or magnesium. The *p*H optimum of the manganese activated synthetase was in the acid range (*p*H 4·5), while that of the magnesium reaction was 7·2. Increase of the cation concentration caused a shift to the left of the *p*H optima of the manganese-activated synthetase and transferase. Thus the same enzyme seemed to catalyse both the synthesis and transfer reactions.

In light of these findings, and in view of the fact that the *M. phlei* transferase showed a specific requirement for manganese, it was of interest to see whether this organism also possesses a manganese-dependent synthetase.

Table 1. TESTING FOR GLUTAMINE SYNTHETASE ACTIVITY OF *M. phlei* EXTRACTS WITH EMPHASIS ON CONCENTRATIONS OF MANGANESE, MAGNESIUM AND HYDROGEN IONS

Reaction mixture No.	$MnCl_2$	$MgSO_4$	*p*H
1	$2{\cdot}25 \times 10^{-2}$ *M*	—	4·5
2	$2{\cdot}25 \times 10^{-3}$ *M*	—	5·0
3	$4{\cdot}5 \times 10^{-4}$ *M*	—	5·5
4	$2{\cdot}25 \times 10^{-4}$ *M*	—	6·0
5		$2{\cdot}25 \times 10^{-2}$ *M*	6·0
6		$2{\cdot}25 \times 10^{-2}$ *M*	6·5
7		$2{\cdot}25 \times 10^{-2}$ *M*	7·0
8		$2{\cdot}25 \times 10^{-2}$ *M*	7·5
9		$2{\cdot}25 \times 10^{-2}$ *M*	8·0

The reaction mixture contained: L-glutamic acid, $2{\cdot}25 \times 10^{-2}$ *M*; hydroxylamine sulphate, $1{\cdot}10 \times 10^{-2}$ *M* (calculated as NH_2OH); ATP, $2{\cdot}5 \times 10^{-3}$ *M*; $MnCl_2$ and $MgSO_4$ as indicated; *p*H as indicated; *p*H values below 7·0 were obtained by the use of acetate buffer, 0·1 *M*, while *tris* (hydroxymethyl) aminomethane buffer, 0·05 *M*, was used for *p*H values of 7·0 and above; bacterial extract, 9·1 mgm. protein. Final vol., 2 ml. All solutions added were adjusted to the appropriate *p*H with sodium hydroxide. Experiments were performed in the absence and presence of L-cysteine, 0·04 *M*, which was added before sonication. The mixtures were incubated at 37° for 90 and 180 min.

The experiments were performed with cell-free extracts at various manganese, magnesium and hydrogen-ion concentrations as indicated in Table 1. All the experiments invariably gave negative results: no synthetase activity could be detected under any of these conditions employed. With manganese, but not with magnesium, these extracts showed high glutamotransferase activity as already reported[1]. The results confirm our previous assumption, that the glutamotransferase of *M. phlei* is not associated with any synthetase activity.

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PHYSIOLOGY

Flow of Portal and Hepatic Blood in Normal and Cirrhotic Liver

HEPATIC blood-flow measured by the bromsulphthalein method, the colloidal radiogold method, or other methods is the sum of both portal blood-flow and hepatic arterial blood-flow. As to the relative portal and arterial blood-flow, one-third to one-fourth of the total hepatic blood-flow was estimated, in animal experiments, to be derived from the hepatic artery under basal conditions[1]. In some cases with cirrhosis of the liver, in which portocaval shunting operations were performed or portal venous collaterals were accessible, 25–75 per cent or 54 per cent of the total hepatic blood-flow was estimated to be from the hepatic artery[2,3]. It is desirable to develop a new method which enables the actual measurement of the portal blood-flow and the hepatic arterial blood-flow in normal subjects and cirrhotic patients, but there has been no report except that of Ueda[4].

Ueda was the first to describe a method of measuring portal and hepatic arterial flow of blood in clinical cases. His method was based on the fact that the hepatic arterial blood was diluted with the portal blood when the hepatic arterial blood appeared in the hepatic veins, and that a dye concentration curve of the peripheral arterial blood could be a substitute for that of the hepatic arterial blood. Relative portal and arterial flow of blood was calculated from T_{1824} concentration curves of the peripheral arterial blood, and of the hepatic venous blood after the injection of T_{1824} into the cubital vein. The ratio of the hepatic arterial blood-flow to the total hepatic blood-flow was calculated as the ratio of the area of the first (hepatic arterial) component of the hepatic venous concentration curve to that of the peripheral arterial concentration curve.

This method is theoretically attractive. However, it involves a difficulty in the actual measurement. According to our experience, the differentiation between the first (hepatic arterial) component and the second (portal) component in the hepatic venous curve, which is needed for the calculation, is impossible by the original method. Hence, we attempted to improve the method, and were successful in the actual measurement of the portal and hepatic arterial flow of blood by our improved method.

Instead of the original method of injecting a T_{1824} solution into the cubital vein, 50–100 μc. of radioactive iodinated human serum albumin is injected into the pulmonary artery by means of a venous catheter, and blood is taken at successive intervals of 2–3 sec. from the hepatic vein through a hepatic venous catheter and also from an arterial needle placed in the brachial artery. Injection into the pulmonary artery serves to reduce diffusion of the injected material before it reaches the hepatic vein. Radioactive iodinated human serum albumin is used, because a major part of T_{1824} which is not yet bound with serum albumin binds with proteins of liver cells and does not appear in the hepatic vein.

The portal flow of blood (P) and the hepatic arterial flow of blood (A) are calculated from the first half-area of the curve of radioactivity in the brachial artery (a), the first half-area of the first (hepatic arterial) component in the hepatic venous radioactivity curve (h), and the total hepatic blood-flow (T), according to the method of Ueda (Fig. 1).

$$\frac{P}{A} = \frac{a}{h} - 1$$

$$P + A = T$$

The total hepatic flow of blood is determined by the method of Bradley *et al.*[5].

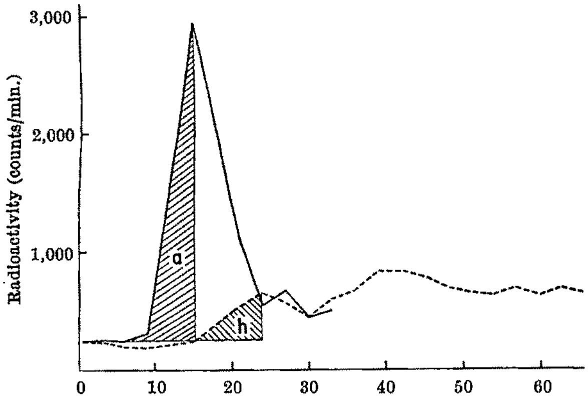

Fig. 1. Radioactivity in blood of a brachial artery (——) and hepatic vein (– – –) in a case with mild cirrhosis of the liver

In five normal subjects the portal flow of blood was 606–915 c.c./min./m.^{2} (66–83 per cent of the total hepatic blood-flow), and the hepatic arterial blood-flow 142–418 c.c./min./m.^{2} (17–34 per cent). In seven cases with cirrhosis of the liver, on the other hand, portal flow of blood was 203–802 c.c./min./m.^{2} (41–79 per cent), and the hepatic arterial blood-flow 179–355 c.c./min./m.^{2} (21–59 per cent).

Thus about one-fourth of the total hepatic flow of blood is derived from the hepatic artery in normal subjects, and the decrease of the total hepatic flow of blood in cirrhosis of the liver is ascribed mainly to the decrease of the portal flow of blood. These results are in agreement with the previous assumption based on the animal experiments and on the results of special cases with cirrhosis of the liver.

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Purification of the Toxic Fractions from *Ascaris lumbricoides* and their Effect on the Guinea Pig

Shinamura and Fuji[1], Bier[2] and Rocha e Silva and Graña[3] were able to isolate from *Ascaris lumbricoides* an extract very active in producing an anaphylactic-like shock in guinea pigs and in dogs. On the other hand, Machebouef and Mandoul[4] showed a similar activity in extracts from *Ascaris megalocephala*. Nakajima[5], after heating at 80° C. an aqueous extract of *Ascaris lumbricoides*, purified it by alcoholic precipitation, obtaining an active fraction containing N and P. Recently, Högberg *et al.*[6], using 'Amberlite *IRC*–50' (*XE*–64), obtained a quite active fraction which released histamine from the perfused paw of the cat. The present communication describes the further purification and chemical analysis of the active fractions, and the effect of these fractions on the guinea pig.

Ascaris lumbricoides, collected from hogs, was treated by the method described by Rocha e Silva and Graña[3]. This extract, called fraction SB_1, was further purified by precipitation with ammonium sulphate to saturation at pH 5·2. The supernatant fluid was dialysed against distilled water for 48 hr., and alcohol then added to 95 per cent. The alcoholic precipitate, dissolved in distilled water, was run through a column of hydroxyapatite. The most active fraction was eluted with phosphate buffer, pH 7·5, 0·01 M ; under these conditions the column did not retain the active fraction. Elution with higher ionic strengths gave other protein peaks which contained some activity, and these fractions are still being studied in our laboratory. After dialysis for 48 hr. against distilled water, the active fraction obtained from the column was precipitated by adding alcohol to 95 per cent and dried *in vacuo*. This fraction was named SB_4. The chemical analysis of these two fractions is shown in Table 1. Passing it again through a column of 'Amberlite *IRC*–50' or zone electrophoresis on starch showed only one protein peak.

Table 1. CHEMICAL ANALYSIS OF FRACTIONS SB_1 AND SB_4 FROM *Ascaris lumbricoides*

	Protein (per cent)		Reducing sugars (per cent)*	Nitrogen (per cent)	Phosphorus (per cent)
	(Lowry)	280 mμ/ 260 mμ			
SB_1	27·4	33·3	21·3–23·3	7·7–8·7	1·18
SB_4	7·6	7·7	42	3·4–4·3	1·80

* Measured as glucose

From our results the active fraction of the *Ascaris lumbricoides* seems to be a complex substance containing sugars and protein.

For the biological assay of the *Ascaris* fractions, guinea pigs weighing 200–400 gm. were used. Intravenous injection of the active fractions caused in the majority of the animals an anaphylactic-like reaction. The activity of the fractions was determined by recording the ratio of deaths to survivals in a group of 6 animals, as shown in Table 2. Table 2 also shows an increase in the carbohydrate with a simultaneous decrease in the protein content when the SB_1 is purified.

Histological examination of the lungs of the animals which died in shock showed a reduction of 70–80 per cent in the number of the mast cells when compared with control animals. Furthermore, mast cells damage very similar to that noted by Mota and

Table 2. Relation of Dose (mgm./100 gm.) to Mortality for Guinea Pigs injected with SB_1 and SB_4 Fractions

Ascaris fractions	Total weight (μgm.)	Protein (μgm.)	Residue including glucide (μgm.)	Mortality ratio
SB_1	50 133 200	15 40 60	35 93 140	0/6 3/6 5/6
SB_4	132 264	10 20	122 244	4/6 5/6

Vugman[7] in anaphylaxis was observed. Perfusion of the isolated guinea pig lungs with 20–30 μgm. of SB_4 released histamine ranging from 1·35 to 5·85 μgm. The addition of 1–5 μgm. of the fraction SB_4 to the perfusing bath containing the guinea pig ileum evoked a strong contraction. A second addition of the same dose of SB_4 was either ineffective or produced a smaller effect, followed by a refractory state on further addition of the fraction. These experiments suggest that the ileum was desensitized by the first addition of SB_4. This desensitization was observed in 27 out of 30 different guinea pig ilea tested. Fig. 1 illustrates one of these experiments. On the other hand, when 7 guinea pigs were injected with sub-lethal dose of SB_4, and two days later the ilea were suspended in an organ-bath and tested with SB_4 as described above, none of them showed any response when the *Ascaris* fraction was added to the bath. On the basis of these findings, it is suggested that the guinea pigs are naturally sensitized to *Ascaris lumbricoides*.

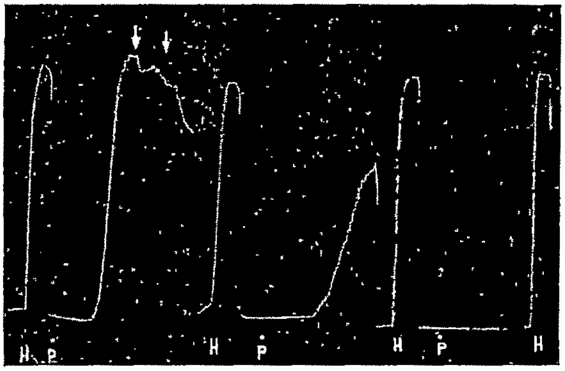

Fig. 1. Guinea pig ileum suspended in 10 ml. atropinized Tyrode solution. Effect of 5 μgm. fraction SB_4 (P) and 0·1 μgm. of histamine (H). The arrows indicate washings with Tyrode solution

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Cholinergic Activity of Hyoscyamine

Many former and recent investigations, employing various clinical and experimental tests[1-4], have already revealed the synergic action of small atropine doses with acetylcholine. We were able to demonstrate quite an unexpected experimental correlation between hyoscyamine and cholinergic activity.

Fig. 1 shows an illustrative example of this correlation selected from a lot of thirty similar experiments. The 2 m. recti abdominis of a frog are suspended in Ringer fluid and their motor reactions are recorded on a slow-moving drum, one above the other, with a pair of vertical writing, isotonic levels having a two-fold amplification. The lower muscle only receives every 15 or 30 min. successive geometrically increasing, hyoscyamine concentrations which increase in geometrical progression with ratio 10 starting with C_{20} (C_{20} stands conventionally for $3{\cdot}16 \times 10^{-20}\ M$ and generally C_n for $3{\cdot}16 \times 10^{-n}\ M$; D_n stands for $5{\cdot}62 \times 10^{n}\ M$; 3·16 and 5·62 are the antilogarithms of 0·500 and 0·750 respectively). The first slightly active concentration (mark 7) is C_{14}. The next one gives a contraction of 5 mm., the third one 1·3 mm., the fourth only 0·1 mm. The concentration C_{10} produces no effect (mark 11). The same concentration, C_{10}, is also added to the upper muscle, also with no effect. At mark 12, the two muscles are washed three times and their responses to acetylcholine concentrations C_8 and C_6 tested. Both muscles show normal reactivity.

They are again washed and, after relaxing to their initial length, both receive paraoxon in concentration D_6 at mark 16, to block their acetylcholine esterase. At mark 17, the lower muscle receives again the series of increasing hyoscyamine concentrations, starting with C_{17}. The first contractile reaction arises at C_{15} and the higher concentrations increase the contraction

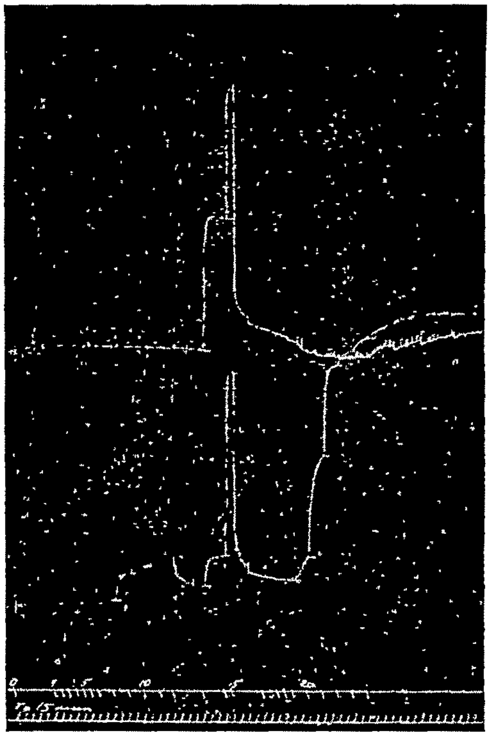

Fig. 1. Cholinergic activity of hyoscyamine

which is maximal at C_{10} (mark 24). At the same mark, the upper muscle receives hyoscyamine C_4 with practically no effect.

In my experiments the lowest active dose of hyoscyamine was C_{17}. It can be seen that hyoscyamine in very low concentrations contracts the frog's rectus, whereas concentrations of ten thousand up to one billion times greater fail to do so.

The amplitude of the maximal contraction produced by hyoscyamine increases by 6–10 times after paraoxon and is generally higher than that elicited by acetylcholine in concentration C_6 on untreated muscle. Concentrations of hyoscyamine, one million to one billion times higher than the threshold ones, are ineffective on treated, as well as on untreated, rectus muscles.

The contractions produced by small hyoscyamine concentrations are relaxed by stronger concentrations of this same alkaloid, by D-tubocurarine, in concentration C_6, and by magnesium chloride of concentration C_3.

These results suggest strongly that hyoscyamine acts on the rectus muscle as a contracting drug through a cholinergic mechanism, possibly by setting free an amount of acetylcholine at the level of its action. The closer pharmacological analysis of the site of this effect, details of which will be published elsewhere, demonstrates that these small concentrations of hyoscyamine act at the level of the neuromuscular junction and not on the contractile substance itself.

Methylatropine and scopolamine have no such effects. Other drugs may also act by releasing acetylcholine through a similar mechanism. I demonstrated it for barium chloride in concentrations between C_5 and C_3 and for neostigmine, in concentrations C_6 and C_5.

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Serum Changes in the Rat following Oral Administration of Cadmium

The comparatively sudden and pronounced drop in hæmoglobin-levels and the resultant anæmia following oral administration of low doses of cadmium to rats has been reported by several workers[1–4]. Starch-gel electrophoresis was performed on the serum proteins of rats similarly dosed, using the apparatus described by Smithies[5], and a modification of the discontinuous buffer system described by Poulik[6], in which low melting point *tris*(hydroxymethyl)-aminomethane (m.p. 125° C.) was used instead of the usual form (m.p. 171° C.).

Sixteen male rats, Chester Beatty strain, aged three weeks and weighing 80–100 gm., were fed on diet 41*B* and allowed unlimited drinking water, containing 50 p.p.m. of cadmium as cadmium chloride. Blood specimens were taken from the tail at fortnightly intervals by rota, and severe anæmia was confirmed after four weeks. Despite continued oral administration of cadmium for a further three months, the degree of anæmia remained constant. Certain modifications of the normal serum protein pattern were observed after a similar time, but again, despite continued dosing, no further change was observed.

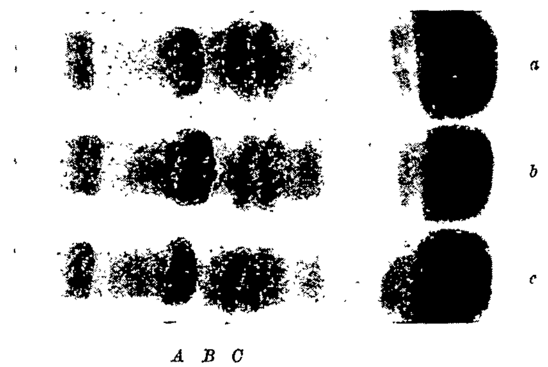

Fig. 1. Starch gel-electrophoresis pattern of the sera of rats given drinking water containing 50 p.p.m. of cadmium as cadmium chloride for four weeks. *a*, Cadmium rat serum; *b*, normal rat serum; *c*, cadmium rat serum. *A*, Unknown; *B* and *C*, transferrin bands

Two abnormalities in the serum protein pattern were seen to occur (Fig. 1). The component at point *A* was considerably diminished in the cadmium-dosed rats, compared with the control rats. The components at points *B* and *C* were noticeably enhanced by comparison with the controls and the possibility that they might be transferrin bands was examined, using the method of Giblett, Hickman and Smithies[7]. 1 μc. of radioactive iron-59 (as ferric chloride) was added to 0·2 ml. of normal rat serum and the specimen was subjected to starch-gel electrophoresis as before. The gel was sliced into two wafers, one slice being stained with a saturated solution of naphthalene black 10*B* 200 in methanol/water/glacial acetic acid (5:5:1) and the other slice, sealed in 'Saran Wrap', was placed in contact with Kodak 'No Screen' X-ray film for 12 hr., followed by development for 3 min. in Kodak *D*8 developer. Comparison of the stained gel and the final X-ray film showed that bands *B* and *C* had bound the radioactive iron exclusively. The result was confirmed by sectioning the starch block into the various bands, digesting the sections in 50 per cent nitric acid containing added carrier iron, and finally assaying the activity using an *M*6*H* liquid counter.

Benzidene staining of the gel revealed the presence of hæmoglobin in the space between the bands *A* and *B*, and further investigation indicated a 'ghost' band in this area, which was apparently the component which reacted positively to the stain.

Estimations of the copper oxidase-levels of the sera by the method of Ravlin[8] indicated no significant difference between the cadmium-dosed rats and the controls.

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Absorption of Amniotic Fluid in the Gut of Fœtal Sheep

SWALLOWING movements are known to be made by the fœtus *in utero*, but the ability of the gut to absorb material from the amniotic fluid has received little attention. This latter aspect has been investigated in fœtal sheep over the age-range 80–145 days (full term).

Under spinal anæsthesia and sedation with sodium thiopentone the fœtus was exposed by Cæsarian section and its abdomen opened. Electrical potential differences were measured between gut contents and fœtal extracellular fluid at various levels, using a Vibron 33*B* electrometer, calomel electrodes and 3 *M* potassium chloride in agar salt bridges. Representative segments of the gut were used to study the absorption of amniotic fluid to which polyethyas a glycol, mol. wt. 3,300–4,000, was added to serve lene volume marker[1]. The segments used were abomasum, jejunum, ileum and colon; approximately 8 cm. lengths of these latter three segments were used. Each segment, isolated by ligation, with intact blood supply, was flushed and filled with the labelled amniotic fluid and then left unexposed for 1–3 hr. The initial volume was calculated from the final weight of the contents and the change in concentrations of polyethylene glycol.

All significant potential differences (greater than ± 3 mV.) showed the gut lumen to be electrically negative with respect to the fœtal extracellular fluid at all ages studied. The abomasal potential difference was 14 mV. at 80 days, 21 mV. at 107 days and 26–30 mV. after 120 days. The potential difference across the small intestine was close to zero prior to 120 days, after which values of 4–10 mV. were obtained. The potential difference across the colon was close to zero at 80 days. From 120 days the transcolon potential-difference was 4–24 mV. These values may be compared with those obtained in the adult which were of the same sign: abomasum 40 mV., small intestine 10–14 mV., colon 15 mV.

Control experiments on single segments showed that the recovery of polyethylene glycol using a turbidimetric method[2] was of the order of 60–80 per cent over the experimental period. Calculations based on an assumed recovery of 60 per cent showed a net absorption of water, sodium and fructose from all segments at all ages studied. The amount of water absorbed from any segment was greater than could be accounted for by the hypotonicity[3] of the amniotic fluid. Sodium absorption occurred against a gradient of concentration and electrical potential. Absorption of fructose took place down a concentration gradient (140–500 mgm./100 ml. in amniotic fluid, 80–120 mgm./100 ml. in fœtal plasma), the fructose concentration in the gut decreasing. The results obtained for water absorption were extrapolated to the whole gut. From this it was computed that the 100–110 day fœtus absorbed fluid at a rate of 120 ml. per day. At 120 days this was about one litre, falling to 500 ml./day at term. The total volume of fluid absorbed from 80 days to term is about 32 litres, which is of the same order of magnitude as the volume of urine produced during this time (about 40 litres[4]). At 100 days fructose absorption occurred such that 6·7–10·7 per cent of the fructose introduced into the small intestine was absorbed in 1 hr., 12·6 per cent/hr. being absorbed from the colon. At 107 days the rate of fructose absorption was 9·0 per cent/hr. from the abomasum, 26–40 per cent/hr. from the small intestine and 46–47 per cent/hr. from the colon.

The results suggest that absorptive powers of the gut are retained in this species from at least 80 days. The gut is capable of absorbing a considerable volume of amniotic fluid with active sodium absorption probably accounting for much of the water absorption. Fructose absorption occurs at a high rate, and this may be of some nutritional significance to the fœtus. The fœtal abomasum appears to bear a functional similarity to the stomach of the fœtal rabbit in its capacity to absorb sodium actively[5].

This work was aided by an M.R.C. grant to Prof. A. St. G. Huggett and a grant to one of us (G. H. W.) from the Central Research Fund, University of London. We thank the Shell Chemical Co., Ltd., for donating the polyethylene glycol.

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Spontaneous Potentials from Explants of Chick Cerebral Hemisphere (Telencephalon) in Tissue Culture

USING techniques and supernatant similar to those used to demonstrate spontaneous potentials from explants of chick embryo cerebellum in tissue culture in Kahn tubes[1], spontaneous potentials have been demonstrated in explants from the telencephalon of 11-day chick embryo brain. The explants were thin slices of the full thickness of the lateral aspect of the telencephalon. The control used was dead chick cerebellum tissue (explanted into a Kahn tube in the same manner as the telencephalic tissue and then killed by heat). The Kahn tube containing the control tissue was placed side by side with the living culture material in the same incubator with identical amplification and recording apparatus. The living brain tissue gave the same form and magnitude of potentials on every recording channel which was tried; whereas the control tissues gave no potentials on any channel.

The form of the potentials are shown in Fig. 1 (26 hr. in culture). The typical activity is a combination of series of spikes of varying complexity. The simplest of these spike potentials arises sharply from the background and has a relatively uncomplicated form and an amplitude of between 20 and 30 μV. The time-interval between them varies somewhat but many recur at nearly 16½ sec. intervals. A second and more complex form is seen to occur almost independently of this first type and consists of a group of smaller potentials having amplitudes between 10 and 15 μV. Each group lasts about 7 sec. and they are about 2 min. apart. This behaviour is unlike the behaviour of spontaneous potentials already reported from explants of cerebellum and medulla oblongata[1,2], but has some similarity to that reported from explants of human cerebellum[3]. The explant continued to show this type of spontaneous electrical potentials until

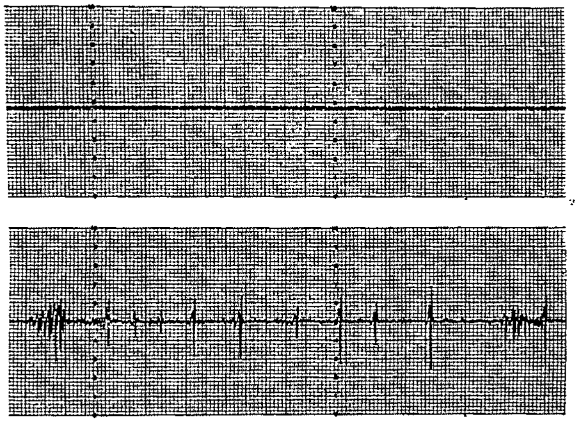

Fig. 1. Lower trace. Spontaneous potentials from an explant of 11-day chick telencephalon 26 hr. after explantation. Vertical lines, ½ sec. apart. Amplitude scale, distance between two horizontal lines equals 1½ μV. The photograph embraces one of the groups of potentials described in the text. Potentials at right-hand side of trace occurred before those at left-hand side. Upper trace. Control of dead cerebellar tissue under identical conditions with identical time and amplitude scales

after 72 hr. in culture. No record of spontaneous potentials from foci in the telencephalon in tissue culture has been found in the literature. Further investigations on these potentials are now in progress.

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RADIOBIOLOGY

Increase of Aldolase Activity in Blood Serum following Irradiation

Sahasrabudhe, Nerurkar and Baxi[1] have found that 2 hr. after whole-body irradiation with 600 r., the aldolase activity of rat liver and scelet muscle decreased by 25–30 per cent.

Experiments were conducted to investigate whether this decrease of aldolase activity in liver and muscle makes any difference to the aldolase activity of blood serum.

As decrease of aldolase activity in the liver and muscle may influence aldolase activity in serum only after some time has elapsed and, as Albaum[2] described, decrease of aldolase activity in rabbit serum 6 hr. after whole-body irradiation with 750 r., the aldolase activity in the serum of guinea pigs was determined 2, 4, and 6 hr. after irradiation with 500 r. (Siemens Stabilivolt apparatus, 180 kV., 10 m.amp., 0·5 copper, 50 cm. distance without a tube, 36·3 r./min. dose-rate). The determinations were undertaken only in serum—in some cases in plasma—samples without any visible sign of hæmolysis by the method of Tovarnicky and Voluyskaya[3] modified by Ananyev and Obouchova[4] using a Unicam *SP.* 500 spectrophotometer. Blood was withdrawn from the heart to avoid any admixture of tissue fluids.

The aldolase activity in the serum of 29 healthy guinea pigs was found to be 34·1 units (ranging from 22·7 to 45·0). In the plasma of 20 healthy animals it was found to be 33·1 units (ranging from 23·7 to 45·1).

The aldolase activity of serum samples 2. 4 and 6 hr. after irradiation was determined in the serum fo 44, 21 and 22 animals. It was stated that the activity reached its maximum 4 hr. after irradiation (Table 1).

Table 1. Serum Aldolase Activity in Healthy and Irradiated Animals

Healthy animals	After irradiation of 2 hr.	4 hr.	6 hr.
34·1 (22·7–45·0)	34·9 (23·1–52·8)	64·4 (41·9–109·3)	53·3 (41·0–72·3)

These results show that aldolase released from liver, and perhaps muscle cells damaged by irradiation appears in serum 1–2 hr. later, that is, 3–6 hr. after irradiation. Thus the determination of aldolase activity in serum may help to detect radiation damage.

Our preliminary investigations on persons suffering from malignant tumour diseases and receiving therapeutic whole-body irradiation gave similar results with the remarkable difference, however, that while in guinea pig the same aldolase activity was found both in whole blood and in plasma, in man the aldolase activity in whole blood was several times greater than in plasma.

Results of investigations to elucidate the cause of this difference and to establish the connexion between dose of irradiation and increase of aldolase activity in serum will be published later.

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Effect on Biological Nitrogen Fixation of Nitrogen Gas treated with Ultra-violet Radiation

Many different theories on nitrogen fixation by *Azotobacter* cells have been presented, among them the proposition of Wilson and Burris[1] that the nitrogen molecules, which are absorbed and take part in the reactions leading to nitrogen fixation, are in an activated state. These authors also suggest that this activated form might comprise a molecule possessing an energy greater than some unknown critical value.

If this suggestion is valid, it might be possible to increase the degree of nitrogen fixation by increasing artificially the energy of the nitrogen gas.

We are recording here some experiments based on this assumption, using ultra-violet light as a means of bringing about an increase in the energy of the nitrogen gas.

The organism used was *Azotobacter vinelandii*, strain *O*. The bacteria were grown in a nitrogen-free medium[2], modified to contain 30 gm. of glucose instead of 10 gm. of sucrose. 0·05–0·2 l./min. of air was passed into the cultures through sintered glass cylinders 20 mm. long and 10 mm. thick (maximal pore diameter 40–60μ). The rate of oxygen transfer for all the cultures was 150 mM O_2/l./hr., as measured by the sulphite method[3]. Separately, 0·4 l./min. of nitrogen was passed into the cultures through a

Table 1. THE EFFECT OF IRRADIATED NITROGEN GAS ON FIXATION OF NITROGEN IN CULTURES OF *Azotobacter vinelandii*, STRAIN *O*

In each experiment eight cultures were run simultaneously under identical conditions except for the variance in the irradiation of the nitrogen gas. All the cultures were gassed with the same amount of nitrogen and at the same oxygen transfer-rate. The nitrogen fixed by the bacteria is expressed as mgm. nitrogen fixed per gm. glucose consumed. The increase of the nitrogen fixation obtained in cultures gassed with irradiated nitrogen gas is expressed in per cent of the mean value of the controls

Experiment	Control		Lamps								
			6 W.			15 W.			30 W.		
	mgm. N per gm. glucose	Mean value	mgm. N per gm. glucose	Mean value	Per cent increase of N	mgm. N per gm. glucose	Mean value	Per cent increase of N	mgm. N per gm. glucose	Mean value	Per cent increase of N
1	8·9 9·1	9·0	10·8 11·4	11·1	23·3	9·5 9·8	9·65	7·2	10·1 10·6	10·35	15·0
2	5·2 5·6	5·4	6·2 6·8	6·5	20·4	5·7 5·9	5·8	6·9	6·0 6·3	6·15	13·9
3	4·1 4·2	4·15	5·4 4·9	5·15	24·1	4·3 4·7	4·5	7·8	4·5 5·0	4·75	14·4
4	4·5 4·9	4·7	5·8 —	5·8	23·4	4·9 5·1	5·0	6·4	5·3 5·6	5·45	16·0
Total mean					22·8			7·1			14·8

capillary of 1·5 mm. diameter. Arrangements were made to wash the nitrogen with pyrogallol and water, and to dry the gas with calcium chloride before it entered the cultures.

Eight cultures were run simultaneously. In six of them the washed and dried nitrogen gas passed through glass-mantled ultra-violet lamps before entering the cultures. The ultra-violet lamps used were Philips' 6 W., 15 W. and 30 W. germicidal lamps marked *TUV* 6, *TUV* 15 and *TUV* 30. The distance travelled by the nitrogen through the capillary from the glass mantle to the open end was 150 mm. The end of the capillary tube was 50 mm. below the surface of the culture. Precautions were taken to avoid direct or indirect irradiation of the cultures by ultra-violet light. The cultures were run for 30 hr. and the growth estimated by dry-weight determinations. The glucose was determined by the anthrone method[4,5]. After digesting 1 ml. of each culture in sulphuric acid and oxidizing the solution with perchloric acid, the nitrogen was determined colorimetrically[6].

Using nitrogen gas, treated with ultra-violet radiation, as a partial nitrogen source, the nitrogen uptake increased by 7–23 per cent.

Table 1 shows that the 6-W. lamp increased the nitrogen uptake by 23 per cent, the 15-W. lamp by 7 per cent and the 30-W. lamp by 15 per cent, compared to the non-irradiated control cultures. Controls run in tubes fitted with the ultra-violet equipment—lighted but not flushed with nitrogen—gave roughly the same values as the ordinary growth tubes. In this case the nitrogen was added through an extra capillary.

Further work on the influence of irradiated gases on bacterial cultures is in progress in this laboratory and will be reported later.

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HÆMATOLOGY

Hæmoglobins of Fœtal *C57BL*/6 Mice

THE existence and nature of differences within species between adult and fœtal hæmoglobins have attracted much attention[1-7], but until very recently a distinct fœtal hæmoglobin type had not been demonstrated in the mouse. Filter paper[8,9], starch block[10] and starch-gel electrophoresis of hæmoglobins of adult mice from different inbred strains and inter-strain crosses have shown inherited strain specificity for either of two general types of electrophoretic pattern, 'diffuse' (two or more components) or 'single' (discrete single band). Tests of hæmoglobin of new-born mice from several different inbred strains have given electrophoretic patterns indistinguishable from those of adults of the same strain[11,12]. However, starch-gel electrophoresis of adult and fœtal hæmoglobins in the *CBA* strain of mouse has shown the presence in early fœtal samples of a slowly moving component which disappears after 15 days gestation[11]. The *CBA* adult mouse has 'diffuse' hæmoglobin (three bands on starch-gel)[11], which renders interpretation of variations in the pattern rather difficult. The concept of a change in hæmoglobin type in mid-fœtal life rather than at birth is sufficiently novel to warrant repetition of the experiment with more favourable genetic material. Starch-gel electrophoresis of hæmoglobin of adult *C57BL*/6 mice reveals the presence of only one component, which provides a simple background against which to analyse variations in pattern. The present communication is a comparison of the starch-gel electrophoretic pattern of hæmoglobin from early and late fœtal and adult *C57BL*/6 mice.

In obtaining timed fœtuses, duration of the mother's pregnancy was timed from observation of vaginal copulation plugs. Blood collected from each fœtus of a litter, by cutting across the umbilical cord, discarding the placenta, and transecting the thorax, was pooled in a Petri dish containing heparinized saline. The adult and fœtal samples thus obtained were subjected to identical means of preparation: the cells were washed twice in 0·9 per cent saline and the packed cells lysed with their own volume of distilled water. One volume of molar phosphate buffer, *p*H 6·8, was added to precipitate the stromal proteins and other contaminants. The mixture was then centrifuged and the clear hæmoglobin solution removed and stored in the cold until required. Each sample was used on the day on which it was prepared,

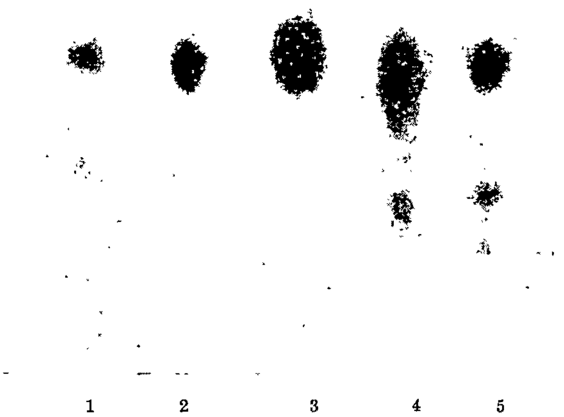

Fig. 1. Starch-gel electrophoresis (Poulik's discontinuous buffer system) of cyanmethæmoglobins prepared from 14-day fœtal and maternal *C57BL/6* blood and from a mixture of these two. 1, Fœtal; 2, mixture; 3, adult; 4, mixture; 5, fœtal

though results using hæmoglobins stored over several days in the cold showed that there was no appreciable alteration in the electrophoretic patterns.

The hæmoglobins were converted to the cyanmethæmoglobin forms and starch-gel electrophoresis carried out as described in a previous communication[11], except that a stronger solution of hæmoglobin was run for 4 hr. The gel was stained as before with an *o*-dianisidine–hydrogen peroxide mixture.

The electrophoretic patterns of hæmoglobins obtained from several groups of fœtuses of all ages from 12 days of intra-uterine life to birth were studied. At 12 days each of four tested litters showed three components, with the slowest moving present in a concentration (judged by intensity of spots) at least equal to that of the most advanced component. At 13 days (four litters) the fastest moving component was increased in intensity while the slowest moving one was diminished ; at 14 days (three litters) this slowest moving spot was disappearing (Fig. 1), and at 15 days (three litters) it was not discernible. The second spot, which had also been diminishing, though more slowly, was still seen at 15 days but was scarcely visible at 16 days. From 17 days on to birth and into adult life, only the first rapidly moving component was seen. In order to determine whether the fastest-moving component observed in these early fœtal samples corresponded in any way with the single hæmoglobin found in the adult animal, an additional sample, composed of two parts of fœtal to one part of adult hæmoglobin, was applied to a gel together with the standard fœtal samples and maternal control. It was felt that if one of the fœtal components corresponded with the adult hæmoglobin, three spots would be visible in the mixture, whereas non-correspondence might result in an indistinct blur or the presence of four components. The mixture separated well into three components with the first component in line with the single adult spot of the control (Fig. 1). It seems, therefore, reasonable to assume that the first fœtal component has the same electrophoretic mobility as the adult component ; however, such mobility may be due to interaction between the two hæmoglobins and does not in itself demonstrate complete correspondence of the two.

These results are similar to observations previously made on the *CBA* strain of mouse[11] in that the adult pattern replaced the fœtal pattern in late intra-uterine life of the mouse rather than at birth as in many other species. The observed transition in hæmoglobin patterns occurred at a time when the proportion of nucleated red cells in the fœtal circulation falls rapidly. From the 14th day to the 16th day the numbers of nucleated erythrocytes have been reported to fall from 21 to approximately 1 per cent[13]. Although at the present time it is not possible to state any specific physiological reason for a shift in hæmoglobin pattern in mid-fœtal life, it is tempting to conjecture some relationship with the shift in proportion of nucleated erythrocytes.

We wish to thank Drs. E. S. Russell and K. B. Roberts for their generous help and advice. This investigation was supported by grant *E*–76 to the Roscoe B. Jackson Memorial Laboratory from the American Cancer Society, and much of the work was carried out while one of us (J. B.) was holding a pre-doctoral fellowship from U.S. Public Health Service Grant *CRT*–5013 to the Roscoe B. Jackson Laboratory.

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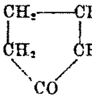
PATHOLOGY

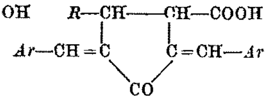
Anti-tumoral Action of New Sarcomycin Derivatives

The anti-tumour effect of sarcomycin-2 methylen *cyclo*-pentanon-3 carboxylic acid was reported so long ago as 1953 (ref. 1), and since then this substance has been widely employed for its broader chemotherapeutic effect on cancer. Recently one of us[2] proposed new procedures for the synthesis of compounds of sarcomycin type, and the preparation of several new sarcomycin derivatives is now possible.

In this communication we report on the action of some sarcomycin derivatives on the growth of the Ehrlich ascites tumour. The tetraploid Lipschutz IV strain of Ehrlich tumour has been used. The following procedure was adopted : from a donor mouse selected from mice with rapidly growing ascites, at 10 days after inoculation of cancer cells, 4 ml. of milky ascitic fluid were withdrawn. The cells were counted in a Spencer chamber and diluted with physiological saline buffered at *p*H 7. 0·2 ml. of the fluid

Table 1. EFFECT OF DIHYDROSARCOMYCIN AND ITS DERIVATIVES ON THE GROWTH OF EHRLICH ASCITES TUMOUR
Amount of compound administered by a single injection: 4 mgm./20 gm. body-weight. Column *a*: survival number on 10 injected animals. Column *b*: percentage increase in body-weight. Figures in black type refer to animals of the control group


(I)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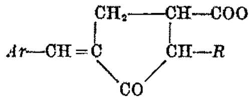
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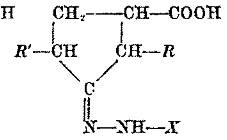
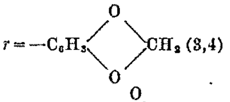
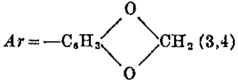
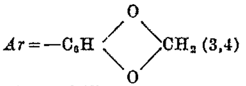
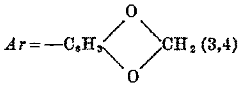

(IV)

Compounds	Days after injection: 7		10		13		16	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Dihydrosarcomycin: I, $R = CH_3$	6 **8**	20 **16**	5 **7**	28 **30**	3 **4**	43 **49**		
SB_1: II, $R = H$; $Ar =$ 	7 **8**	13 **10**	4 **6**	47 **32**	2 **4**	47 **50**		
SB_2: II, $R = CH_3$; $Ar =$ 	8 **7**	12 **9**	4 **5**	30 **29**	1 **3**	50 **47**		
SB_3: II, $R = H$; $Ar = -C_6H_6-OCH_3$ (4)	8 **8**	7 **10**	8 **7**	33 **29**	2 **4**	43 **46**		
SB_4: II, $R = CH_3$; $Ar = -C_6H_4-OCH_3$ (4)	8 **7**	13 **9**	7 **6**	33 **31**	2 **3**	54 **50**		
SB_5: III, $R = C_2H_5$; $Ar =$ 	8 **9**	12 **18**	8 **7**	20 **20**	6 **3**	21 **35**	4	16
SB_6: III, $R = C_2H_5$; $Ar = -C_6H_5$	8 **8**	13 **9**	8 **7**	19 **20**	7 **3**	22 **40**	6	29
SB_7: III, $R = C_2H_5$; $Ar = -C_6H_4-OCH_3$ (4)	9 **8**	12 **10**	9 **6**	15 **20**	9 **4**	30 **33**	9	44
SB_8: III, $R = C_2H_5$; $Ar = -C_6H_4-N(CH_3)_2$ (4)	10 **7**	10 **13**	9 **6**	15 **27**	9 **3**	35 **40**	9	44
SB_9: omodihydrosarcomycin: I, $R = C_2H_5$	6 **8**	29 **33**	5 **6**	46 **40**	 **3**	 **46**		
SB_{10}: III, $R = CH_3$; $Ar = -C_6H_5$	6 **7**	24 **30**	6 **5**	50 **41**	 **2**	 **50**		
SB_{11}: III, $R = CH_3$; $Ar =$ 	6 **8**	22 **19**	6 **6**	57 **50**	 **3**	 **59**		
SB_{12}: III, $R = CH_3$; $Ar = -C_6H_4-OCH_3$ (4)	7 **7**	21 **15**	7 **6**	57 **37**	 **2**	 **50**		
SB_{13}: III, $R = CH_3$; $Ar = -C_6H_4-N(CH_3)_2$ (4)	7 **7**	23 **30**	7 **6**	23 **32**	7 **3**	23 **39**		
SB_{14}: IV, $R = H$; $R' = C_2H_5$; $X = -CONH_2$	9 **7**	16 **13**	8 **6**	20 **22**	7 **3**	23 **28**		
SB_{15}: IV, $R = C_2H_5$; $R' = H$; $X = -CSNH_2$	6 **7**	4 **19**	5 **5**	5 **28**	4 **2**	5 **36**	3	7
SB_{16}: IV, $R = C_2H_5$; $R' = H$; $X = -C_6H_3(NO_2)_2$ (3,4)	7 **7**	30 **19**	6 **6**	41 **30**	4 **4**	62 **51**		
SB_{17}: IV, $R = H$; $R' = C_2H_5$; $X = -CSNH_2$	6 **8**	10 **20**	6 **4**	42 **35**	 **1**	 **68**		
SB_{18}: IV, $R = CH_3$; $R' = H$; $X = -CSNH_2$	8 **7**	21 **21**	4 **4**	26 **30**	 **1**	 **49**		
SB_{19}: II, $R = C_2H_5$; $Ar = -C_6H_4OCH_3$ (4)	8 **8**	21 **19**	6 **5**	48 **37**	 **3**	 **40**		
SB_{20}: II, $R = nC_3H_7$; $Ar = -C_6H_4OCH_3$ (4)	9 **8**	18 **20**	6 **4**	58 **35**	 **2**	 **56**		

containing approximately 3 million cancer cells was injected intraperitoneally into the lower abdominal region into each of a number of young Swiss albino mice, weighing about 20 gm.

In each set of experiments the animals were divided in two groups, one treated with the compound, and the other used as control. The compounds to be tested were dissolved as calcium salt with saline and the resulting solution was adjusted to *p*H 7. Before the inoculation, the solutions were filtered through a Berkefeld candle, and aseptic precautions were maintained during the procedure. Each animal received one single administration of 4 mgm. immediately after the injection of cancer cells. The effectiveness of the compounds against the growth of the ascites tumour was recorded by the determination of survival-rate of treated animals, by measurements of the amount of ascites fluid after treatment (weighing the mice), and by cytological examinations of ascites tumour cells.

Table 1 shows the main results of the treatment with dihydrosarcomycin and with sarcomycin derivatives.

After early administration of the compounds, inhibition of ascites tumour is clearly shown, in some cases, on the basis of survival-rate and of increase of body-weight. The most effective compounds were found to be SB_5, SB_6, SB_7, SB_8 and SB_{15}, in each of which the CH_3CH_2 radical had been introduced; these are derivatives of omodihydrosarcomycin[3].

Further to clarify the chemotherapeutic action of those compounds, inhibition of the ascites tumour has been also evaluated by means of other parameters, as reported in Table 2.

The results in Table 2 clearly indicate that the sarcomycin derivatives really affect the growth of

Table 2

Compound	Mitotic rate	No. of cells/μl.	Mean total volume of fluid	Mean percentage living cells (Schrek)
Dihydro-sarcomycin	24	$5 \cdot 2 \times 10^4$	4·8 ml.	96
SB_5	20	$4 \cdot 6 \times 10^4$	4·2	94
SB_6	18	$5 \cdot 0 \times 10^4$	4·0	80
SB_7	14	$3 \cdot 1 \times 10^4$	2·5	46
SB_8	17	$4 \cdot 0 \times 10^4$	3·1	50
SB_{15}	15	$2 \cdot 9 \times 10^4$	2·9	28
Control	28	$7 \cdot 3 \times 10^4$	6·2	100

Ehrlich ascites tumour. It is also evident that the dihydrosarcomycin is less active than its derivatives.

It must be emphasized that in the evaluation of chemotherapeutic results, the dose and time of administration of the drug are very important. Under our conditions, one single dose only was given, whereas it is well known that a bigger effect can be obtained when several doses are given after the injection of cancer cells.

Detailed results of the anti-tumoral action of sarcomycin derivatives will be reported elsewhere.

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Reversible Nature of Liver Cell Damage due to Carbon Tetrachloride as demonstrated by the Use of Phenergan

Rees, Sinha and Spector[1] have suggested a possible sequence of events in the rat liver following the administration of carbon tetrachloride. Thus at 6 hr. there is a large increase in liver fat and liver cell damage verging on necrosis. In addition, the hepatic enzymes, malic and *iso*citric dehydrogenase, can be detected in the blood. By 12 hr., histological examination shows early hepatic necrosis and massive fatty change, and glutamic dehydrogenase can be detected in the serum. As glutamic dehydrogenase is wholly a mitochondrial enzyme, this latter observation is indicative of mitochondrial injury. By 24 hr. the concentration of hepatic enzymes in the blood is maximal, as is the quantity of the fat in the liver. Histologically, there is widespread parenchymal cell necrosis. Recknagel and Anthony[3] have emphasized that the accumulation of fat in the liver occurs before mitochondrial injury becomes demonstrable by *in vitro* enzymological study, and have suggested that the initial effect of carbon tetrachloride on the liver occurs at about 3 hr.

In previous experiments it was found that the administration of phenergan ('Promethazine HCl') at 0 and at 6 hr. to animals poisoned with carbon tetrachloride modifies considerably the above sequence of events[4]. In rats receiving both phenergan and carbon tetrachloride, there was no significant liberation of hepatic enzymes into the blood and hepatic necrosis was almost absent in the first 24 hr. On the other hand, the hepatic fatty change was unaltered both in appearance and in quantity.

In the present investigation, these observations have been extended. It was found that if animals given carbon tetrachloride and phenergan at 0 and 6 hr. were given no further doses of phenergan in the period 24–48 hr., liberation of hepatic enzymes into the circulation was demonstrable from 24 hr. onwards, and by 48 hr. there was hepatic necrosis of the same order as found in the liver of a rat not treated with phenergan 24 hr. following poisoning with carbon tetrachloride. However, if the poisoned animals received additional treatment with phenergan during the 24–48 hr. period, hepatic necrosis and release of hepatic enzymes into the blood were prevented almost completely, and even at 72 hr. these changes were not observed (Table 1).

Table 1. Degree of Liver Necrosis and Level of Serum *iso*citric Dehydrogenase following Carbon Tetrachloride Poisoning in Rats with and without Phenergan Treatment

Time after carbon tetrachloride poisoning (hr.)	Time of phenergan treatment (hr.)	Serum *iso*citric dehydrogenase (μl. oxygen per ml. per hr.)	Degree of necrosis	Comment
0	nil	1 (0·6–1·7)	0	—
24	nil	153 (144–180)	+ + + +	Active necrosis
24	0 and 6	12 (9–15)	+	—
48	nil	35 (28–40)	+ + + +	Healing necrosis
48	0 and 6	78 (60–84)	+ + + +	Active necrosis
48	0, 6, 24 and 30	11 (2–17)	+	–
72	nil	3 (1·5–4)	+ + +	Healing necrosis
72	0, 6, 24 and 30	1·0 (0·6–1·7)	+	–

The animals were dosed with 1·25 ml. carbon tetrachloride/kgm. body-weight. When phenergan treatment was given, the dose at 0 hr. was 25 mgm./kgm. body-weight and all subsequent dosage was at 12·5 mgm./kgm. body-weight level. Albino female rats (120–200 gm. body-weight) were used.

Active necrosis denotes areas of eosinophilic coagulative necrosis of liver cells. Healing necrosis denotes extensive infiltration of these areas by chronic inflammatory cells.

Since phenergan fails to prevent fatty accumulation in the liver, it is clear that the drug does not act by detoxicating carbon tetrachloride. In addition, the development of hepatic necrosis at 48 hr. after administration of carbon tetrachloride to animals given only two doses of phenergan would also suggest that phenergan had not detoxicated the carbon tetrachloride but rather prevented a secondary 'necrogenic' reaction in the liver cell.

Carbon tetrachloride reaches its maximum concentration in the liver about 3 hr. after administration, and thereafter the concentration falls rapidly[4]. 24 hr. following poisoning with carbon tetrachloride, chemical estimation discloses that the concentration of the latter in the liver has dropped to zero, and in the present investigation using carbon-14 tetrachloride it was not possible to detect any radioactivity in the livers of animals 24 hr. after poisoning.

Thus, the delayed appearance of hepatic necrosis 24 hr. after administration of carbon tetrachloride in rats given phenergan at 0 and 6 hr. only occurs in the absence of carbon tetrachloride as demonstrable by chemical and radiochemical techniques.

The simplest explanation of these results is to assume that carbon tetrachloride irreversibly damages a reaction that is non-essential in the short term for the survival of the cell, and that as one consequence of this primary injury there is a secondary disturbance, for example, the entry of sodium and water into the cell. It is this secondary disturbance which is prevented so long as phenergan is present. It seems that the primary injury caused by carbon tetrachloride will continue in force, after its concentration has dropped, until the vital constituents of the damaged system are re-synthesized. This process appears to take up to 48 hr.

The hypothetical secondary disturbance could be associated with the breakdown of phosphoprotein, a system which Judah[5] has shown to be sensitive to the action of phenergan and has postulated as part of the mechanism controlling cellular water and electrolyte movements.

The reason for stating that the primary site of attack of carbon tetrachloride is a system not immediately essential for the life of the cell is that, in the presence of phenergan, the damage caused by carbon tetrachloride is reversible. It is clear that this conclusion carries with it some important implications, including therapeutical possibilities.

We are grateful to Sir Roy Cameron and Prof. C. Rimington for their advice and encouragement. One of us (K. R. R.) is indebted to the Medical Research Council for a grant for assistance.

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Alloxan and Hypersensitivity

During the course of another investigation, it was found that a diabetogenic dose of alloxan (175–200 mgm./kgm.)[1] protects approximately 50 per cent of rats sensitized to horse serum and later injected intravenously with this antigen. The time of death was lengthened in those rats suffering lethal shock. Controls consistently showed 0–10 per cent survivors.

Rats were of Sprague–Dawley strain, sensitized by a single intraperitoneal injection of a mixture of horse serum and *H. pertussis*. Alloxan was injected subcutaneously into the sensitized rats just prior to, or four days before, challenge with horse serum. Of the various routes tested, the subcutaneous route for injection of alloxan proved the least toxic. The purpose of this communication is to describe some aspects of the effect of alloxan on hypersensitivity reactions in the rat and guinea pig.

One unit of insulin, injected subcutaneously into alloxan-treated sensitized rats 1 hr. before challenge, partially reversed the protective effect of the alloxan. This amount of insulin also increased the speed with which death occurred in untreated sensitized controls (Fig. 1). Ganley[2] recently reported similar findings in mice.

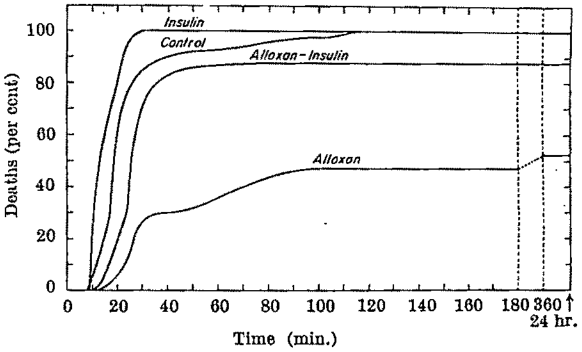

Fig. 1

In the guinea pig, alloxan injected either intraperitoneally or subcutaneously at 300 mgm./kgm. body weight four days prior to challenge reduced the size and intensity of the skin reaction to PPD in animals sensitized by injection of killed *M. tuberculosis* in paraffin oil emulsion. Long, Miles and Perry found a similar action of alloxan in guinea pigs infected with living *BCG* [3]. Alloxan did not protect against either 'immediate' or 'delayed' type anaphylaxis. However, it did appear to give protection during the first 24 hr. to the type of shock induced by intraperitoneal challenge with old tuberculin. Failure to protect from anaphylactic shock in the guinea pig may be due to the very swift occurrence of emphysema of the lungs and death from bronchospasm.

The protective effect of a diabetogenic dose of alloxan in rat anaphylaxis and delayed skin hypersensitivity in the guinea pig, with its reversal in the rat by insulin, suggests the involvement of a lack of insulin or an excess of glucose. We have evidence that large amounts of glucose (4 gm./kgm. × 2) injected intraperitoneally prior to challenge gives at least some degree of protection.

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ANATOMY

Function of the Fœtal Thyroid of the Rabbit with regard to Cholesterol Metabolism studied by Fœtal Thyroidectomy

In earlier communications, it was reported that the fœtal pituitary–thyroid system influenced cholesterol and fat metabolism. Following removal of this system, a significant rise in the serum cholesterol occurred[1], and also in the fat content of the fœtal liver[2]. The technique for removing the fœtal pituitary and thyroid involves decapitation of the fœtus either through the neck to remove both the thyroid as well as the pituitary, or through the mouth to preserve the thyroid. It has already been shown that this operation has no significant effect on the weight development of the fœtus[2–4], but it may be objected that the metabolic changes may be the result of removing structures other than the pituitary and the thyroid, such as the brain.

To overcome this objection and to investigate this problem further, the fœtal thyroid was removed surgically on the twenty-second to twenty-third day of pregnancy and each operated fœtus then allowed to continue development within the uterine cavity. These experimental fœtuses with their litter mates for controls were recovered just before term on the twenty-ninth to thirtieth day of pregnancy by Cæsarian section. The serum cholesterol-levels were determined (as indicated in ref. 1) and the results are shown in Table 1.

Table 1. SERUM CHOLESTEROL IN THYROIDECTOMIZED AND CONTROL RABBIT FŒTUSES

Fœtuses	Age at investigation	No.	Mean serum cholesterol (mgm./ 100 ml. ± *S.E.*)	
Thyroidectomized (at 22–23 days)	29–30 days	7	129*	± 7·7
Litter-mate controls	29–30 days	32	86*	± 3·5

* These differences are significant, $P < 0.001$.

There is no significant difference in weight development of thyroidectomized and control fœtuses. The operated fœtuses all show a significant increase in serum cholesterol, and this indicates, therefore, that the fœtal thyroid plays a part in fœtal cholesterol metabolism. In addition, these results indicate that the maternal thyroid secretions are unable to compensate for the absence of the fœtal thyroid.

One of us (J. G. B.) expresses his indebtedness to Prof. A. Jost for his help in developing techniques for intra-uterine surgery of the fœtus, and to the Medical Research Council for a grant towards apparatus.

We are indebted to Prof. E. W. Walls and Prof. A. C. Dornhorst for their interest and guidance in this investigation.

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BIOLOGY

Transport of Carbon in Fungal Mycelium

INTEREST in the problem of translocation by fungi has been stimulated by Schütte's paper[1], and this communication is concerned chiefly with the movement of carbon compounds through an established mycelium.

We have investigated three aspects of translocation by fungi:

(1) Ability to grow from a 'complete' nutrient medium on to a 'deficient' medium (distilled water-agar) using the dish method of Schütte. Of 20 species representing all classes of the fungi, 10 were able to grow on to the deficient medium ('translocating' fungi *sensu* Schütte), 6 were unable to do so ('non-translocating' fungi) and 4 were indeterminate.

(2) The ability of growing mycelium to transport labelled carbon compounds using the dish technique with sucrose-^{14}C in the centre dish. All 'translocating' fungi were capable of moving compounds labelled with carbon-14 as the mycelium grew on to either deficient or complete media. However, 'non-translocating' species were also able to move labelled compounds when their mycelium grew out on to a complete medium; the possibility of respired $^{14}CO_2$ being re-absorbed by the growing mycelium was examined and rejected. This observation is at variance with Schütte's hypothesis that growth on to a deficient medium was evidence for translocation by a fungus.

(3) Movement of compounds labelled with carbon-14 through an established mycelium. Three species were used; two were selected as exemplifying the behaviour of 'translocating' fungi (*Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind. and *Pellicularia filamentosa* (Pat.) Rogers) and one as a 'non-translocating' fungus (*Saprolegnia ferax* (Gruith.) Thuret) in the sense of Schütte.

The dish technique was used, there being a distance of approximately 4·6 cm. between the edge of the centrally placed, inner dish and the circumference of the outer dish. The complete medium, potato dextrose +0·1 per cent peptone, was chosen because it supported better growth of a wide range of fungi than any simple, synthetic medium; replication was achieved by using only a single batch of Oxoid dehydrated potato extract. The deficient medium was distilled-water agar containing Oxoid 'Ionagar No. 2'.

Complete liquid medium (approx. 15 ml.) was placed into the inner dish, and the outer dish poured with either deficient or complete agar medium. After inoculation of the inner dish and incubation at 25° C. for 14 or 16 days, some of the medium was withdrawn from the inner dish and replaced with 5-ml. fresh, complete liquid medium containing glucose-^{14}C having an activity of 5 μc. By this time the mycelium had usually completely covered the medium in the outer dish.

Incubation was continued, and at intervals the mycelium on the agar medium was sampled by cutting 3 disks (area 1 cm.^{2}) along a radius, the innermost close beside the central dish, the outermost at the circumference of the outer dish, and the third sample at a point midway between these two. The samples were counted and the results are presented in Table 1.

Some activity could be detected in the mycelium one hour after the glucose-^{14}C had been supplied. Thereafter, activity increased progressively at the innermost and central sampling sites up to about 78 hr., but there was little indication of accumulation at the outermost site, possibly because of more rapid catabolism in the physiologically younger part of the mycelium. The aerial mycelium of *R. stolonifer*

Table 1. MOVEMENT OF COMPOUNDS LABELLED WITH CARBON-14 THROUGH ESTABLISHED MYCELIUM

Elapsed time (hr.)	*P. filamentosa*						*R. stolonifer*						*S. ferax*		
	Deficient			Complete			Deficient			Complete			Complete		
	I	*M*	*O*	*I*	*M*	*O*	*I*	*M*	*O*	*I*	*M*	*O*	*I*	*M*	*O*
1	0	1	1	0	1	—	2	0	0	0	0	4	12	2	1
6	9	1	2	6	1	—	3	0	2	2	0	29	9	1	1
24	23	11	4				9	0	0				40	8	9
54	26	17	17	55	22	—	33	10	6	54	9	7	64	17	2
78	64	23	16	69	28	—				80	16	10	56	16	3
120							56	17	2				50	19	13

I, Innermost; *M*, middle; *O*, outermost sampling site; results expressed in counts/cm.^{2}/min. above background.

on the lid of the dish at the edge of the colony was also active.

Recently, Lucas[2] showed that compounds labelled with phosphorus-32 are not transported through an established mycelium, and suggested that their movement was normally concomitant with growth of the hyphal tips. Possibly a sink for carbon is maintained by respiration in the younger part of the mycelium, whereas a similar sink for phosphorus would not exist. Consequently, we do not consider our results are in conflict with those of Lucas, but that they reflect different metabolic patterns for carbon and phosphorus. However, when considered together, Lucas's results and our own are contrary to a 'mass flow' hypothesis of translocation in mycelium.

This work is being extended to include a study of the movement of phosphorus, and the forms in which carbon and phosphorus move in the mycelium.

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Prevention of Manganese Deficiency in Oat by soaking the Grain in Solutions of Manganous Chloride

TRACE element deficiencies in crops are normally overcome by applying the required element to the soil or by spraying the plant with a solution containing it. However, it has been found that soaking seed in a solution of a salt of the required element is an effective way of preventing the development of deficiency symptoms in the crops grown from soaked seed[1–4]. Grey speck of oat, caused by manganese deficiency, is prevalent in many areas of west Scotland due usually to induced manganese deficiency associated with high soil *p*H. To our knowledge, the possibility of preventing this condition by seed soaking has not been reported before. The following investigations were carried out to determine the possible usefulness of such treatments.

The effects of different soaking treatments on the germination capacity of oat were determined. 5-gm. samples of oat grains (*Avena sativa* L. var. Victory) were soaked in 100 ml. of water or solutions of manganous chloride ranging in concentration from 0·2 per cent to 10·0 per cent w/v. After surface drying, the grains were germinated at 10° C. on moist filter paper in Petri dishes. With all soaking treatments longer than 36 hr., there were reductions in the rate of germination, and the final germination percentage. The magnitude of the effects was proportional to the concentration of the manganous chloride solution. With soaking periods less than 24 hr., and solutions less than 2 per cent manganous chloride, there was little adverse effect on germination.

The full requirement of manganese for a crop of oats has been reported to be 7 ounces per acre[5]. To meet this requirement, it is necessary for the grain to have a manganese content of 1,450 mgm. per lb. at the time of sowing, on the basis of an effective sowing-rate of 140 lb. per acre. The manganese content of the grains used in our experiments was 21·5 mgm./lb. In order to be successful there would need to be a considerable uptake of manganese during the soaking period, and at the same time, germination must not be affected adversely. An estimate of the amounts of manganese taken up during soaking was obtained by measuring the amount of manganese left in solution at the end of treatment using the colorimetric method of Sideris[6]. Some of the results obtained are given in Table 1.

Table 1

Length of soaking period (hr.)	Concentration of manganous chloride solution (percentage w/v)	Manganese content of grains (mgm./lb.)	Final germination (percentage)
0	0	21·5	100
8	2	1,290	94
24	2	2,290	91
24	10	5,920	15

From these figures it can be seen that the manganese content of the grain can be raised to a level which, without compromising germination, provides the full crop requirement. This was tested by growing plants from treated and untreated grain in nutrient solutions with and without manganese.

Twenty plants grown from grains treated by soaking in water or manganous chloride solution were supplied with modified Hoagland's solution[7] with or without manganese. Half of each sample was gathered after 63 days, and the remainder at maturity, after 114 days. The average dry weights of the plants are shown in Table 2.

Table 2

Length of soaking period	Soaking solution	Nutrient solution	Average dry weight (gm.) at 63 days	Average dry weight (gm.) at 114 days
24 hr.	distilled water	Complete	0·59	5·05
24 hr.	distilled water	manganese-free	0·26	3·27
8 hr.	2 per cent $MnCl_2$	manganese free	0·40	4·99
24 hr.	2 per cent $MnCl_2$	manganese-free	0·73	4·41

Grey speck was observed on the control plants grown in manganese-free solutions before the 63-day stage, but was not observed at any time on any of the plants in the other treatments. The dry-weight figures bear out the contention that the method is a feasible way in which to provide the oat crop with its manganese requirement.

The possible use of seed-soaking treatments to overcome manganese deficiency under field conditions was investigated on a preliminary basis during 1959 using soil rich in shell sand from the Island of Tiree. Manganese-deficiency symptoms were not obvious in plants from treated grain, while plants from untreated grain showed typical grey speck and considerably reduced growth. Full-scale trials will be carried out this year and reported elsewhere.

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Nucleic Acids and Sexual Differentiation in *Allomyces*

THE finding that thymine is the most effective nucleic acid base in inducing proliferation of male gametangia in *Allomyces*[1] is of special significance for an understanding of the biochemical basis of sex differentiation in these water-moulds.

A first step towards understanding the nature of the thymine effect in *Allomyces* can be derived from recent demonstrations that this nitrogen base induces a selective, stimulatory effect on nuclear multiplication, with concomitant increase of the deoxyribonucleic/ribonucleic acid (DNA/RNA) ratio in the related mould *Blastocladiella*[2]. Consequently, we could expect a similar effect in *Allomyces* and safely predict that in promoting increased DNA synthesis relative to RNA (higher DNA/RNA ratios) in the cytoplasm of differentiating hyphæ, thymine automatically favours male differentiation, with its attributes of high rate of nuclear multiplication and low RNA basophily.

The gametophytic strains of *Allomyces* have been grown 8–10 days on Emerson's 'YpSs'[3] agar medium (Difco) in Petri dishes at 25° C. and the gametes collected by mild centrifugation 2–3 hr. after flooding the plates with Machlis's 'DS' solution[4]. Pellets of gametes were carefully washed twice with distilled water before their dispersal in 0·4 *N* potassium hydroxide for a 1 hr. extraction at 100° C. Two aliquots were then withdrawn and used according to McIntire and Sproull's[5] analytical procedure : (1) for selective DNA precipitation with salamine (protamine sulphate, supplied by Gen. Biochem. Inc., U.S.A.) 0·05 per cent at 4° C. overnight, followed by sodium chloride 10 per cent–sulphuric acid 0·1 *N* hydrolysis (30 min.) of the precipitate, previously washed with ice-cold distilled water ; (2) for direct, sodium chloride 10 per cent–sulphuric acid 0·15 *N* hydrolysis at 100° C. (30 min.) to get the total purines and pyrimidines fraction. Measurements of both fractions at 268 and 330 mμ with a model *DU* Beckman spectrophotometer gave the basis for final calculations of the quantities of DNA and total purines and pyrimidines, using McIntire and Sproull's formulæ. RNA (ribonucleic acid) was considered as total purines and pyrimidines less DNA (negligible, soluble pool of purines and pyrimidines in the RNA nuclear cap-containing gametes of Allomyces). Proteins were determined with the biuret reaction[6].

As shown by my results summarized in Table 1, I have measured consistently a higher DNA/RNA ratio in the mixed populations of gametes liberated from the gametophyte of *Allomyces macrogynus* Emers. grown in the presence of additional thymine. Of importance is the fact that uracil, the corresponding RNA base, was ineffective in *Allomyces* as previously shown in *Blastocladiella*[2]. Such higher DNA/RNA ratios measured in the gametes of thymine-treated *Allomyces* conform to the expected higher proportion of male gametangia (minimal 75 per cent) differentiated under such conditions, and to the relatively smaller RNA nuclear caps[7] of the predominantly male gametes liberated. Incidentally, these determinations provide chemical confirmation of earlier microscopic data on the smaller value of the nuclear cap/nucleus volume ratio in the male compared to the female gametes in *Allomyces*[8]. Similar verification was obtained : (1) for the gametes of a male colchicine mutant[1] in comparison with the composite population of female and male gametes of the wild-type of *A. macrogynus* ; (2) for the gametes of a male, hybrid strain (*A. macrogynus* × *A. arbuscula*[9]) compared with the gametes of a female, similar hybrid strain (Table 1).

Table 1

Cultures (8–10 days, at 25° C.)	♂/♀ gametangia	DNA/RNA* in gametes liberated
1. *Allomyces macrogynus* Emers.:		
Wild-type on 'YpSs' 'Difco' medium	1/1	9·6
Wild-type on peptone-glucose† ('PYG Cantino' 'Difco' medium)	1/2	8·4
Wild-type on 'YpSs' + thymine (2×10^{-3} *M*)	4/1	10·9
♂ Colchicine mutant on 'YpSs' medium	3/1	10·7
2. *A. macrogynus* × *A. arbuscula:*		
♀ hybrid strain	2/98	6·9
♂ hybrid strain	92/8	12·8

* Average values from three experiments.

† Feminizing medium.

Moreover, the higher proportion of DNA in the male gametes could also be verified on a protein basis :

male hybrid strain	12·0 μgm. DNA/mgm. protein
female hybrid strain	6·4 μgm. DNA/mgm. protein

The use of nucleic acid antagonists has tended to confirm the importance of the DNA/RNA balance in the biochemical basis for sex differentiation in *Allomyces* : at relatively high concentrations (10^{-2}–10^{-3} *M* in solid 'YpSs' 1/2 medium) all compounds favouring an increased DNA/RNA ratio in *Blastocladiella*[2]—that is, thiouracil, bromouracil, *iso*cytosine and (paradoxically) aminopterin—induced a significant increase in the male/female ratio (up to 75 per cent males on 10^{-2} *M* thiouracil) in *A. macrogynus*. Inversely, pteropterin, which tended to lower the ratio in *Blastocladiella*, showed a feminizing effect in *Allomyces*.

In conclusion, it appears as if differentiation of male sex organs in *Allomyces* is associated with the genetically controlled, polar accumulation of a biochemical system favouring more active DNA synthesis in the prospective male territory of the cytoplasm of differentiating hyphæ. Conversely, the prospective female territory is characterized by polar segregation, in this region of the cœnocytic cytoplasm, of a more 'vegetative' type of nucleic acid metabolism which favours RNA synthesis and—with its potential role in protein synthesis—confers on the future female gametes their well-known capability for parthenogenetic development.

I am indebted to Prof. Ralph Emerson, University of California at Berkeley, for his generous gift of the two hybrid strains of *Allomyces*. Thanks are also expressed to Prof. E. C. Cantino in whose Laboratory at Michigan State University, East Lansing, my first nucleic acid determinations were made.

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Determination of the Volume and Weight of Living Animals

WHEN accurate measurements of the weight of aquatic animals are necessary, most authors remove the adherent water by towelling the body with filter paper and weigh quickly and repeat until a constant weight is obtained. The method is defective since, apart from possible damage to the animals, the degree of dryness reached may not be the same each time. These difficulties increase when the animals are small and several have to be used at a time. Similarly, determinations of volume necessitate an elaborate procedure or the use of complicated apparatus.

For small marine Crustacea I find the following procedure better than the volumometer which I described[1]. A basket is suspended by a nylon thread from one arm of a balance and immersed in a definite volume of sea-water in a beaker. The apparent weight of the basket and the thread is determined. The animals are then transferred to the basket and weighed. Next, a fourth of the sea water in the beaker is removed and distilled water is added, stirred and the animals weighed again. The animals are removed and the empty basket is weighed in the 75 per cent sea-water. The difference in apparent weights of the animals in sea-water of two densities will thus help to calculate the volume of sea-water displaced and also the weight in air.

It has been found that determinations of apparent weight are not vitiated by osmotic transport, since most Crustacea take more than 10 min. to respond to changes in media. This method is particularly suitable for making continuous observations on the rate of water transport in animals left in anisotonic media.

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β-Galactosidase Changes in the Developing Intestinal Tract of the Rat

IN some micro-organisms β-galactosidase (lactase) is an adaptive enzyme, that is, its biosynthesis is induced by external agents such as lactose or lactose-related substances[1]. Apparently this is not the case for the intestinal β-galactosidase of the rat, since a lactose-rich diet does not modify the intestinal specific activity[2]. In the work reported here, the behaviour of this enzyme has been investigated in the albino rat, starting from the last third of the intra-uterine life until adult age.

Homogenates of the small intestine from the duodenal flexure to the ileo-cæcal valve were used. The enzyme was determined by the *ortho*-nitrophenol released from the *ortho*-nitrophenyl-β-galactoside, at 38° C. and *p*H 3·5[4]. The age of fœtuses was estimated according to the histo-differentiation shown in the duodenal, ileo-cæcal and umbilical regions[3], independently of the biochemical values. This technique allows a very close correlation between histological and biochemical data.

The changes in β-galactosidase activity during the end of pregnancy, new-born period and adult age of the rat are plotted in Fig. 1.

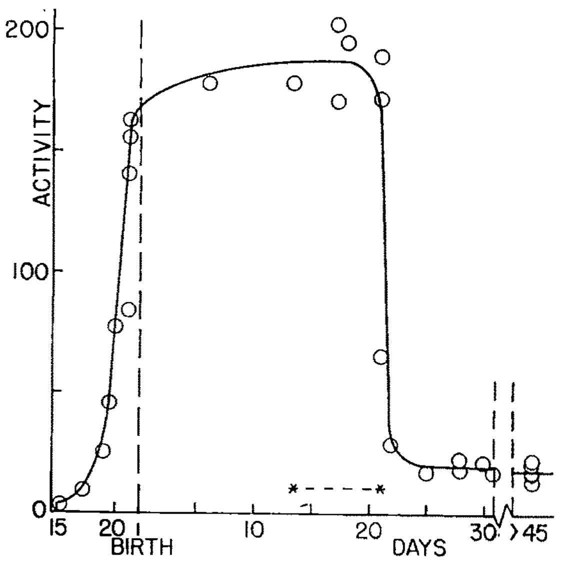

Fig. 1. Intestinal β-galactosidase activity expressed as mμmoles of *ortho*-nitrophenol released per mgm. of tissue (wet weight) in 1 hr. *----* Transition from lactation to adult diet; gestation, 22 days

The enzyme activity was also examined in different physiological and dietetic conditions: in adults on a lactose-rich diet, in pregnant rats, in nursing mothers, and in young rats kept after weaning on a complete diet with lactose (25–30 per cent) as the only carbohydrate (lactose-rich diet). The results are recorded in Table 1 together with the values for the normal adult and suckling rats.

Table 1. INTESTINAL β-GALACTOSIDASE ACTIVITY EXPRESSED AS MμMOLES *ortho*-NITROPHENOL RELEASED PER MGM. OF TISSUE (WET WEIGHT) PER 1 HR.

Diet	Condition	No. of animals	β-galactosidase activity	Increase
Normal	Adult	5	18 ± 2·9	×1·00
Normal	Pregnant	4	18·6 ± 1·0	×1·03
Normal	Nursing	3	18·4 ± 2·4	×1·02
Mother's milk	1–20 days old	14	183 ± 8·8	×10·0
Lactose*	Adult	3	19·3 ± 2·1	×1·07
Lactose	25–27 days old	3	19·8 ± 3·0	×1·10

* 'Lactose' means a complete diet with lactose 25–30 per cent as the only carbohydrate.

The features which became apparent from these results are: (1) The specific activity does not change on the diet rich in lactose (no induction in a strict sense), confirming discoveries of other authors[2]. (2) There are no significant changes either in the pregnancy or in the nursing period. (3) Activity increases rapidly at the end of fœtal life until birth, parallel to the very fast histo-differentiation of the digestive tract[3]. The high level remains rather constant in the new-born. (4) At the time of weaning (21–22 days) the activity decreases abruptly to the low adult-value. (5) This net decrease in the activity also occurs when the new-born is kept on the lactose-rich diet.

Several authors have shown that lactose hydrolysis is necessarily previous to its intestinal absorption; the somewhat low β-galactosidase adult activity makes its action a limiting step in lactose assimilation, and therefore laxation and diarrhœa occur[2]. An activity ten times higher in the new-born, as compared with the adult animal, is in agreement with the expected great efficiency as regards milk lactose assimilation in the sucklings. The 'physiological immaturity of the new-born' seems to be in this case rather a physiological adaptation (in a broad sense) to a milk diet. In any event, β-galactosidase belongs to the few biochemical systems showing an activity in this period, generally marked by enzyme deficiencies[5], higher than in adult age.

Conchie *et al.*[4] have shown a considerable increase for this and other mammalian glycosidases in the epididymis, apparently associated with sexual development. The enzyme activity from other tissues remains about the same during and after growth, and this finding has been confirmed by us for the pancreas and liver in all the conditions mentioned here.

It should be noted that the fall in β-galactosidase activity is observed at the time of weaning, where other authors have found a loss of permeability to macromolecules, such as insulin and anticorps[6]. This age seems to be peculiar in the evolution of the gastro-intestinal tract of the rat.

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Avian Schistosomes in Hertfordshire

Limnaea stagnalis collected from three sources in the vicinity of Winches Farm Field Station of the London School of Hygiene and Tropical Medicine were found to be shedding furcocercous cercariæ. On emergence from the snail, the cercariæ swam freely for a short time with either the head- or tail-end forward and then attached themselves to the side of a beaker nearest the light. The cercariæ were apharyngeal, possessed a pair of eyespots and had six pairs of flame cells in the body and one pair at the base of the tail. The posterior part of the body was taken up by five pairs of penetration glands, the two anterior pairs having coarsely granular and the three posterior pairs having finely granular contents. In its behaviour and morphological characters the cercaria is identical with that described as *C. ocellata* by Rees[1] and Taylor and Bayliss[2], who have given a detailed description of the cercaria which will not be repeated here. At one site (Verulamium Lake) a small percentage of *Limnaea ovata* were occasionally found to be shedding this cercaria. In both hosts the sporocysts were located in the digestive gland.

Several attempts were made to infect domestic ducks, by single and multiple infections, by cutaneous and oral routes with pooled cercariæ from a large number of infected snails. Droppings were examined for eggs at weekly intervals, and at 4–6 months post-mortem examinations for worms were made. All results were negative. It has been observed that the birds common to all three locations are duck, moorhen and coot.

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Diapause, Neurosecretion and Hormones in Copepoda

DESPITE the great advances made during the past decade in the investigation of the endocrinology of the Crustacea Malacostraca[1], little or nothing has been published on the endocrine organs or hormones of the lower Crustacea. Neurosecretory cells have been observed in barnacles[2], but nothing is known for certain of any hormonal emanations from these cells.

Calanus finmarchicus and *C. helgolandicus* pass through three generations a year, in southern populations at least, overwintering as the last larval instar (the fifth copepodid stage)[3]. It has recently been shown (Smyly, W. J. P., personal communication) that *Cyclops leuckarti* has a similar life-history, and that, in this species, the overwintering copepodid penetrates to a depth of some 15 cm. into the mud of lake bottoms, passing the cold season in a quiescent state. This condition has obvious analogies with the diapause of many species of insects, which is known to be controlled by the endocrine system, especially by the neurosecretory system of the brain. We have therefore embarked on an investigation of the equivalent system of copepods.

The endocrine structures of the cerebral region of *Calanus* include a pair of lateral groups of neurosecretory cells at the front edge of the brain. From these the neurosecretory fibres pass forward and outward, skirting round the anterior blood sinus to reach the frontal organ. In adult and juvenile copepodids taken during the summer months these neurosecretory cells contain an abundant granular secretion. In the overwintering larvæ, however, this secretion is grossly depleted or even absent. By analogy with insects, therefore, we conclude that the secretion of these cells is probably playing some part in the control of the diapause, moulting and the overwintering metabolism and behaviour.

In the Crustacea Decapoda the same endocrine structures which are concerned with the control of moulting and metabolism also govern colour change. It therefore seemed reasonable to look for chromactivating hormones in copepods, even though they may possess no chromatophores. Accordingly, extracts of whole *Calanus finmarchicus* and of whole *Euchaeta norvegica* were assayed for chromactivating substance *A* (ref. 4) on *Leander adspersus*. One unit of this substance was found in 7·2 whole *Calanus*, while 6·5 units were found in one whole *Euchaeta*. The whole of the activity was found to be present in the cerebral region of the body in *Euchaeta*, and in the brain plus frontal organ of *Calanus*. It seems likely, therefore, that this hormone is secreted, as in decapods, by the neurosecretory cells of the brain.

This work is continuing, and a fuller account will be published elsewhere. We are grateful to Dr. F. S. Russell, Dr. S. M. Marshall, Mr. W. J. P. Smyly, Dr. B. Swedmark, the National Science Foundation and

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Verification of the Gastropod Golgi Cycle with an Electron Microscope

THE Golgi cycle of the gastropod (*Navanax*) was detailed[1], based on osmicated preparations and studies of living embryos vitally stained with methylene blue. However, because of the scepticism that has surrounded vital-dye observations, particularly since the neutral red controversy of the early 'thirties, these studies with methylene blue have never enjoyed complete acceptance by cytologists and have been severely criticized in some quarters.

It is particularly important, therefore, to report that our recent investigations of gastropod (*Crepidula*) development, by means of the ultra-thin sectioning technique, confirm substantially the earlier findings with methylene blue. Taken together, these two independent approaches to the Golgi body problem offer what we consider to be strong evidence that :

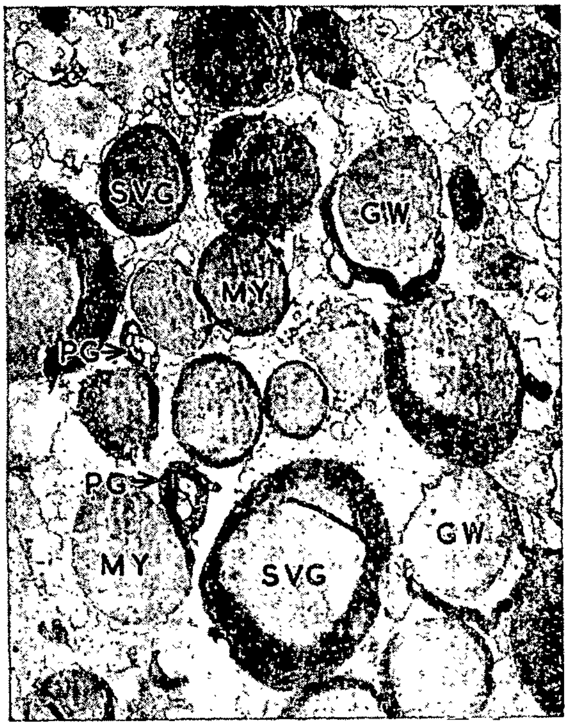

Fig. 1. Electron micrograph (× 3,400) of yolk-laden cell of *Crepidula veliger* showing three stages in the elaboration of protein yolk. Simple vesicular Golgi bodies (*SVG*), consisting of chromophilic cortices and chromophobic centres, eventually release the latter (*GW*) as mature secondary yolk spheres (*MY*). Following this withdrawal of the chromophilic envelope (*GW*), the Golgi material lies temporarily at the side of the yolk sphere as a hollow, pycnotic Golgi remnant

(1) The Golgi 'apparatus' in the gastropod veliger exhibits a cycle in which certain stages can be identified consistently by both methods; these stages are Golgi granules (droplets), simple vesicular Golgi bodies (Fig. 1, *SVG*); compound vesicular Golgi bodies, pycnotic Golgi bodies (*PG*), as well as disintegrating plates of primary yolk and newly formed mature secondary yolk spheres (*MY*).

(2) The Golgi system engages in the elaboration of protein yolk which makes its initial appearance as the chromophobic component of the Golgi body, increases greatly in size during the embryonic stages, and is eventually released as a mature yolk plate by virtue of the withdrawal from its surface of the chromophilic Golgi envelope (Fig. 1, *GW*).

(3) The Golgi complement of these cells is a series of spheres, presumably chiefly phospholipoidal in chemical composition[2], but such phospholipid spheres, through their ability to release their cores as formed cell products, play a dynamic part in the economy of the cell.

(4) Under appropriate control the microscopically visible chromophilic components of the Golgi elements in these living cells are selectively stained by methylene blue and are thus revealed in essentially unaltered form comparable with identical stages observed by means of the electron microscope.

These observations, which will be reported in detail elsewhere, indicate that in these, and perhaps in many animal cells, the function of the Golgi 'apparatus' is the continual building up of formed energy reserves from the raw nutritive materials present in the cytoplasm.

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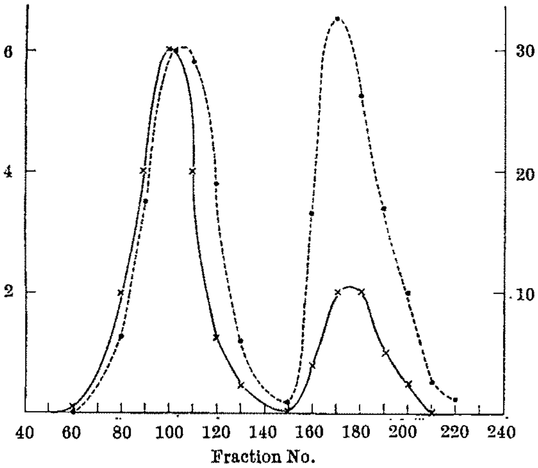
A 'Natriferic' Principle other than Arginine–Vasotocin in the Frog Neurohypophysis

IT has been shown that neurohypophyseal extracts from amphibians and teleosts stimulate the active sodium transport across frog skin more markedly than do extracts from mammalian pituitaries, and this led to the hypothesis that they contain a new hormone, differing from either oxytocin or vasopressin[1,2]. Its chemical constitution being unknown, this principle has been provisionally called 'natriferin'. Its possible identity with Heller's 'water-balance principle'[3] was of interest.

A synthetic peptide, arginine–vasotocin, prepared by Katsoyannis and du Vigneaud[4], has been shown to possess the pharmacological properties ascribed to the 'water-balance principle'[5,6], and to exhibit a high natriferic activity[7,8]. Moreover, a hormone having the same amino-acid composition and the same pharmacological properties as arginine–vasotocin has been extracted from the neurohypophysis of the chicken[9], the frog[10], and a teleostean[11].

According to Sawyer[7], the natriferic activity of the frog neurohypophysis should be entirely attributed to arginine–vasotocin. The results described below, however, indicate that arginine–vasotocin accounts only for approximately one-half of the total natriferic activity of the neurohypophysis of the frog, *Rana esculenta*. The remaining half must be ascribed to an active principle, differing from either oxytocin or arginine–vasotocin and of as yet unknown chemical constitution; this principle has not been found in the chicken.

The neurohypophyseal hormones of the frog, *Rana esculenta*, were purified by a method described previously[5,9,10,12], and treated by means of a gradient elution technique with an 'Amberlite *IRC*–50' column[12]. Fig. 1 shows the chromatographic profile obtained for the oxytocic activity (tested on the rat uterus) and the natriferic activity (tested on the 'short-circuited' frog skin[1]) of the eluate fractions. Natriferic activity was tested using synthetic oxytocin ('Syntocinon', Sandoz) as a standard. By definition, one natriferic unit corresponds to the effect produced by one I.U. of oxytocin. The complete analysis was carried out three times with separate batches of glands and comparable profiles were obtained.



←(0·1 *M*, *p*H 5 →0·5 *M*, *p*H 7·7)→ (0·75 *M*, *p*H 7·7) →

Fig. 1. Chromatographic profile of the frog neurohypophyseal principles. The hormones are separated on 'Amberlite *IRC*-50' by a gradient (of *p*H and ionic strength) elution technique (ref. 12). Concentrations and *p*H of the ammonium acetate buffer are shown below the figure. The oxytocic (left-hand scale) and natriferic (right-hand scale) activities of the effluent fractions are expressed in units/ml. Note that different scales are used. The right-hand natriferic peak corresponds to arginine–vasotocin and the left-hand natriferic peak to an unknown principle
×, Oxytocic activity; ●, natriferic activity

A study of the effluent fractions corresponding to the right-hand side of the chromatographic profile reveals coincident peaks of oxytocic and natriferic activities (25 per cent and 50 per cent of the total recovered activities respectively), corresponding to a substance which exhibits also a strong vasopressin activity[10]. This substance has the same amino-acid constitution and the same pharmacological properties as arginine–vasotocin[10], which is also present in the chicken neurohypophysis[9].

The left-hand side of the profile shows other peaks of oxytocic and natriferic activities (75 per cent and 50 per cent of the recovered activities, respectively). The peak of natriferic activity must necessarily be attributed to an unknown principle for the following reasons: (1) The strong natriferic activity as compared with the oxytocic activity (3–15 times higher, according to the region of the peak), eliminates the possibility that this substance is oxytocin, although oxytocin appears as a rule in this region of the chromatogram. (2) Its position on the profile and the absence of associated vasopressic activity[10] distinguish it from arginine–vasotocin. (3) The 'water-balance' activity of the pooled effluent fractions number 110–130, determined by means of Sawyer's gravimetric technique[6] on *Rana esculenta* bladder, was found to be ninety times higher than oxytocin itself and about 2·5 times less active than arginine–vasotocin. (4) The pooled effluent fractions, number 110–130, have no antidiuretic activity in both the rat and the frog. This contrasts with the high antidiuretic activity exhibited by arginine–vasotocin[8].

In the chicken, however, the effluent fractions corresponding to the left-hand side of the profile show only a low peak of natriferic activity coincident and equivalent to the peak of oxytocic activity. Oxytocin has been characterized chemically in this region of the chromatogram[12]; its presence accounts entirely for the observed natriferic activity.

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Enhancement of Intraperitoneal Staphylococcal Virulence for Mice with Different Bile Salts

The intraperitoneal injection of a 1 per cent mixture of equal parts of sodium taurocholate and sodium glycocholate followed by an inoculum of *Staphylococcus aureus* has been previously found to enhance virulence of the latter for mice[1]. In the light of this observation, effectiveness in this respect of other individual bile salts, as well as the minimal effective concentration of those salts possessing such activity, was investigated.

Sodium taurocholate, sodium glycocholate, sodium glycotaurocholate and sodium dehydrocholate were obtained from Nutritional Biochemicals Co., sodium cholate from K. and K. Laboratories and sodium deoxycholate from the Fisher Scientific Co. All were readily soluble in distilled water and were sterilized by autoclaving at 15 lb. for 15 min. 0·5 ml. of the indicated concentration of each bile salt was injected intraperitoneally into groups of 10 albino, male Swiss mice weighing 15–20 gm., followed immediately by 0·3 ml. of an 18-hr. culture of a strain of *Staphylococcus aureus* grown in trypticase soy broth at 37° C. The latter had been isolated from a purulent lesion and was highly pigmented, hæmolytic, coagulase-positive, and fermented mannitol. All deaths occurring within 48 hr. as well as the number of positive post-mortem heart-blood cultures for *Staphylococcus aureus* in the animals that died were noted. In addition, one group of 10 mice received 0·5 ml. of 5 per cent gastric mucin and another group of 10, 0·5 ml. of physiological saline immediately prior to the intraperitoneal bacterial injection, as controls. A mortality of 10 out of 10 deaths within 48 hr. with 10 positive post-mortem heart-blood cultures was produced with the gastric mucin adjuvant whereas there were no fatalities with the saline solution. Intraperitoneal toxicity of 0·5 ml. of different concentrations of each bile salt *per se* was also determined. Sodium deoxycholate, 0·25 per cent, elicited a mortality of 3/10 with all post-mortem heart-blood cultures negative ; 0·1 per cent was innocuous. 4 per cent was found to be the maximum non-lethal concentration of sodium dehydrocholate. No fatalities occurred with a 1 per cent concentration of the remaining bile salts.

The number of fatal bacteræmias produced by the intraperitoneal staphylococcal inoculum with the aid of different concentrations of each bile salt investigated is contained in Table 1. As may be observed from Table 1, complete to almost complete mortality accompanied by bacteræmia was obtained with 0·25 per cent sodium deoxycholate and with a 1 per cent concentration of the remaining bile salts tested except sodium dehydrocholate. Lesser concentrations produced lower mortalities. The same intraperitoneal bacterial inoculum without bile salts, on the other hand, had resulted in no deaths. The maximal tolerated concentration of sodium dehydrocholate, 4 per cent, failed to elicit any mortality. Since the failure of the latter might conceivably have been due to a bactericidal activity on its part against the injected *Staphylococci*, the following experiment was performed. 0·3 ml. of an overnight culture of the test strain was distributed into each of two series of tubes. 0·5 ml. of 4 per cent sodium dehydrocholate was added to the tubes of one series, 0·5 ml. of physiological saline to the other. All were placed in a shaker water bath at 37° C., oscillating at approximately 120 per minute and comparative colony counts upon similar aliquots from both series at intervals of 2, 4, 6, 8 and 24 hr. were performed. No significant reduction in the number of viable bacteria was observed following exposure to sodium dehydrocholate as compared with physiological saline even up to 24 hr., so that the ineffectiveness of sodium dehydrocholate as a virulence-enhancing substance could not be attributed to any sterilizing effect upon the bacterial inoculum in the peritoneum.

Many chemically diverse substances, including certain anionic and non-ionic surfactants, have been found capable of converting ordinarily innocuous intraperitoneal staphylococcal infections in mice into rapidly fatal ones associated with septicæmia[1,2]. All possess one property in common, namely, the ability to lower surface tension. A substance possessing this characteristic might conceivably interfere with contact between peritoneal phagocytes and the bacteria so that the former are no longer able to envelop, engulf and destroy the latter. Furthermore, permeability of the peritoneal barrier between the abdominal cavity and the blood might also be increased by the surfactant and thus facilitate passage of the intraperitoneal organisms directly into the blood stream, thereby permitting them to circumvent the protective action of peritoneal phagocytes and minimize their exposure to the bactericidal action of peritoneal fluid. The surface tension-lowering ability of each bile salt was determined by means of the capillary tube method[3], with the following results: values expressed in dynes/cm. at 25° C. for physiological saline was found to be 66·2 ; that of 1 per cent sodium taurocholate, sodium glycocholate, sodium glycotaurocholate and sodium cholate 41·6, 40·4, 38·0 and 45·3 respectively ; of sodium deoxycholate 0·25 per cent, 45·3 ; while that of 4 per cent sodium dehydrocholate was 61·3. The lack of effectiveness of the last bile salt provides additional evidence to indicate that an association exists between the ability of a substance to lower surface tension and its ability to enhance virulence of intraperitoneally injected *Staphylococci* for mice.

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Table 1. MOUSE MORTALITY/10 AND POSITIVE POST-MORTEM HEART BLOOD CULTURES ELICITED BY INTRAPERITONEALLY INJECTED *Staphylococcus aureus* WITH THE AID OF VARIOUS CONCENTRATIONS OF DIFFERENT BILE SALTS
(Mortality with 5 per cent gastric mucin—10 (10); with physiological saline—0)

Bile salt		Concentratuon of bile salts				
	4 per cent	1·0 per cent	0·5 per cent	0·25 per cent	0·1 per cent	0·05 per cent
Sodium taurocholate		10 (10)*	0	0	0	
Sodium glycocholate		8 (8)	4 (4)	1 (0)	0	
Sodium glycotaurocholate		10 (10)	6 (6)	2 (2)	0	
Sodium cholate		10 (9)	3 (3)	3 (3)	0	
Sodium deoxycholate				10 (10)	7 (5)	1 (1)
Sodium dehydrocholate	0	0				

* No. of post-mortem heart-blood cultures positive for *Staphylococcus aureus*.

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Paper Chromatography of Natural Saponins

APART from an early attempt by other authors[1–3] to adapt a method of paper chromatography for the examination of natural saponins, the potentialities in this field do not seem to have been investigated. The present communication deals with the application of paper partition chromatography to the identification of natural saponins and the separation of the constituents present, with particular reference to saponins of the important medical plants.

Such a separation was found to be readily achieved using the system *n*-butanol/acetic acid/water (6 : 1 : 3) as mobile phase. Good separation requires a 3-hr. conditioning of the Whatman No. 1 filter paper strip with substances applied in the chromatograph chamber, saturated with vapour of the solvent (the walls are lined with the paper). About 0·2 mgm. of the saponin dissolved in 50 per cent ethanol (primulic acid in 95·5 per cent ethanol) is spotted at the origin, and an ascending chromatogram is run at 19–20°. The solvent front moves slowly, usually about 30 cm. in 15–18 hr.

The strips are then exposed to a stream of warm air for 5 min. After evaporation of excess acetic acid, the chromatograms are heated at 105° for a further 30 min.

The detection reagent which yielded the best results was a 25 per cent ethanolic solution of phosphotungstic acid[4,5]. Other solvent systems and reagents are being tested. Characteristic colours reach optimum intensity in about 2 min., by which time very little background colour has developed. Each of the saponins examined so far yields a characteristic chromatogram distinguishable by the number, colour and R_F values of the coloured zones.

The colours obtained are: blue in daylight and ultra-violet light with *Herniarias glabra* saponosides; blue in daylight and pink in ultra-violet light with saponosides of *Saponaria officinalis* L. and *Gypsophila paniculata* Fisch. (Caryophyllaceae); magenta with primulic acid; cherry-red with the saponosides of *Calendula officinalis* L. (Compositae); violet with saponosides of *Polygala senega* L. (Polygalaceae); grey-green with escinol from *Aesculus hippocastanum* L. (Hippocastanaceae) and saponosides of *Quillaja Saponaria molina* (Rosaceae). The saponosides yield spots of the same colours as their aglycones when treated with phosphotungstic acid[4–6].

Full details will be published in *Dissertationes Pharmaceuticae*, Cracow, Poland (in English).

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MICROBIOLOGY

A Diurnal Cycle for Holotrich Protozoa of the Rumen

In the last decade the study of the ciliate protozoa of the rumen has emphasized the investigation of individual species, rather than the ciliate population as a whole.

Several outstanding biochemical differences between Oligotrichs and Holotrichs have now been established *in vitro*[2–4]. However, *in vitro* work can delineate biochemical capabilities only. It cannot predict with certainty the activity of these organisms *in vivo*, and therefore if any attempt were made to estimate the value of the organism of the host animal, some knowledge of the ecological factors concerned is necessary.

Initial investigations into factors affecting the concentration of protozoa *in vivo*[5] have demonstrated a diurnal cycle for *Entodinium*. This cycle is in agreement with that obtained by other workers[6,7]. In the work described here a diurnal cycle for the holotrich protozoa was demonstrated.

Two merino wethers, fitted with rumen fistulæ, were fed a ration consisting of 500 gm. oaten chaff and 150 gm. lucerne chaff daily at 0900 hr. After 25 days on this ration, rumen samples were taken at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16 and 20 hr. after feeding for 3 consecutive days. Samples were taken from the bottom of the rumen, and protozoal counts were made by the method described by Purser and Moir[5]. The holotrich species consisted entirely of *Dasytricha*.

Variations between sheep and between days were not significant, but statistical analysis revealed the highly significant ($P<0{\cdot}001$) regression $y=2132{\cdot}8-192{\cdot}3x+5{\cdot}1x^2$ between holotrich concentration (y) and the time in hours after feeding (x). This curve and the mean values are presented in Fig. 1.

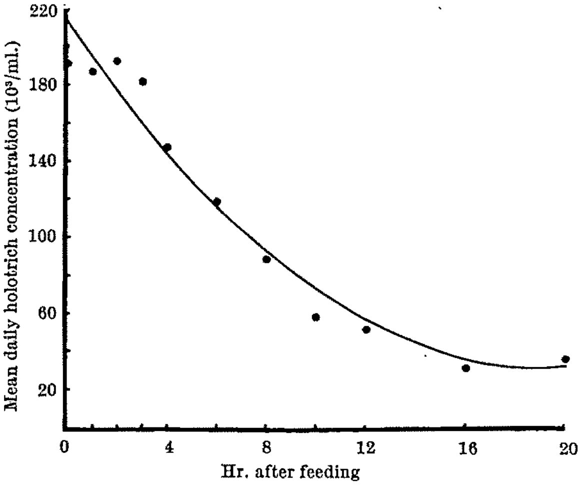

Fig. 1. Mean daily holotrich concentration (●) and the regression $y = 2132{\cdot}8 - 192{\cdot}3x + 5{\cdot}1x^2$ ($P < 0{\cdot}001$) between holotrich concentration (y) and the time in hours after feeding (x)

This diurnal cycle differs substantially from that exhibited by *Entodinium*. Whereas the concentration of holotrichs diminishes gradually until 20 hr. after feeding and then builds up rapidly to the original concentration, the recovery of *Entodinium* begins only 8 hr. after feeding. It has been suggested that the *Entodinium* cycle is controlled largely by physical dilution factors such as those due to feeding and salivation[5]. This would not appear to be the case for the holotrichs.

It is possible that the holotrich cycle is a reflexion of site differences, for the cycle implies a five-fold increase in the concentration in 4 hr. Boyne *et al.*[8], however, have failed to demonstrate a site difference and it is difficult to understand why a sudden change in site distribution should occur immediately before feeding.

This cycle has been confirmed afterwards in experiments under different nutritional régimes, but the factors governing it have not been explained. It is important, however, that this difference between holotrichs and oligotrichs *in vivo* is recognized, when samples are taken from animals for population analysis or for *in vitro* work.

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Multiplication of Hansen's Bacillus in Complex Symbiosis *in vitro*

The work reported here followed a chance observation made while attempting, without success, to grow *Mycobacterium leprae* in a culture of *Entamoeba terrapinae*.

A fragment of leproma (*L*.II), about 1 c.c. in size, had been incubated for eight days, at 25° C., in saline buffered at *p*H 7·0 with phosphates. It was thereafter inoculated into the following culture medium, called *M-E-R* : (*a*) 30 gm. of powdered skimmed milk were suspended in 200 ml. distilled water ; this was autoclaved at 115° C. for 20 min. and cooled. (*b*) A whole egg (about 60 ml.) was aseptically homogenized either by hand-shaking in a flask containing glass beads or by the use of a mechanical mixer. (*c*) Milk (*a*) and egg (*b*) were mixed together and the mixture aseptically distributed (18 ml.) into sterile test tubes (dia. 25 mm.). (*d*) 2 ml. of an autoclave-sterilized solution of reductose (2·5 per cent in water) were aseptically added to each tube.

(A variant of medium *M-E-R*, used later on and called *M-E-R-M*, differed from it only in that solution (*a*) contained, in addition to the milk powder, 0·6 gm. gastric mucin.)

After incubation at 25° C. for several weeks, the microscopic examination of medium *M-E-R* inoculated with leproma *L*.II revealed the presence of an exceedingly large number of bundles of acid-fast bacilli recalling the typical 'globes' seen in the cells of lepromatous lesions.

However, only one out of three identical tubes inoculated likewise with fragments of the same leproma *L*.II gave such positive results. This suggested that contaminating microflora might be involved. From the single tube in which a multiplication of *M. leprae* had apparently occurred, four micro-organisms were isolated by standard procedures. Two were aerobes, an anthrax-like bacillus and a species of *Neisseria*. Two were anaerobes, an agar-liquefying organism and a gas-producing *Clostridium*.

My initial observation was repeated with three other specimens (*L*.I from the same patient as *L*.II, *L*.III and *L*.IV from two other patients) using the following procedure : (*a*) incubation of the fragment from leproma (about 1 c.c.) at 25° C. during at least 8 days in buffered saline (*p*H 7·0) ; (*b*) inoculation of the four micro-organisms together in a tube of medium *M-E-R* (or *M-E-R-M* in the case of specimen *L*.IV) and incubation during about 24 hr. (the medium is then clotted) ; (*c*) introduction of the macerated fragment (*a*) on the surface of (*b*) and incubation at 25° or 34° C., the tubes being tightly capped.

After one week, the clotted medium was liquefied and the proliferation of the symbionts could be stopped by the addition of either 'Sigmamycine' (a platinum loop of the dry product) or malachite green (1 ml. of a 0·5 per cent sterile solution in water) or both. However, this step was optional since it did not influence at all the evolution of *M. leprae* as observed monthly in Ziehl-stained smears.

In primary cultures, bundles of acid-fast bacilli increased and diminished in number alternately several times. They were preferably localized at the surface of the medium and in the ring which was progressively formed on the glass just over the surface. When occurring singly, bacilli were granular in appearance, but this happened very rarely as they were mostly seen in three-dimensional dense bundles or in one-plane clumps. Sometimes the agglomerates were rather loose and sometimes disintegrated into separate individuals in the immersion oil. There was no macroscopic difference between a culture in which *M. leprae* were multiplying and one inoculated with the symbionts only. Some observations, which, however, required further confirmation, led me to believe that the anthrax-like bacillus is the organism responsible for making medium *M-E-R* suitable for the growth of *M. leprae*.

Secondary cultures from the primary tubes were obtained in two ways. Either in medium *M-E-R*, using then a large inoculum representing at least one-fifth of the volume of culture, or on agar-media of various composition containing malachite green, using such an inoculum that, after partial desiccation, it left a heavy and creamy layer on the surface. Further sub-cultivation has so far been unsuccessful.

I now have on hand four suspensions very rich in bundles of *M. leprae* and aged respectively 12 (*L*.II), 11 (*L*.I and *L*.III) and 8 (*L*.IV) months. The possibility of subcultivating from them on an agar-medium gives us the hope of devising a method for the easier preparation and standardization of large amounts of lepromin.

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Rhamnose for Detection and Isolation of *Azotobacter vinelandii* Lipman

During investigations on the utilization of pentoses by *Azotobacter* spp., *A. vinelandii* proved to be the only species able to utilize rhamnose as sole carbon source. The experiments were carried out with a nitrogen-free nutrient solution containing mineral salts, 0·1 per cent agar and 1·0 per cent rhamnose as source of carbon and energy. The growth was estimated by comparison with check cultures without carbon source, and in a few cases the nitrogen fixation was determined by Kjeldahl analysis.

Twenty-eight strains of *A. chroococcum* and nine strains of *A. beijerinckii* were tested, and all were found completely unable to utilize rhamnose. Of *A. vinelandii* ten strains were tested, and nine grew as well or better with rhamnose as sole source of carbon as with glucose. One strain showed only slight, but unmistakable, growth with rhamnose as carbon source. In the nitrogen fixation experiments 14·1–15·8 mgm. nitrogen was fixed per gm. of rhamnose decomposed, and 13·3–14·4 mgm. per gm. of glucose by the same strains.

This exclusive ability of *A. vinelandii* can be used for enrichment and isolation of this species from soils and other sources, where other *Azotobacter* species are present at the same time. In the presence of *A. chroococcum*, *A. vinelandii* cannot be detected or isolated by means of the ordinary enrichment cultures with glucose or mannitol as carbon source, because *A. chroococcum* always will be entirely dominant in such cultures. In nitrogen-free enrichment cultures with rhamnose as sole carbon source, however, *A. vinelandii*, when present, will develop profusely in 3–5 days. In the absence of *A. vinelandii* other *Azotobacter* species may develop, but only after a considerably longer incubation period (1–2 wk.).

A considerable number of cultivated soils has been tested by this technique, but *A. vinelandii* was found only in a few garden soils, and always together with *A. chroococcum*. It seems to be restricted to the most fertile soils, and to occur much more sparsely than the other *Azotobacter* species. It was not possible, however, to establish more precisely the factors determining the occurrence.

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VIROLOGY

Mutation in Tobacco Mosaic Virus induced by a New Carcinogen, 4-Nitroquinoline N-Oxide

SEVERAL reports on the mechanism of mutation induced by nitrous acid in tobacco mosaic virus (TMV) phages T_2, T_4, $\varphi \times 174$, and in transforming deoxyribonucleic acid (DNA), have been published. These indicate that, with nitrous acid treatment, inactivation and mutation occur at the same time, and therefore either may be due to a single chemical alteration in nucleic acid.

Recent experiments on the effects of 4-nitroquinoline-N-oxide (4-NQO) on TMV have demonstrated that this new type of powerful carcinogen[1,2], within a certain wide range of concentrations: (1) does not inactivate TMV, although inactivating its ribonucleic acid in proportion to the concentration; (2) induces mutation in both TMV and TMV–RNA, but at a much higher rate in the latter than in the former. That 4-NQO may induce mutation without involving a change in the infectivity of TMV suggests the possibility of considering inactivation and mutation separately, and leads us to believe that the mutagenic mechanism of 4-NQO may be basically different from that of nitrous acid.

Inactivation tests. Aliquots of 1 γ/ml. TMV solution and of 290 γ/ml. solution of TMV–RNA extracted with phenol, both in 10^{-2} *M* phosphate buffer of *p*H 7·4, were each mixed with equal volumes of 4-NQO solutions in such final concentrations as shown in Table 1, and the mixtures were allowed to stand at room temperature for 60 min., after which infectivity of each mixture was assayed. For the assay 20 half-leaves of *Nicotiana glutinosa* were used, and the infectivity was judged by the number of typical lesions per half leaf. TMV used in this experiment was the ordinary mosaic strain of the Hatano Tobacco Experiment Station. The purification of the virus was made by three cycles of differential centrifugation (Spinco model *L*) of expressed sap from the diseased leaves of *Nicotiana tabacum* L. variety White Burley, which were gathered 3–4 weeks after the virus inoculation.

The results of this experiment are shown in Table 1, in which changes in infectivity according to the concentrations of 4-NQO are expressed by the variation of the mean number of local lesions per half leaf.

Table 1. EFFECTS OF 4-NQO ON THE INFECTIVITY OF TMV AND TMV–RNA

		Concentration of 4-NQO (mol.)				
		0	0.5×10^{-6}	1×10^{-6}	0.5×10^{-5}	0.5×10^{-4}	0.5×10^{-3}
TMV	4-NQO		46·0		46·0	38·4	42·0
	Solvent only (vol. per cent ethanol in water)	44·5	43·1 (0·002)		45·2 (0·02)	37·2 (0·2)	38·2 (2)
RNA	4-NQO	57·6		68·7	40·5	19·4	6·7

In another experiment, at a fixed concentration of 4-NQO (0.5×10^{-4} *M*), the incubation time was changed from 0 to 1 and 3 to 6 hr., and the infectivity expressed by the average number of local lesions produced in 16 half leaves was found to be almost constant, being 22·5, 22·4, 28·3, and 28·5 respectively.

Mutagenic effect. In this experiment, the mutagenic action of 4-NQO was estimated by the number of necrotic lesions produced in the leaf of *Nicotiana tabacum* L. variety *xanthi*. It is well established that the necrotic lesion is specifically produced by a mutant of TMV.

Aliquots of 0·45γ/ml. TMV solution and 400γ/ml. TMV–RNA solution, both in 10^{-2}*M* phosphate buffer of *p*H 7·4, were each mixed with 4–NQO in such final concentrations as listed in Table 2, kept at room temperature for 60 min., and then assayed. The total number of necrotic lesions produced in 15 leaves varies according to the concentrations of 4-NQO as shown in Table 2.

Table 2. PRODUCTION OF MUTANT LESIONS IN TOBACCO LEAVES BY 4-NQO

	Concentration of 4-NQO (mol.)			
	0	1×10^{-6}	0.5×10^{-4}	0.5×10^{-3}
TMV*	0	3	5	2
TMV	0	4	3	5
RNA	3	18	22	15

* Refers to an experiment in which 0·45 mgm./ml. TMV solution was incubated with 4-NQO for 60 min. and assay was made by diluting the mixture to 1 : 1,000 in order to avoid any possible effect of 4-NQO itself on the leaf

Control experiments in which 4-NQO solution alone was applied to tobacco leaves proved that no necrotic reaction was caused by this substance.

From the chemical point of view, it may be stated that the carbon at position 4 of the 4-NQO molecule forms a highly reactive centre, through the polar effect of the N-oxide and nitro groups, and can easily substitute with nucleophilic reagents with production of nitrous acid. Therefore, deamination by this nitrous acid also might be considered as a possible mechanism in the mutation of RNA induced by 4-NQO. As has been already reported[3,4], however, 4-NQO reacts rapidly only with SH groups at neutral *p*H and at room temperature, and under these conditions the ultra-violet absorption of this chemical is not affected when kept mixed with any other possible cellular nucleophilic group.

Also, when TMV–RNA is treated with 4-NQO, no change could be detected in ultra-violet absorption of either substance up to 24 hr. after mixing. Further investigations on the interaction between RNA and 4-NQO are under way.

It is well known that mutagenic and carcinogenic actions are often found in one and the same substance, and the fact that 4-NQO, besides its potent carcinogenic action, possesses also remarkable mutagenicity on *Aspergillus niger*[5] and *Streptomyces griseoflavus*[6] has been reported. 4-NQO, with its characteristic chemical reactivity, may eventually serve as a unique tool in the analysis of the mechanism of mutation on the molecular basis.

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Hæmagglutinating Sub-units released from Newcastle Disease, Para-influenza 1 and Mumps Viruses by Treatment with Ether

IT was shown originally by Hoyle[1] that influenza viruses can be split by treatment with ether into sub-units, hæmagglutinin and soluble (nucleoprotein) antigen. Recently, a study on the sub-units of Newcastle disease and mumps viruses has been published, which indicated that these viruses do not liberate hæmagglutinin on treatment with ether, but only filamentous particles of nucleoprotein nature, exhibiting complement-fixation activity[2]. Working with the Blacksburg strain of Newcastle disease, the Sendai strain of para-influenza 1 and the Enders strain of mumps viruses, we found that these viruses, on treatment with ether, released both nucleoprotein antigen and hæmagglutinin.

The viruses were isolated from infectious allantoic fluids by two cycles of alternate low- and high-speed centrifugations. Concentrated preparations containing 10^4–10^5 hæmagglutination units per ml. were stirred and treated with a half volume of ether at 15° C. for 90 min. The aqueous phase was separated by low-speed centrifugation and, after removal of the water-soluble ether, clarified by centrifugation at 17,000g for 45 min. to remove the intact or only partially split virus. On electron microscopic examination of the supernatant, predominantly spherical particles of about 30 mμ diameter and, in the case of Newcastle disease and mumps viruses, also long filaments, similar to those described by Schäfer and Rott[2] and Horne *et al.*[3], were seen. The highest yield of hæmagglutinin was obtained with Newcastle disease virus, the number of the hæmagglutination units recovered indicating that the particle of this virus contains at least four hæmagglutinating sub-units.

The hæmagglutinins were isolated from the suspensions of sub-units by repeated adsorption on to fowl erythrocytes and by subsequent elution. In the case of Newcastle disease virus, however, this procedure did not assure the complete removal of hæmagglutinins from the suspension of sub-units. The hæmagglutinin could be concentrated and further purified by sedimentation at 145,000g for 90 min.

The hæmagglutinins of the viruses investigated were non-infectious; they agglutinated fowl and guinea pig erythrocytes to the same dilution, although the virus preparations, from which they were derived, showed higher hæmagglutination titres with fowl than with guinea pig erythrocytes. They exhibited neuraminidase activity and liberated from virus hæmagglutination inhibitor, isolated from bovine serum, about the same amount of sialic acid, calculated per hæmagglutination unit, as the corresponding viruses. They did not exhibit hæmolytic properties, although the viruses investigated are all hæmolytic[4]. They were precipitated quantitatively on dialysis against distilled water, but the precipitate could not be re-dissolved in the original solvent (phosphate buffered saline pH 7·2). They could be stored at 3° C. for several months without any decrease in hæmagglutination activity. They were not pelleted under conditions (36,000g for 45 min.) sufficient for almost quantitative sedimentation of the viruses. On electron microscopic examination of samples of hæmagglutinin, spherical particles of about 30 mμ were predominant (Fig. 1).

Immune guinea pig sera were prepared against the three viruses investigated, their hæmagglutinins and, in the case of the Sendai strain of para-influenza and mumps viruses, also against the nucleoprotein antigens, respectively. The viruses did not show any relationship by cross-neutralization, hæmagglutination inhibition and complement-fixation tests. The

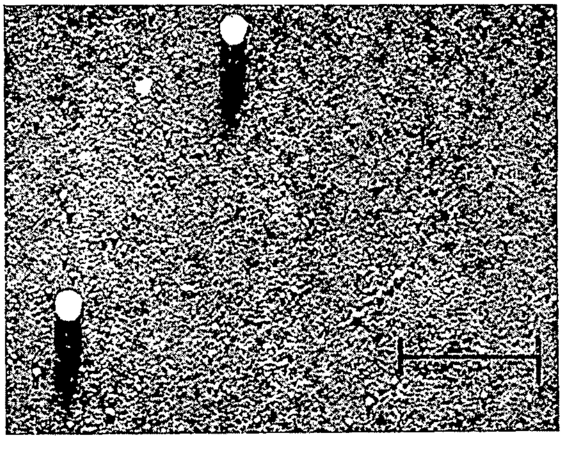

Fig. 1. Electron micrograph of para-influenza 1 virus hæmagglutinin. Scale, 1μ

same holds for their sub-units, as estimated by cross complement-fixation reactions, and in the case of hæmagglutinin also by cross-hæmagglutination inhibition tests.

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CYTOLOGY

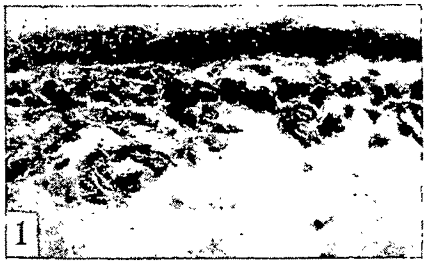
The Stainable Mucin of Joint Tissues and Synovial Fluid

Joint synovial fluid is considered to be a dialysate of blood with added mucin, which consists of hyaluronic acid[1]. The source of the latter is uncertain, but the belief is current that the synovial membrane is the likely site of formation. Articular cartilage is said to contain no hyaluronic acid, and the sulphated mucopolysaccharide it does contain is not found in appreciable quantity in synovial fluid. Synovial membrane is not lined by an epithelium, but is composed of fine, compact connective tissue of variable cellularity[2], the cells being indistinguishable from fibroblasts by electron microscopy[3]. Claims have been made that the 'membrane', especially in its superficial part, stains with mucicarmine[4-6], and also reacts with the periodic acid – Schiff technique[3]. Metachromasia, which might indicate the presence of hyaluronic acid in the membrane, has not been unequivocally demonstrated; some claim that the membrane is metachromatic[6,7], but others deny it[3,5].

In the present work, specimens of synovial membrane were removed immediately after death from the parapatellar region of the knee-joint of 5 rats, 10 guinea pigs, 10 rabbits, 10 cats, 5 dogs, 5 sheep and 4 monkeys, and from 4 human subjects at post-mortem. In addition, specimens of articular cartilage were taken from the distal femoral condule, and of fibrocartilage from the articular disks of rats, guinea pigs, rabbits, cats, dogs and monkeys. The fixative most often employed was 80 per cent alcohol, but Heidenhain's 'susa' was also used; the results were similar with both fixatives. Paraffin sections from each specimen were stained with hæmatoxylin and eosin, Southgate's mucicarmine and 0·5 per cent aqueous toluidine blue, whereas others were treated by the periodic acid – Schiff technique. A hyaluronidase (bovine, testicular) control was used with all the mucin-staining methods and a saliva (diastase) control as well with the periodic acid – Schiff technique.

The results obtained with each technique were precisely the same in all species examined. The distribution of mucinous material in the tissues, as shown by mucicarmine staining, was similar to that of the metachromatic material revealed with toluidine blue, but was different from the mucopolysaccharide distribution shown by the periodic acid – Schiff technique.

In synovial membrane, the only staining observed with mucicarmine was a faint coloration of the intercellular material, similar to that of connective tissue fibres generally, and with toluidine blue no metachromasia was observed, except in mast cells in the deeper part of the membrane. With the periodic acid – Schiff technique, some specimens of mucous membrane showed a weak reaction in the fibrous groundwork between the cells; but it was no greater than that given by connective tissue fibres in other parts of the section, and normally was weaker than the reaction seen in the walls of blood vessels. In a few instances, a fairly thick layer of precipitated synovial fluid was present, in contact with, or slightly separated from, the synovial membrane, and this material stained strongly with mucicarmine (Fig. 1), and stained metachromatically with toluidine blue. Only a weak reaction was given with the periodic acid – Schiff technique. The results obtained with all three techniques were uninfluenced by pre-treatment of the section for 24 hr. in buffered hyaluronidase at *p*H 6·9.



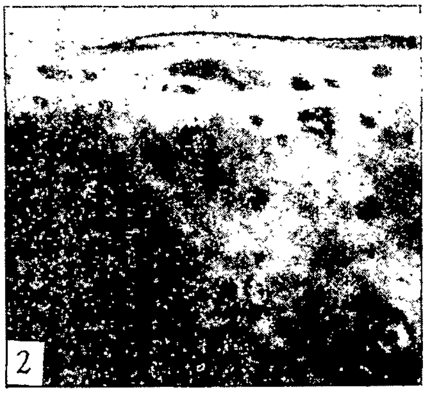


Fig. 1. Mucicarmine and hæmatoxylin preparation of human synovial membrane. Layer of precipitated synovial fluid is strongly stained with mucicarmine. (× 360)

Fig. 2. Mucicarmine and hæmatoxylin preparation of rabbit articular cartilage. General matrix is irregularly stained, but perilacunar haloes strongly stained; less staining near free surface. (× 460)

It is generally appreciated that articular cartilage contains stainable mucin, and that the distribution of the material shown with the periodic acid – Schiff technique differs from that revealed with mucicarmine. This was confirmed in the present work. With the periodic acid – Schiff technique, the whole matrix reacted moderately and evenly throughout, but no reaction was seen in the shrinkage spaces within the lacunæ. The cartilage cells, as is well known, contain glycogen, and it was confirmed that they give a positive periodic acid – Schiff reaction, which is abolished by pre-treatment with saliva. The distribution of material stainable with mucicarmine was found to be similar to that of metachromatic material, but was quite different from that of periodic acid – Schiff positive material. The matrix immediately around the lacunæ stained strongly, in the form of haloes, but the staining of the general matrix was blotchy and irregular: towards the free surface of the cartilage there was a marked decline in the amount of stainable material in both the general matrix and in the perilacunar haloes, but small amounts were present here and there, even adjacent to the free surface (Fig. 2). The claim that cartilage cells contain stainable mucin was not confirmed; under oil immersion, it was observed that the material stainable with mucicarmine and metachromatic with toluidine blue occupies the shrinkage space between the cell and lacunar margin, and is often seen to be drawn into strands where the cell has shrunk away from the lacuna. The histological picture suggests that the cell secretes a mucinous material into the lacuna, whence it is incorporated into the perilacunar matrix, and thence passes into the general matrix, where it has an irregular distribution, and declines in amount towards the free surface of the cartilage. In articular disks, the cartilage is usually of the hyaline variety towards their free margins, and here the stainable mucin showed the same distribution as that described here. As in synovial fluid, pre-treatment with hyaluronidase did not influence the periodic acid – Schiff reaction, nor the mucicarmine or the metachromatic staining.

From the present work, it is concluded that there is insufficient evidence to show that the stainable mucin of synovial fluid is produced, at least in any quantity, from normal synovial membrane. Regarding articular cartilage, it can be said that it contains an appreciable quantity of material which stains with mucicarmine and is metachromatic with toluidine blue, and also contains material with a different distribution, which gives a positive reaction with the periodic acid – Schiff reagent. Precipitated synovial fluid also contains such material, and in neither site was the staining result influenced by hyaluronidase. Although cartilage may not contain hyaluronic acid, it seems conceivable that mucinous material of some kind may be added to synovial fluid from the wear and tear of cartilage, though present chemical evidence is not in accord with this view.

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Nucleic Acid Content of Haploid and Diploid Cells of *Protomyces inundatus*

Protomyces is a fungus of uncertain taxonomic position and its life-history is incompletely known. In particular, previous investigators[1,2] have been unable to determine satisfactorily the position of meiosis. In the course of a taxonomic investigation into the genus *Protomyces*, a number of standard staining techniques was employed in an attempt to demonstrate meiosis, but up to now none has proved satisfactory. In view of the recent demonstration of a linear relationship between deoxyribonucleic acid (DNA) content and ploidy in a polyploid series of *Saccharomyces*[3], we decided to use DNA estimation as the basis of a method for distinguishing between haploid and diploid stages in *Protomyces*.

Stock cultures of *P. inundatus* were established on its natural host *Apium nodiflorum*, in the greenhouse: chlamydospores formed readily in such infections. Single-spore cultures were established by means of a dummy objective from: (*a*) unfused endospores obtained from germinating chlamydospores; (*b*) fusion bodies formed by the conjugation of two endospores; (*c*) cultures of budding cells obtained from mycelium within the host by plating out sterilized fragments of leaf tissue containing only vegetative mycelium.

Cultures obtained by all three methods were grown on a basal semi-synthetic liquid medium[4] with glucose as carbon source on a shaker at 20° C. The results were converted to a per cell basis by counting the cells in a small aliquot by means of a hæmocytometer. Nucleic acids were determined by the method of Ogur *et al.*[3]. RNA, together with metaphosphate, was extracted in 10 per cent perchloric acid overnight at 3° C. Labile and total phosphate were determined colorimetrically using ammonium molybdate and stannous chloride, and from the values obtained metaphosphate and RNA phosphate[5] were calculated. RNA was also determined by the orcinol method of ribose estimation[6] and gave results in good agreement with those obtained by phosphorus estimation. DNA was extracted in 5 per cent perchloric acid at 70° C. for ten minutes and determined as total phosphate. Insoluble nitrogen was determined by the semi-micro Kjeldahl's method. Cell volume was calculated from measurements of major and minor axes of a hundred cells, assuming the shape of the cells to be that of an ellipsoid.

The results obtained are presented in Table 1. In a second experiment, confined to the determination of nucleic acids, the relative amounts of DNA and RNA in unfused endospores and fusion bodies were similar to those found in the first experiment. Different values on a per cell basis were obtained, however, possibly because the cultures were gathered 12 days after inoculation as opposed to 25 days in the first experiment; the cells were probably in a more actively dividing condition than those analysed in the first experiment.

Table 1. NUCLEIC ACID CONTENTS AND OTHER CHARACTERISTICS OF CELLS FROM THREE STAGES OF *P. inundatus*

	Cultures derived from:		
	(*a*) Unfused endospores	(*b*) Fusion bodies	(*c*) Mycelium in host
DNAP/cell (μgm.)	$1{\cdot}42 \times 10^{-9}$	$2{\cdot}85 \times 10^{-9}$	$2{\cdot}84 \times 10^{-9}$
RNAP/cell (μgm.)	$1{\cdot}70 \times 10^{-8}$	$1{\cdot}85 \times 10^{-8}$	$1{\cdot}84 \times 10^{-8}$
Meta-P/cell (μgm.)	$2{\cdot}19 \times 10^{-8}$	$2{\cdot}35 \times 10^{-8}$	$2{\cdot}34 \times 10^{-8}$
Insoluble N/cell (μgm.)	$1{\cdot}59 \times 10^{-7}$	$1{\cdot}97 \times 10^{-7}$	$1{\cdot}77 \times 10^{-7}$
Dry weight/cell (μgm.)	$5{\cdot}77 \times 10^{-6}$	$5{\cdot}68 \times 10^{-6}$	$5{\cdot}62 \times 10^{-6}$
Vol./cell (μ^3)	53·21	55·43	54·77

The above results indicate that there is a doubling of the DNA content following fusion and that this is maintained throughout the rest of the life-cycle up to chlamydospore formation. There is thus a strong suggestion that meiosis occurs in the chlamydospores, in the stage preceding the formation of the endospores. Having now ascertained where meiosis is likely to occur, and by concentrating cytochemical investigations on this stage, it should be possible to find out if this is the case or not. The metaphosphate content of the cell is high, and this may be the reason why most nuclear stains tried, stained the cytoplasm as well as the nucleus. By growing single spore colonies on a medium devoid of phosphorus it has been possible to show the nucleus. One of the striking features of the results is that the RNA content of diploids is only very slightly greater than that of haploids. This situation is in contrast to that found by Ogur *et al.*[3] in yeast, where the RNA content, like that of DNA, was an indication of ploidy. The values of metaphosphate, dry weight, insoluble nitrogen and volume, like that of RNA, are all similar in haploid and diploid cells of *P. inundatus* and the only significant difference detected is in the DNA content.

The measurement of DNA content appears to be a valuable method of determining the position of meiosis in cases where cytological methods give unsatisfactory results. While the DNA content in both *Protomyces* and yeast appears to be a reflexion of ploidy, the RNA content appears to be related to the metaphosphate, insoluble nitrogen, dry weight and volume of the cell. *Allomyces*[7] and *Enteromorpha*[8] like *Protomyces* are examples of natural diploids the cells of which are about the same size as those of haploids. In most cases where diploids are produced artificially, for example *Aspergillus*[9] and *Aspidium*[10], they are double that of the haploids. Yeast is one of the few cases found in Nature in which diploids are twice the size of haploids. A knowledge of the RNA contents of haploid and diploid cells of other types of organisms would be of great interest.

Giant cells have been obtained in our cultures by the use of camphor[11] and are being examined biochemically. By comparing these giant cells, which may be diploids, with naturally occurring ones we hope to elucidate the contrast between diploid cells of *Protomyces* and those of yeast.

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GENETICS

Pulmonary Tumours, Group VII and Sex in the House-Mouse

Although the exact number of factors concerned in the inheritance of susceptibility to pulmonary tumours in the house-mouse is still unknown, experiments by various workers[1-4] have suggested the presence of one major factor in linkage group VII: this factor is here termed *Tm*. A series of experiments has been undertaken to confirm the existence of this factor and to obtain an estimate of its position in group VII. The following is a preliminary report of the results of the first experiments in the series.

Mice of the following stocks were used: high susceptibility—strain *A/Cam*; low susceptibility—vestigial tail–wavy-2 (*vt–wa*-2); homozygous Rex (*Re*); *CBA/FaCam*

Strain *A* animals of both sexes were mated to the two mutant stocks, and the F_1, again of both sexes, were back-crossed to multiply recessive, low susceptibility stocks. The offspring, which totalled more than 1,000, were injected intraperitoneally at eight weeks of age with 0·5 mgm. 1 : 2 : 5 : 6-dibenzanthracene suspended in 0·5 ml. horse serum. They were killed six months later and their lungs placed immediately in Tellyesniczky's fluid. The number of nodules present on the surface was counted 24 hr. later. This number was taken as a measure of susceptibility. For a preliminary analysis all animals without tumours were classified as non-tumourous and animals with tumours, whether one or more were present, were classified as tumourous.

wa-2, *vt* and *Re* were chosen as markers to represent group VII as they have minimal effect on weight. This is of importance, because weight is known to affect susceptibility, and interaction between weight and susceptibility can mimic linkage in linkage experiments that only involve one of the linkage phases.

The recombination values obtained for *wa*-2–*Tm* and *vt–Tm* were different from 50 per cent at the 1 per cent level of probability (Table 1). These values, and the others involving *Tm*, are calculated from coupling-phase data only. Further experiments in this series will provide repulsion-phase data, thus giving balance and allowing corrections for disturbed viability and misclassification. However, the data related to *Tm* and sex are balanced (that is, there are approximately equal quantities from both phases), and controls are available in the form of data from females heterozygous for *Tm*. The significantly low value for *Tm*–sex† is heterogeneous from a 'linkage' point of view and is due to the homogeneously higher susceptibility of females[4].

Table 1. Recombination Values involving *Tm*

Pairs of factors	Phase balance	n	r	χ^2 (1 : 1)	$\frac{r}{r+n}$
vt — *Tm*	*U*	415	334	8·76*	44·593
wa-2 — *Tm*	*U*	421	328	11·55*	43·792
Tm — sex	*B*	134	159	2·13	54·266
Tm — sex †	*B*	255	201	6·39‡	44·079
Tm — sex	*U*	58	69	0·95	54·331
Tm — *Re*	*U*	60	67	0·39	52·756

n, non-recombinants; *r*, recombinants; *U*, unbalanced data; *B*, balanced data.

* Significant at the 1 per cent probability-level.

† Significant at the 5 per cent probability-level.

‡ Data from heterozygous females.

The significant recombination values confirm the association between susceptibility and group VII and clearly demonstrate the existence of a susceptibility locus (or loci) in this region of the linkage group. As the order in group VII is known to be *wa*-2–*vt*–*Re*, and as *Re* gives a value close to 50 per cent with *Tm*, *Tm* is best accommodated on the opposite side of *wa*-2 to *Re*: the tentative order being *Tm*–*wa*-2–*vt*–*Re*. The order is tentative because misclassification of *Tm* certainly occurs to some degree in these results. An excess of *Tm* can occur when mice with any number of tumours are classified as *Tm*+, and this may be severe enough to mask the true position. This diffi-

Table 2. RECOMBINATION VALUES FOR THE GROUP VII MARKERS AND SEX

Pairs of factors	Phase balance	n	r	χ^2 (1 : 1)	$\frac{r}{r+n}$
vt — *wa*-2	*U*	1,346	459	435·88*	25·429
vt — sex	*B*	386	383	0·01	49·805
vt — sex†	*B*	606	594	0·12	49·500
wa-2 — sex	*B*	370	353	0·40	48·824
wa-2 – sex†	*B*	525	557	0·95	51·479
Re — sex	*U*	78	90	0·86	53·571

The data not involving *Tm* are twice as large as the rest because they include all the mice that were classified at birth for sex, *wa*-2, *vt* and *Re* and which died before classification for *Tm* was possible.

* Significant at the 1 per cent probability-level.

† Data from heterozygous females.

culty should be cleared up when repulsion-phase linkage data are available.

The experiments also give data on the partial sex-linkage of group VII (Table 2), a matter about which there has been some doubt. There is no indication of partial sex-linkage such as Wallace[5] found for *wa*-2 and shaker-2 (*sh*-2) or as Carter and Phillips[6] found mimicked in the offspring of heterozygous females.

More detailed discussion and analysis of these results are to be published elsewhere.

I thank Dr. M. E. Wallace for constant advice and encouragement and much helpful discussion. Part of this work was supported by a scholarship from the Medical Research Council.

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Existence of Multiple Sex Genotypes in the Hermaphrodite Polychaete Worm *Ophryotrocha puerilis siberti*

IT was demonstrated in 1951 that *Ophryotrocha puerilis* shows a marked sex variability, as the age when each individual passes from the male to the female sex phase is highly variable. *O. puerilis* can thus be considered as an unbalanced hermaphroditic species[1].

It was shown later that individuals of *Ophryotrocha puerilis* collected in the Bay of Naples and individuals of the same species collected in Plymouth Sound belong to two different sub-species[2]. Small oocytes first appear in isolated Naples individuals at a mean size of eighteen chætigerous segments, whereas in isolated Plymouth individuals they appear at a mean size of twenty chætigerous segments and therefore the two sub-species differ in the relative duration of the male and female phases. The former hypothesis of a genetical basis for sex variability inside each population is thus strongly supported.

The genetic nature of such variability was later demonstrated by the successful selection for the prolongation of the male phase and for the anticipation of the female phase[3]. Individuals that never passed to the female phase or which began to show oocytes at the mean length of 25 segments only were obtained at the fourth generation from a strain which passed to the female phase at a mean length of about 18 segments in the first generation. Selection against duration of the male phase produced individuals which began to show oocytes at 11 segments in a generation the individuals of which passed to the female phase at a mean length of 13 segments.

This series of experiments, which was interrupted in 1955, lead to a revision of the traditional interpretation of sex determination in hermaphrodites, and it has been resumed using the same technique but examining groups of more numerous individuals in each generation. Individuals have been examined three times a week.

Experiments are still going on, and their results are confirming and extending results obtained in the first series of experiments.

Crosses have started from one pair of individuals which were collected at Roscoff and thus belong to the Atlantic sub-species *O. puerilis siberti*. Their sons ($n = 148$), which form the 0 generation, pass from the male to the female phase at a mean length of 18·8 segments, which is a little lower than the mean which is characteristic for the sub-species. In the following generation the sons of the specimens that were selected for maleness ($n = 350$) begin to show oocytes at mean values of 20·9 segments, the sons of the specimens selected for femaleness ($n = 340$) pass to the female phase at about 18 segments and the mean falls a little below 17 segments in the second generation ($n = 285$, $\overline{m} = 16{\cdot}85$). Selection for the anticipation of the female phase at generation 3 has given a mean value of 16·44 in 170 individuals examined so far.

The mean lengths for the appearance of oocytes in the groups selected for the duration of male phase rise in generation 2 to about 22 segments ($n = 291$, $\overline{m} = 21{\cdot}75$) and they rise again very nearly 23 segments at generation 3.

Crosses between individuals of the second generation, which showed oocytes at 15 segments, and individuals of the first generation, which showed oocytes at 23 segments, produced groups of individuals (370 individuals altogether) which passed to the female phase at mean lengths between 18·64 and 18·69 segments, which scarcely differ from the mean obtained in the 0 generation (18·79).

This last result was to be expected on the basis of the crosses published in 1955. It falls in line with results obtained in classical research work on polygenic determination. Present experiments are showing the efficiency of selection in the next generations, which are now being investigated.

The various types of crosses and the various results inside the two lines selected for maleness and for femaleness, which may be called arrhenogenous and thelygenous lines[4], will be discussed in detail in a forthcoming paper. The existence of multiple sex genotypes is nevertheless wholly confirmed in *Ophryotrocha*. The so-called polyfactorial sex determination was demonstrated some time ago for some gonochoric cyprinodont species by Breider and by Kosswig[5], for the hermaphrodite polychæte worm *Ophryotrocha* in 1955. This mechanism now awaits application to the phenomena of sex variability in heterogonic cycles[4], and also to the phenomena of relative sexuality as defined by Hartmann.

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AGRICULTURE

Amines in Silage

In 1908 Russell, referred to by Watson[1], reported that silage contained "in addition to ammonia, amines such as pentamethylene diamine, betaine, adenine, and other unidentified purin bodies". Barnett, in his book about silage fermentation[2], expressed the opinion that putrescine and cadaverine would be present in very poor silages as products of putrefaction.

In the course of work on the chemical composition of grasses and legumes during the ensiling process, silage was prepared in plastic jars (each jar containing about 2 kgm. fresh material) from fresh green maize and lucerne. After 83 days, the maize silage had a *p*H of 3·8 and showed all the characteristics of normal fermentation. The presence of tyramine was established by chromatography[3]. Further proof of its identity was obtained by cutting out an area corresponding to the tyramine spot on the chromatogram which was not developed by ninhydrin, and eluting with warm 95 per cent ethanol. The ethanol was evaporated *in vacuo*, and the residue tested for tyramine by the 1-nitroso-2-naphthol reagent[4].

When fresh green maize was tested for amines before ensilage, no tyramine was found.

Silage from fresh green lucerne was prepared under the same conditions with the further addition of 1·0 per cent sugar. In addition to tyramine at least two more amine spots appeared to be present. One had R_F 0·81 and is possibly phenylethylamine (blue colour when developed with ninhydrin); the other one had R_F 0·50 (brown colour), and might be serotonin. The eluting solvent used was butanol/acetic acid/water (120 : 30 : 50).

Tyramine and serotonin are known to exert strong physiological effects in the animal body. The possibility that these amines might interfere with consumption of silage by cattle is under investigation.

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MATHEMATICS

Relationship between the True and Mean Anomalies

For an elliptical orbit, we have the following relations[1]:

$$r = \frac{a(1 - e^2)}{1 + e\cos\nu} \qquad \frac{\mathrm{d}M}{\mathrm{d}\nu} = \frac{r^2}{a^2\sqrt{(1 - e^2)}}$$

whence,

$$\frac{\mathrm{d}M}{\mathrm{d}\nu} = \frac{(1 - e^2)^{3/2}}{(1 + e\cos\nu)^2} \tag{1}$$

where r, a, and e have their usual meanings, and ν and M are the true and mean anomalies, respectively.

The solution of equation (1) has been obtained in various ways[2,3] giving[2]:

$$M = \nu + 2\sum_{n=1}^{\infty}(-1)^n\left\{\frac{1}{n} + \sqrt{(1 - e^2)}\right\}\varkappa^n \sin n\nu \tag{2}$$

where $\varkappa = \frac{1}{e}\left\{1 - \sqrt{(1 - e^2)}\right\}$, which gives[4], by Lagrange's Expansion Theorem[5],

$$\varkappa^n = \left(\frac{e}{2}\right)^n\left\{1 + n\sum_{\lambda=1}^{\infty}\frac{(2\lambda + n - 1)!}{\lambda!(\lambda + n)!}\left(\frac{e}{2}\right)^{2\lambda}\right.$$

As far as terms in e^4, equation (2) gives[3]:

$$M = \nu - 2e\sin\nu + \left(\frac{3e^2}{4} + \frac{e^4}{8}\right)\sin 2\nu - \frac{e^3}{3}\sin 3\nu + \frac{5e^4}{32}\sin 4\nu$$

A simplified version of the solution to equation (1) enables this expansion to be derived rather quickly.

This solution may be obtained as follows:

We have, from equation (1),

$$\frac{\mathrm{d}M}{\mathrm{d}\nu} = (1 - e^2)^{3/2}(1 + e\cos\nu)^{-2}$$

$$= (1 - e^2)^{3/2}\left\{1 + \sum_{n=1}^{\infty}(-1)^n(n + 1)e^n\cos^n\nu\right\} \tag{3}$$

Now

$$2^{n-1}\cos^n\nu = \sigma + \sum_{\lambda=0}^{\left[\frac{n}{2}\right]}\binom{n}{\lambda}\cos(n - 2\lambda)\nu,\ n > 0$$

$$\text{where, } \sigma = \tfrac{1}{2}\binom{n}{\frac{n}{2}}, \text{ for } n \text{ even}$$

$$= 0, \text{ for } n \text{ odd}$$

and $\left[\frac{n}{2}\right] \equiv$ integral part of $\frac{n}{2}$.

Hence, equation (3) becomes:

$$\frac{\mathrm{d}M}{\mathrm{d}\nu} = (1 - e^2)^{3/2}\left[1 + 2\sum_{n=1}^{\infty}(-1)^n(n + 1)\left(\frac{e}{2}\right)^n \left\{\sigma + \sum_{\lambda=0}^{\left[\frac{n}{2}\right]}\binom{n}{\lambda}\cos(n - 2\lambda)\nu\right\}\right] \tag{4}$$

But,

$$1 + 2\sum_{n=1}^{\infty}(-1)^n(n + 1)\left(\frac{e}{2}\right)^n\sigma = (1 - e^2)^{-3/2}$$

Hence, equation (4) becomes:

$$\frac{\mathrm{d}M}{\mathrm{d}\nu} = 1 + 2(1 - e^2)^{3/2}\sum_{n=1}^{\infty}\sum_{\lambda=0}^{\left[\frac{n}{2}\right]}(-1)^n(n + 1)\left(\frac{e}{2}\right)^n\binom{n}{\lambda}\cos(n - 2\lambda)\nu$$

Integrating with respect to ν, we obtain the solution in the form:

$$M = \nu + 2(1 - e^2)^{3/2}\sum_{n=1}^{\infty}\sum_{\lambda=0}^{\left[\frac{n}{2}\right]}(-1)^n(n + 1)\left(\frac{e}{2}\right)^n\binom{n}{\lambda}\frac{\sin(n - 2\lambda)\nu}{n - 2\lambda} \tag{5}$$

since M and ν vanish together.

From this equation, it can be seen that the coefficient of $\sin\nu$ is:

$$2(1-e^2)^{3/2}\sum_{n=1}^{\infty}(-1)^n(n+1)\left(\frac{e}{2}\right)^n\sigma' \qquad (6)$$

$$\text{where, } \sigma' = \begin{pmatrix} n \\ \frac{n-1}{2} \end{pmatrix}, \text{ for } n \text{ odd}$$

$$= 0, \text{ for } n \text{ even.}$$

Extracting $\left(\frac{e}{2}\right)$ from equation (6) we obtain the expression :

$$e(1-e^2)^{3/2}\sum_{n=1}^{\infty}(-1)^n(n+1)\left(\frac{e}{2}\right)^{n-1}\sigma'$$

But,

$$\sum_{n=1}^{\infty}(-1)^n(n+1)\left(\frac{e}{2}\right)^{n-1}\sigma' = -2(1-e^2)^{-3/2}$$

Hence, the coefficient of sin ν is $-2e$ for all n, and the solution (5) may be expressed as :

$$M = \nu - 2e\sin\nu + 2(1-e^2)^{3/2}\sum_{n=2}^{\infty}\sum_{\lambda=0}^{\left[\frac{n}{2}\right]}(-1)^n(n+1)\left(\frac{e}{2}\right)^n\binom{n}{\lambda}\frac{\sin(n-2\lambda)\nu}{n-2\lambda}$$

where $n \neq 2\lambda + 1$.

As from equation (2), we obtain as far as terms in e^4, the usual expression :

$$M = \nu - 2e\sin\nu + \left(\frac{3e^2}{4}+\frac{e^4}{8}\right)\sin 2\nu - \frac{e^3}{3}\sin 3\nu + \frac{5e^4}{32}\sin 4\nu$$

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The Complete *n*-Point Graph

A COMPLETE n-point graph consists of n points in a plane (or on the surface of a sphere), each joined to all the others by lines, not necessarily straight. The problem of determining the minimum number of intersections, m, for various values of n is being investigated.

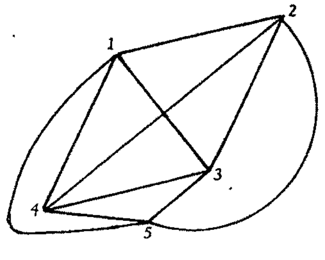

Fig. 1

Fig. 1 shows a complete 5-graph which has 1 intersection, this being the minimum for $n = 5$.

The problem does not appear tractable analytically, so it was programmed for the University of London *Mercury* computer. The programme was written on the assumption that a minimum for $n + 1$ points can be obtained by adding an extra point in a suitable place on a minimum solution for n points. However, the computations have proved that this apparently natural assumption is false : two different minimum configurations for $n = 7$, $m = 9$ were taken and one led to the true minimum $n = 8$, $m = 18$, while the other led to $n = 8$, $m = 19$.

I thank Dr. A. D. Booth for help and encouragement.

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STATISTICS

A Basic Notion in the Theory of Cognitive Systems

IN a previous communication[1] a network was described which would resolve linear relations among continuous input signals. It was indicated that for times greater than the resolution time (for some intrinsic error criterion) this net could follow changing relations and thus, by means of other nets, one could observe the relations between relations and so on. The process described is essentially that of ascending a scale of logical types. To characterize this more generally, consider a binary time series $y(t)$ of discrete time t. If this contains $2^r \leqslant 2^m$ structures of length it can be described by a logical function :

$$y(t+1) = f\{y(t)\ \ldots\ y(t-m),\ x_1(t)\ \ldots\ x_r(t)\} \qquad (1)$$

where it is supposed that the variance $\mu(x)$ has been minimized. The x's then describe, roughly speaking, which of the 2^r structures is under consideration. But this by no means determines the function uniquely; there is an enormous redundancy of something like $2^{2^r}(2^m/m)$ possibilities (as examination of the periodic case, $r = 0$, shows). However, for realization of logical functions it is well known that there are nets which are minimal with respect to number $v(f)$ of connexions required. In this case one may expect to obtain effective unicity by requiring the minimization of a function such as :

$$H(f, x) = \log v(f) + \log \mu(x) \qquad (2)$$

If $H(f, x)_{\text{min.}} < \log \mu(y)$ we say that (1) is a resolving transformation $f^{(1)}$ of type 1, written formally as :

$$y = f^* x^{(1)} \qquad (1')$$

Clearly the process could be repeated on the variables x to obtain higher types, but this requires a notion, in a certain sense dual to resolving transformation, which I call selective punctuation, $y^{(1)}$. This is the insertion of a sequence $y^{(1)}$ of length m into the signal and may be regarded as the formalization of an experiment to test whether the $x^{(1)}$ so generated by the natural continuation of $y^{(1)}$ can be resolved into an $f^{(2)}$, and so on.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, May 29

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. David Attenborough: "Travels in Madagascar".

Tuesday, May 30

Laboratory Animals Centre (at the Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2), at 10 a.m.—Symposium on "Hazards of the Animal House".

Institution of Chemical Engineers (at the Royal Commonwealth Society, Craven Street, Strand, London, W.C.2), at 2.30 p.m.—Symposium on "Biochemical Engineering".

Tuesday, May 30—Friday, June 2

Seventeenth Radio and Electronic Component Show (at Olympia, London).*

Wednesday, May 31

British Association for Commercial and Industrial Education (at County Hall, London, S.E.1), at 9.30 a.m.—Exhibition and Conference on "Successful Instruction".

University of London (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. David B. Sprinson (Columbia University): "The Reactions of Aromatic Biosynthesis".*

Institute of Information Scientists (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Annual General Meeting. 7.30 p.m.—Miss M. Webb: "Some Thoughts on the National Science Reference Library".

Thursday, June 1

Institute of Physics and the Physical Society (in the Physics Department of Imperial College, Prince Consort Road, London, S.W.7), at 2.30 p.m.—Dr. R. W. Twiss: "On Re-designing Michelson's Stellar Interferometer"; Mr. K. Hacking: "Secondary Flare in Lenses".

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. W. Dresser: "A Study of the Adaptive Secondary Response to a Protein Antigen in Mice"; Mr. J. G. Howard and Mr. M. F. A. Woodruff: "Effect of the Graft-versus-Host Reaction on the Immunological Responsiveness of the Mouse".

Thursday, June 1—Saturday, June 3

British Society for Immunology (in the Edward Lewis Theatre of the Middlesex Hospital Medical School, Cleveland Street, London, W.1)—Spring Meeting.

Friday, June 2

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 10 a.m.—Dr. H. E. M. Kay: "Some Relevant Problems of Homotransplantation".*

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. P. D. MacMahon: "Development of Stabilizing Equipment for Helicopters".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Experimental Officers (2) (with a university degree in agriculture or in pure science with main subject botany or bacteriology) in the Department of Agriculture and Fisheries for Scotland, for research work in the field and in the laboratory on potato black-leg disease—The Establishment Officer, Room 73, Department of Agriculture and Fisheries for Scotland, St. Andrew's House, Edinburgh 1 (May 31).

Assistant Lecturers (with particular interests in physical chemistry, inorganic chemistry or organic chemistry) in the Department of Chemistry—The Secretary, The University, Edinburgh (May 31).

Lecturer in the Department of Pathology—The Secretary, The University, Glasgow (May 31).

Lecturer or Assistant Lecturer in Pure Mathematics—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (June 3).

Research Demonstrator in the Department of Physics—The Registrar, The University, Leicester (June 3).

Tutorial Assistant in the Department of Geography—The Registrar, The University, Leicester (June 3).

Assistant Lecturer in Organic Chemistry—The Registrar, The University, Manchester 13 (June 5).

Lecturer (Grade II) in the Department of Organic Chemistry—The Registrar, The University, Bristol 8 (June 5).

Lecturer or Junior Lecturer in Applied Mathematics—The Registrar, Trinity College, Dublin 2 (June 5).

Lecturer or Assistant Lecturer (preferably qualified to carry out research in electrophysiology) in Marine Zoology at the Marine Biology Station—The Registrar, University College of North Wales, Bangor, North Wales (June 6).

Chair of Physics; and Chair of Chemistry (from 1962)—The Registrar, University College of Sussex, Stanmer House, Stanmer Road, Brighton (June 8).

Research Fellow in the Nuclear Physics Group of the Department of Physics, to prepare and participate in experiments on the 7 GeV. proton synchrotron at the Rutherford High Energy Laboratory of the National Institute for Research in Nuclear Science—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 8).

Assistant Lecturer and a Lecturer in Physics—The Registrar, The University, Nottingham (June 10).

Demonstrator in Zoology—The Registrar, The University, Nottingham (June 10).

Lecturer or Assistant in Geology—The Secretary of University Court, The University, Glasgow (June 10).

Research Fellow in the Department of Biochemistry—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (June 12).

Assistant Lecturer or Lecturer (honours graduate) in Mathematics—The Secretary, Bedford College, Regent's Park, London, N.W.1 (June 17).

Lecturer (graduate in science with special qualifications in botany or agricultural botany) in Botany—The Secretary, The West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (June 17).

Lecturer (physicist or physical chemist, with some background in the biological sciences) in Radiation Physics and Biology in the Isotopes Laboratory—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (June 17).

Research Assistant (chemist, preferably with two or three years research experience), for work on volatile inorganic compounds in collaboration with Dr. L. H. Long—The Secretary, The University, Exeter (June 17).

Lecturer in Physical Chemistry at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 22).

Assistant Lecturer or Lecturer in the Department of Mathematics—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (June 24).

Demonstrator or Assistant in the Sub-Department of Pharmacology, University College of the West Indies—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (June 28).

Head (with wide experience of research on plant diseases caused by fungi and bacteria, and preferably a special interest in microbiological problems, for example, as associated with soil or mushroom composts) of the Mycology and Bacteriology Department—The Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex (June 30).

Chair of Philosophy; and Senior Lecturers or Lecturers in Mathematics at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 3).

Lecturer in Geography in the Department of Metallurgy The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

Lecturer in Mathematics; and a Senior Lecturer or Lecturer in Physics at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 15).

Lecturer in Prehistory in the Department of Anthropology, University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 15).

Postgraduate Research Assistant (with a knowledge of X-ray diffraction methods), for studies of liquid structure under the personal supervision of Prof. J. D. Bernal—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

Senior Research Assistant (preferably with a special interest in pharmacology or physiology) in the Animal Laboratory of the Department of Psychology, for work on variables affecting conditioned behaviour—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

Technician for biochemical work in the Department of Cancer Research—The Administrative Officer, Mount Vernon Hospital, Northwood, Middlesex.

REPORTS and other PUBLICATIONS

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PHYSICS DE[illegible]

SCIENTIFIC AND TECHNICAL MAN-POWER IN BRITAIN

IN the recent debate in the House of Commons on the economic situation in Britain, no attention was given to the situation as regards scientific and technical man-power, though this is at least as important as the question of mobility of labour between industry and industry and skill and skill. Nor is it reflected in the report by Political and Economic Planning (see *Nature*, April 15, p. 199), which was so freely quoted in the debate. That report indeed suggests that the chief impediment to a faster growth of the British economy is that we do not want it enough. The British worker is not sufficiently prepared to take risks, to move to a new job and fight for advancement; and in the British business-man also the aggressive, dynamic, restless spirit is less in evidence than the desire for an easy life with safe but small profits.

Political and Economic Planning does not suggest that these natural desires for security should be condemned or ignored; rather they must be faced as valid human needs, which may limit the pace of technological advance and the rate of growth of the economy, but are susceptible to deliberate measures to overcome them. It criticizes the lack of flexibility in industry, and the wasteful hoarding and inefficient use of labour, to which attention was directed in the House of Commons, and it emphasizes the need for Britain to rely on the skill and brain-power of the population to maintain and even improve the standard of living. For that reason alone, social investment, and particularly capital expenditure on education, is regarded as among the most urgent calls on the investment resources of the nation; but in the same connexion, referring to the distribution of public investment, stress is laid on the importance of increasing more rapidly the productivity, and so lowering the cost of coal mining, as well as increasing the efficiency of the railways. Although it is not disputed that heavy taxation does cause some distortion of the economy, and in particular the allowances for depreciation of capital assets are criticized, the PEP study found insufficient evidence to support the more sweeping assertions about the harmfulness of the present taxation system. The allocation of resources, however, is the vital matter, especially where production cannot be immediately increased, and it is admitted that consumption expenditure has not been restrained sufficiently to keep the economy in a healthy condition and to permit a satisfactory steady rate of growth.

One of the reasons for the inadequate rate of growth of the British economy since the Second World War may have been the absence of an objective of growth at which to aim, but PEP can only propose that all efforts should be made by the Government, the employers and the trade unions to increase co-operation in economic life. The rate of growth of productivity could be improved by stepping up the amount of investment; but within this general aim of increasing the level of investment, priority should be given to encouraging labour-saving investments. Attention must also be directed towards increasing the mobility of labour and achieving the maximum flexibility in the labour force; and since the shortage of skilled man-power of all kinds is likely to persist as industry expands, technical education will continue to be a priority and one in which shortage of teachers is likely to be as great a problem as lack of buildings. Apart from this, however, general education at all levels is an important factor in economic progress and the report reiterates that investment in education, apart altogether from its social value, can make a great contribution to production.

What is particularly obvious from the PEP report, without being explicitly stated, is the need for education, scientific research, defence and fiscal policy to be effectively integrated if proper use is to be made of the resources of Britain. It is encouraging to note that there is already some evidence that this is recognized to some extent in the machine-tool industry, where the manufacturers accept that constant work is needed in design, research and development to enable British products to be competitive. The shortage of technically qualified men cannot be overcome by the manufacturers alone; although the industry has provided for scholarships, time will be required before the schools of research and higher training in machine-tool technology now being developed at the Manchester College of Science and Technology, the University of Birmingham, the Royal Technical College, Salford, and the College of Aeronautics, Cranfield, with research grants of more than £100,000 from the Department of Scientific and Industrial Research, or from the special courses at the Birmingham, Loughborough and Salford Colleges of Advanced Technology. Nor can the manufacturers alone solve the problem of improving delivery dates or secure the full benefits of adopting electronic techniques to the control of machine tools without the co-operation of the trade unions.

It is not sufficient, however, for such co-operation and co-ordination to be secured in respect of a single industry. The improved facilities for higher technical education that it needs must form part of a considered policy and programme for expanding technological and technical education in Britain, just as the expansion of its research effort must form part of a considered programme for research and development generally. Further, the demands made for scientific and technological man-power alone interlock the expansion of research and development with that of technological and university education generally.

Obviously many of the necessary decisions have to be taken at the highest possible level. Many of them clearly fall within the sphere of the Minister for Science, but other Ministers are also involved—those of Education, of Power, of Labour, for example, as well as the President of the Board of Trade and the Chancellor of the Exchequer. The ultimate decisions can be taken only at Cabinet-level, but the Minister for Science bears a large responsibility to see that the scientific and technological issues are clearly and forcefully formulated, especially in respect of the demands they make for scientific and technical man-power and provision for its training. The Advisory Council for Scientific Policy, as its last annual report shows, is already clarifying some of the requirements, and the research councils are also examining some of the issues, notably the Council for Scientific and Industrial Research. Some, however, like the Nature Conservancy, appear to lack the resources required even for preparatory exploration, and it should be a first priority on the part of the Minister for Science to see that the task of elucidating all the factors that bear on the distribution of Britain's scientific and technological effort is carried out with the utmost urgency. Only when the results of such a survey are fully available can really sound decisions be taken; in the meantime, it is only too likely that action may have to be taken to strengthen the country's economic position before the full facts are known. The recent debates in Parliament have demonstrated the critical importance of some of the inquiries now being made into particular aspects of Britain's scientific and technological effort, and the urgent need to complete these inquiries at the earliest possible moment.

Mr. Albu, in a recent broadcast talk, suggested that one reason for the backwardness of some sections of British industry is their failure to adapt themselves to the changed educational system and to employ university-trained scientists and engineers not only in research but also in design and production. Possibly Mr. Albu makes too light of the contribution which should also be coming from the colleges of technology, and he may rather over-stress the part that the Government could play in changing the situation. Undoubtedly, however, the Government could ensure that the board of every nationalized industry includes a first-class scientist or technologist, and it could stimulate the backward industries by detailed inquiries such as those made by the Department of Scientific and Industrial Research. It has considerable powers which could be exercised through the research associations, and its authority as a large-scale purchaser could also be used to encourage research. These powers, once again, can only be exercised wisely on the basis of knowledge and not on political theory, and Mr. Albu's suggestions once again really illustrate the decisive position of the Minister for Science and of the Advisory Council for Scientific Policy. On the wisdom and urgency with which Lord Hailsham and his advisers address themselves to these problems depends more than on any other single factor the success of Britain's effort to adjust itself to the world situation of to-day.

THE. AGE OF CONFERENCES

Communication or Conflict

Conferences—Their Nature, Dynamics, and Planning. Edited by Mary Capes, with the collaboration of A. T. M. Wilson. Pp. xi + 228 (London: Tavistock Publications, 1960.) 30*s*. net.

EVERY year, tens, and possibly hundreds, of thousands of people congregate in groups, both large and small, in many different lands. For the most part the purpose of these meetings is the exchange of knowledge and ideas, but some others are held to attempt agreement between delegates whose vital interests may be influenced by any resolutions which may be adopted. Most participants arrive in various conditions of hope and apprehension and depart in varying degrees of despair, stimulation or exhaustion, each with his own opinion of the success or otherwise of the conference.

The book under review is concerned with the factors which make for success or failure. It seems that an "international conference on the functioning of international conferences" was held at Eastbourne during 1956 at some unspecified date, which, from the reference in the foreword to prevailing snow and ice, I can only conclude was within the period January–April. This omission is characteristic of the imprecision of much of the text. The meeting was convened by the World Federation for Mental Health, presided over by Dr. Brock Chisholm (formerly director-general of the World Health Organization) and attended by a mixed bag of social scientists, numbering twenty in all, in which psychiatrists were the most numerous. Three pre-circulated working papers were prepared for the conference entitled: "The Cultural Perspective" (by Margaret Mead), "The Appreciation of Individual Idiosyncrasies" (by Otto Klineberg) and "Historical Development of Group Work" (by T. A. Ratcliffe) and these form Part 1 of the book. Discussion of these papers in plenary sessions led to the setting up of four groups, each having five members who were responsible for leading discussions on a particular topic of the conference. The record of these discussions comprises Part 2. Part 3 consists of four appendixes dealing chiefly with the organization and running of conferences for different purposes.

It is difficult to know what purpose this book will serve. The stated object was "not to provide a rigid set of blue-prints on conference management but to sensitize those interested in small conferences, whether as sponsor or participator, to the subtleties involved when people come together in discussion". Some of the supposed subtleties are so obvious as to be axiomatic, as for example, the cautionary tale on p. 53 of which the moral is to avoid large dormitory accommodation (30 to a room). Some are so masked by verbiage as to be barely discernible, while others are plainly nonsensical. To a physical scientist the notion (p. 14) that "In any conference it is desirable to discount as many as possible of the disadvantages of previous experience or unequal experience—this means that it will be *desirable to equalize language background by having each participant speak in a language not his own*" (my italics) is patently silly.

Scientists are notoriously avid conference goers, and have had to devise semi-empirically sets of rough-and-ready rules appropriate to the successful conduct of their meetings, whether these be large jamborees or small groups of experts discussing narrow fields, as in the Gordon research conferences, or the informal Faraday Society Discussions, or international delegate conferences charged with specific duties such as agreement on nomenclature or atomic weight standards. The learned societies of Britain and other countries have, among their senior officials, persons with very wide experience of all these types of meetings and of the problems of mechanics, procedure, differences of language and cultural background of the participants, and it is therefore all the more remarkable that apparently not one of these officials was invited to participate in this meeting. One is tempted to think that it would have been difficult for such an official to sit patiently through the "widely exploratory discussion", extending over *three* of the seven days of the meeting, which was apparently necessary before 21 participants could frame the agenda and "draw up an integrated programme of work acceptable to every member". Even then the device of setting up four groups had not been agreed on.

The organizers of international scientific conferences who peruse this book will, despite its many shortcomings, be able to learn some lessons—not all of which may be those intended by the author and contributors. In particular, it is useful to be reminded that each participant arrives with his own characteristic attitudes and preconceptions and his own particular responses towards external problems with which he is presented and which may include a strange language, strange procedures and the establishment of understanding with conference-members of different characteristics and cultural background, and that a prime function of the organizing committee must be to devise machinery which will effectively minimize the barriers which may thereby be created. It might be a salutary exercise for a conference-organizing committee at its first meeting to make a précis of these and related problems and the devices which have been, and might be, adopted to solve them. As a preliminary to this exercise the committee members might read this book with some profit, though, at the conclusion of their reading, they might well turn to the opening sentence of the foreword in which it is written that the international conference of which this book is the outcome "was perhaps unique" and be forgiven for hoping that it will remain so.

F. S. Dainton

CELLULAR ORGANIZATION

The Organization of Cells and other Organisms

By Laurence Picken. Pp. xxxvii + 629 + 34 plates. (Oxford : Clarendon Press ; London : Oxford University Press, 1960.) 84*s*. net.

The investigation of the biological cell has received in the past ten years a great impetus, primarily because of the insight that new techniques have given into the structure of its constituent macromolecules. This scholarly book is therefore most opportune, and its relation to present-day trends in cell biology needs careful consideration.

As the author indicates, this is an attempt to integrate the structural aspects of virology, bacteriology and immunology with cytology, and it is the great merit of the book that one author has been able to consider viruses, bacteria, the protozoa and the various structures inside and outside the cells of animals and plants from a single point of view. It must be unique to find an interesting discussion by the same author of, for example, bacteriophage genetics and the cuticle of isopods, and to find an attitude which invites the reader to study not only the observations but also the concepts which inform our attitude to the cell. In this breadth of approach the book compares with the great cytologies of the past and in its emphasis on structure and shape with D'Arcy Thompson's classic work.

There is, however, one major defect, which must be mentioned. This is the refusal, albeit deliberate, to examine, except in general terms, the chemical basis of the fine structures discussed. We thus have the study of macromolecules independently of the molecules which comprise them and with little mention of detailed aspects of their biosynthesis and replication. For example, while Gale's early work on protein synthesis and ribonucleic acid is mentioned, that published by Zamecnik, Hoagland and their colleagues since 1955 is not. A consideration of the latter would surely have modified the sections on cytoplasmic ribonucleic acid, and might perhaps have altered a balance in which three pages are devoted to the endoplasmic reticulum under the heading of "Other Cytoplasmic Membrane Systems", compared with the seventeen pages each devoted to the Golgi apparatus and the mitochondria.

It is also possible to criticize the rather naïve template hypothesis of protein synthesis discussed here, and the absence of any mention in this context of possible correlations between base sequence in deoxyribonucleic acid and the composition and structure of proteins. It would also have been most interesting to have had some discussion on the structure of myoglobin in relation to the other protein structures illustrated.

Inevitably, of course, in such a complex book as this, some at least of the interpretations will have been modified even before it reaches the printers. Even so, it is clear that these omissions and others like them arise from the basic philosophy which permeates it, and it is necessary to ask whether it is indeed possible to discuss the organization of cells without a more detailed consideration of their chemistry. Such a requirement might well make an already immense task prohibitive, but there is the alternative of treating a narrower range of biological structures in greater detail. One does not have to hold the extreme view that all biological problems can be solved with the aid of the enteric bacteria and their parasites, to wish that the author had given more time to a discussion of this choice.

This is really the crux of the matter. Imperfect scientists, faced with such a fascinating array of structures, may well be diverted from their immediate problems. May there not be sufficient paradoxes and exceptions in the present narrow fields to stimulate the most lethargic research worker ? Does not the emphasis on the final structure tend to hide an essential unity in ontogeny of everything the cell makes ? It is possible to conceive, and is even probable, that the nematocyst of a cœlenterate is synthesized in the same way as the pancreatic enzymes. It may well be that if we knew the contents of one 'black box', we could predict the properties of all the other cellular 'black boxes'.

Whatever our answers to these questions, they can only affect the emphasis that we would put on this or that aspect of structure, and not its general importance to our understanding of the cell. This careful and well-written account will provide an introduction to a whole range of problems, some of which are in danger of being neglected to-day—an introduction which will be helped by the presence of many plates and diagrams, including a welcome use of X-ray diffraction photographs to illustrate various facets of macromolecular structure. These latter would have been even more valuable, however, if the technique had been briefly described in order that the newcomer might have a better opportunity to appreciate the molecular basis of the identities and differences, which he is asked to see in the illustrations.

The volume with its excellent index and bibliography is, as might be expected, very well produced except for the descriptions which appear under the plates themselves. These are so short as to be misleading in certain cases (for example, Figs. 15·3 and 19·3), and the reader has always to refer to the longer descriptions, collected together at the beginning of the book.

In spite of its shortcomings this is a great personal achievement; in some ways more stimulating because it advances a view of the cell at present unfashionable; if there is one thing to be learnt from this book, it is that fashions change.

P. M. B. WALKER

AVIATION METEOROLOGY

Weather Forecasting for Aeronautics

By Joseph J. George. Pp. ix+673. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 15 dollars.

Handbook of Aviation Meteorology

(Air Ministry: Meteorological Office.) Pp. xvi+404+26 plates. (London: H.M. Stationery Office, 1960.) 25*s*. net.

THE first book is fascinating for the meteorologist whether or not he is concerned with the provision of forecasts for aviation. It is essentially a practical manual of techniques and rules for weather forecasting based on a wealth of experience and extensive study over a long period by a man who has for years been concerned with applying meteorology to airline operations.

The results largely apply to the North American continent and are thus not necessarily always directly applicable elsewhere in the world. Nevertheless, the author's techniques and presentation are sure to tempt many forecasters to try the methods out for themselves and will stimulate ideas on how comparable problems might be tackled elsewhere. This is where the book's value to the meteorologist outside North America lies.

It is unique in the field of meteorological literature to come across a book of such practical application that one cannot help but wonder whether meteorological research effort in general is too much devoted to theoretical studies. Perhaps the author himself had this in mind when he says, in reference to other works on the prediction of the movement and development of maritime cyclones, "the material is interesting . . . but it has the drawback of being mostly subjective and providing no numerical answer." This search for a numerical answer is the keynote of the book, with the result that no attempt is made to justify or explain statements and rules in terms of physical and dynamical meteorology. Some of these may cause a raising of eyebrows among the theorists. Mr. George need not make any apology on this score, however, since it is quite apparent that he is dealing throughout with the application of facts emanating from appropriate studies and their justification, and explanation is neither necessary nor appropriate in a book of this nature.

The book can be regarded as composed of three sections. The first (Chapters 2–11) deals with the movement and development of pressure systems and fronts, culminating with two chapters contributed by R. J. Shafer on the computation of pressure changes (referred to as "the Poor Man's Numerical Weather Prediction System") and an example of the construction of a prognostic chart by applying the rules developed in the preceding chapters. Altogether it constitutes nearly half the book and includes practically 100 pages on the movement, deepening and filling of cyclones.

The second covers the prediction of meteorological elements of particular significance to the operation of aircraft, including very low ceilings and fogs, severe weather conditions (thunderstorms, line squalls, turbulence, hail, icing), upper winds and temperatures. It is most comprehensive and includes many useful empirical formulæ. The section is rounded off by a chapter on the use of radar in forecasting.

The third section devotes some 120 pages to local forecast studies relating to 14 airports in the United States. These could well serve as models of the type of work that should be done at every airport in the interest of improving the accuracy and reliability of aerodrome forecasts within the limits of present-day observing practices.

Every forecaster engaged in work for aeronautics should have a copy of Mr. George's book. Although the production is such that it would make a handsome addition to the bookcase, it deserves more than occasional reference and could well justify a constant place on the forecaster's desk as an additional tool for his job. Perhaps it is not too much to hope that it will inspire others to produce similar practical manuals on forecasting for aeronautics in Europe and elsewhere.

Handbook of Aviation Meteorology is a successor to Dr. R. C. Sutcliffe's well known *Meteorology for Aviators* which was first produced in 1938. Dr. Sutcliffe's book has been regarded as the standard text-book, not only in the United Kingdom but also in many other countries, for the meteorological training of pilots and navigators, both civil and military, for their licencing examinations. With the general extension of aviation to higher altitudes and the advancement of meteorological knowledge during the past two decades, there has been an ever-increasing need for a revised version of Dr. Sutcliffe's book. This *Handbook of Aviation Meteorology* will, therefore, be welcomed, particularly by pilots and others concerned with the applications of meteorology to aviation, as fulfilling a long-felt need.

The book includes much new material. It is gratifying to find mention of so many modern operational factors, such as temperature accountability, engine icing, runway visual range, clear air turbulence, helicopter operations, meteorological aspects of aerodrome design, to name but a few, as well as the inclusion of new knowledge about old operational problems, such as thunderstorms and airframe icing.

The treatment of some of the more difficult concepts is particularly good. They are explained lucidly and in detail, with adequate diagrams and illustrations. For example, the 'Tephigram' (so long an imponderable mystery of doubtful purpose to many) will be much more readily understood by the reader of the *Handbook*, and its many uses and value will be better appreciated by the clear examples of its application. Altogether, the evident objective of tailoring the book to suit the reader for whom it is primarily designed is successfully achieved and, in particular, it was a wise decision to keep the mathematics of the derivation of formulæ in an appendix instead of interspersing it in the text.

The only major criticism of this book is that it is already out of date in certain aspects dealing with the procedures for providing meteorological service to pilots and operators. It is recognized that frequent changes occur of necessity in these procedures in order to keep pace with the rapid development of aircraft and airline operations. Nevertheless, for a book published in the jet age of 1960, it is indeed unfortunate that the chapter on "Meteorological Service for Aviation" should largely reflect the position as it was in the first half of the 1950's when so many changes have occurred and new trends emerged since then. It is considered that as this chapter is short (barely 6 pages) it could have been brought up to date immediately before going to press, or could have been written in more general terms to withstand better the weathering of these years of advancement. At the very least the suggestion that for further reading (Appendix 3) one should consult an International Civil Aviation Organization document published in 1951 (and which has been obsolete and out of print for the past five years) should have been corrected.

Fortunately, it is only a very tiny proportion of the whole book that can be criticized in this way. Otherwise it is an excellent production and very well illustrated. It can confidently be predicted that it will be widely used by pilots and other flight-crew members not only as a basic training manual but also as an interesting and readable book of very great value to their work. This book can also be strongly recommended to anyone who wishes to extend his knowledge of meteorology beyond the elementary stage, without scaling the heights of the more advanced text-books on the subject.

THE LITERARY PRESENTATION OF SCIENCE

The Craft of Technical Writing
By Daniel Marder. Pp. xiv+400. (New York and London: The Macmillan Company, New York, 1960.) 35*s*.

HERE we have one more addition to the seemingly endless ranks of books—a large proportion of them coming from America—on how to write scientific and technical, or more properly technological, papers, articles, reviews, reports and even books, including presumably books about how to write scientific and technical, or more properly technological, papers, articles. . . . Is there here not more than a suggestion of the infinite regress ?

About two years ago I expressed the opinion (*Nature*, 184, 1749; 1959) that the best of these books, in any event for British writers, was Godfrey and Parr's *The Technical Writer*; I see nothing in the one now before me to justify any revision of the earlier statement. At a rough estimate the earlier book, though 60 pages shorter, contains twice as much useful material, justification for its being 10*s*. dearer than the new one.

Plenty of other books of this class have appeared during the post-War years; one cannot help wondering not only just what they are aiming at but also whether we are to see a continuation of this proliferative growth until every university in the United States, and perhaps every scientific and medical department in the larger ones, will have its own and special manual of presentation, alongside its own text-books of biochemistry, physics, statistical methods, etc.

In the general field of text-books there is such an abundance that the would-be student could not escape complete confusion did not the helpful hand of his professor guide him gently to usage of the proper departmental 'Holy Writ'. No such help is available, however, to the British student faced with a multiplicity of these well-meaning aids to the writing of his own language; he will therefore have to rely on such unintentional warnings as that to be found in Dr. Marder's preface, where he is told that "effective writing comes about through the knowledge and application of rhetorical principles . . ." The mind boggles at the thought of some of our less literary-minded colleagues trying to coat the bitter pill of their writing with the jam of rhetoric.

Admonitions like this destroy the value of much good advice given by Dr. Marder. After all, most rules for writing simple (when simplicity is desirable), clear and unambiguous prose are recognized by him and are indeed the same on both sides of the Atlantic. Nevertheless there are to-day legitimate differences between the two major "English-speaking" countries; a witness to this is the highly significant, even though not generally appreciated, fact that Fowler's seminal *Modern English Usage* has now been replaced by Margaret Nicholson's *A Dictionary of American-English Usage*. This, be it noted, is published in New York by the Oxford University Press, though naturally obtainable in Britain. Fowler's book was published by the same Press, but from Oxford.

Among the many deficiencies of Dr. Marder's book is his complete failure to observe, or even to mention, the invaluable distinction drawn originally by Fowler, and still retained by Nicholson, between the relative pronouns 'which' and 'that'. Again, Dr. Mander must with regret be included in the long ranks of those incurring Prof. A. V. Hill's wrath because they will insist on using 'data' as a synonym for such terms as results, figures or values. Mercifully, though, Dr. Marder has not yet joined the all too rapidly increasing band who cannot recognize the word as a plural noun.

There does not appear to be in this book anything of particular appeal to the British scientific or technological writer. In general, what Dr. Marder advocates is in fact common practice for all those who use the English language with reasonable rectitude in their writing; it differs in no important essentials from the advice to be found in the best post-War books on the subject published (and written) in the United Kingdom. They began, I think, with Kapp's *The Preparation of Technical Information*, now nearly thirteen years old, and they might fittingly be allowed to end, in any event for the present, with the comprehensive volume by Godfrey and Parr, to which I have already referred. Apart from anything else, legitimate differences in the usage of language between ourselves and our American colleagues naturally receive the American treatment in Dr. Marder's book, which is sufficient to make its use as a guide to British writers a little hazardous, to put it mildly.

There is a felicity of writing that can hardly be taught, but that may perhaps be hardly, in the other sense, acquired, if by no simply definable process; but the ability to handle the English language as an effective tool in the communication of scientific and technical knowledge and information can surely be derived, if at all, from possession and study of Kapp, Gowers, Godfrey and Parr and, perhaps needless to add, Nicholson, since Fowler is no longer with us. I cannot see that Dr. Marder has anything to offer in addition.

A. L. BACHARACH

ION TRANSPORT: AN UNORTHODOX VIEW

Transport and Accumulation in Biological Systems

By Dr. E. J. Harris. Second edition. Pp. xi + 279. (London: Butterworths Scientific Publications, 1960.) 50*s*.

THE first edition of Dr. Harris's book appeared eleven years after the War, at the end of a decade in which the use of radioisotopes and flame-photometry had led to a very rapid advance in the knowledge of cell permeability. In the four years which have preceded the publication of a second edition progress has been slower, but a great deal of experimental work has been done, and a digested presentation of this new work is very welcome. The plan of the book remains the same: a few introductory chapters on the physico-chemical features of membrane penetration are followed by accounts of the transport of water, ions, and simple organic molecules by a wide variety of animal membranes; a final chapter gives a brief account of ion movements in plant cells, particularly in giant algal cells and in yeasts. The different animal tissues are described separately, and if this sometimes does less than justice to the resemblances between cells of different types, it discourages that too easy transference of results and hypotheses from one tissue to another, which is a constant temptation to anyone working in this field.

A controversial feature of the book is Dr. Harris's interpretation of equilibration curves. In a number of tissues, including muscle and red cells, the kinetics of ion-exchange between the cell phase and the exterior are not always first order. Dr. Harris explains this by supposing that diffusion in the cell interior is a rate-limiting factor. The more usual explanation is that the departure from first order kinetics comes about because one is dealing with a whole population of cells, differing in size, shape, and probably also in permeability, so that what is observed is the sum of a large number of first order processes. Dr. Harris points out that: "Analysis of kinetics assuming several first order processes is no more than the expression of the first two or more terms of the series describing the diffusion process". This is formally correct, but, though it is good for us to be reminded that the same kinetic behaviour may be predicted by more than one model, it remains true that the population of cells exists, that the cells do vary slightly, and that when the exchange of sodium or potassium in a *single* muscle fibre was examined by Hodgkin and Horowicz, first order kinetics were followed.

The question whether exchange is limited by the membrane resistance or by diffusion in the cell interior is important because it affects one's whole approach to the investigation of ion transport. Consider, for example, the distribution of sodium and potassium across muscle or red-cell membranes. In the conventional view, the chief problem to be solved is the elucidation of the way in which energy from cell metabolism is used to shift ions of a particular species across the cell membrane from a lower to a higher electrochemical potential. In Dr. Harris's view, "There is no reason to expect that metabolic energy is directly involved in the ordinary sodium and potassium turnover processes, for these resemble exchanges on an inert ion exchanger". The need for active metabolism, if the normal intracellular ionic composition is to be maintained, is attributed to the effect of metabolism on the affinity or selectivity of the adsorption sites. If Dr. Harris is right, views on ion transport will have to be completely re-oriented. But, whatever the difficulties of the conventional theory may be, it is not likely that many workers will be as ready as Dr. Harris to dismiss the membrane—at least until there is more evidence for the existence of enough specific adsorption sites within the cell.

I. M. GLYNN

LOUIS PASTEUR

Louis Pasteur

A Master of Scientific Enquiry. By Jacques Nicolle. Pp. 196+8 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1961.) 35*s*. net.

JACQUES NICOLLE is well qualified to write about Louis Pasteur. Through family and other connexions he has been able to learn much about the motives and methods of a truly great man whose contribution to science has been far greater than his epoch-making discoveries. This book describes how Pasteur approached and tackled the immediate problem, and, as a result, Nicolle can fairly claim to have produced a great deal more than 'just another biography'.

Louis Pasteur is concerned only with Pasteur the investigator. From his beginnings in stereochemistry to his final essay in human biology, the pertinacious Frenchman approached each investigation in the same way. He never moved until he was steeped in all that was already known; he insisted on examining agronomic and industrial material on the spot; he carried out his researches as near the source of difficulty as possible, even if it meant setting up a makeshift laboratory; he reduced his propositions and his apparatus to the simplest terms; and no tentative claims were made until his results had become self-evident.

In Pasteur's inquiries into the divergent types of tartaric acid, problems of wine- and beer-making, the causes of pébrine and flacherie in silkworms, the nature of anthrax and chicken cholera, the claims of distinguished colleagues to support the truth of abiogenesis (the possibility of which Pasteur never wholly renounced) and the origin of rabies, Nicolle shows how Pasteur's method of approach never varied and still stands as a model for men of science everywhere. It is indeed a tribute to a man whose father served with Napoleon that his methods are now being applied to the solution of industrial problems. Nicolle's book can take its place as a worthy successor to René Vallery-Radot's admirable *La Vie de Pasteur* and *Les Oeuvres de Pasteur*, which owed so much to its editor, Pasteur Vallery-Radot.

T. H. Hawkins

TERPENE PROPERTIES AND STRUCTURES

Die Terpene

Sammlung der Spektren und Physikalischen Konstanten. Von J. Pliva, M. Horák, V. Herout und F. Sorm. Teil 1 : Sesquiterpene. Pp. xxii + 1–228. (Deutsche Akademie der Wissenschaften zu Berlin: Kommission für Spektroskopie.) (Berlin: Akademie-Verlag, 1960.) n.p.

THIS work is not a compilation of analytical data from all available sources. It is designed not only as an aid to the identification of terpenes but also as material for the study of relations between the infra-red spectra and molecular structure. Advisedly limiting the former objective for the better attainment of the latter, the authors include only physical data determined on the identical sample used for the infra-red work. Largely, therefore, this is a report on a consistent plan of laboratory work, over about twelve years, by a single team at the Czechoslovakian Academy of Sciences.

The plan provides for the fractionation of essential oils, by a standardized technique of distillation followed by alumina-column chromatography, into constituents in which further treatment produces no change of physical characters, including infra-red absorption. These isolates are investigated and classified primarily on the basis of infra-red spectra. Most, though not all, are compounds previously known; but the Prague group has been prominent in the work which in recent years has led to the revision or final elucidation of the structures of such long-known compounds as caryophyllene and humulene (to which, respectively, 9- and 11-membered rings are now assigned), as well as the successful investigation of such new terpenes as the acoran series from calamus oil, embodying spiro-linked 5- and 6-membered rings.

The criterion for classification of a compound is the infra-red spectrum of the saturated hydrocarbon formed by its complete hydrogenation, using Adams's catalyst at room temperature. As a means of investigating structure, it is claimed that this technique, compared with Ruzicka's dehydrogenation procedure, has more general applicability, less risk of altering the carbon skeleton, and has higher yields, permitting of work on smaller quantities. From twenty to thirty carbon skeletons are thus recognized among the sesquiterpenes, and these are named by modification of familiar, or at least identifiable, trivial names, for example, caryophyllan from caryophyllene, zieran from zierone. Sub-groups such as vetivan, isovetivan and neovetivan provide for the *cis-trans* isomerism which may arise naturally or in the course of the hydrogenation.

217 natural or artificial sesquiterpenes are described, each on its own card (British Standard *A*4 size). A complete card gives molecular and graphic (though not steric) formulæ, density, refractive index and specific rotation, boiling-point (under low pressure) or melting-point, ultra-violet absorption characters if any, a brief indication of source and means of purification, literature references on isolation and constitution, and an infra-red transmittance curve from 650 to 1,500 or 2,000 cm.$^{-1}$, as appropriate, in a rock-salt prism instrument of resolving power 2–3 at 650 and 7–10 at 2,000 cm.$^{-1}$. Experimental data on film thickness, solvent, etc., are supplied.

Indexing, both by classes and by individuals, is adequate. The type correctly indexed as "eremophilan" appears in the structure-key as "eremophyllan".

Further publication on the monoterpenes and higher terpenes, and on the results of gas-chromatographic or other improved methods of separation, is contemplated.

E. G. Kellett

SERVOMECHANISMS

Control Engineering

(The Van Nostrand Series in Electronics and Communications.) By Prof. Gordon J. Murphy. Pp. xii + 385. (Princeton, N.J.: D. Van Nostrand Company, Inc; London: D. Van Nostrand Company, Ltd., 1959.) 56*s*.

THIS book presents in some detail the mathematical theory of linear servomechanisms, and, without treating any particular topic exhaustively, describes its application to several different types of system. Techniques for non-linear systems are confined to the two final chapters. So many books have now been published in this field it is probably appropriate to describe in some detail the contents of the present text. After a very general introductory chapter, the second deals with the Laplace transform, the transfer function and related stability considerations under the heading of "Time Response". There follows an excellent chapter on design in the *s*-plane which concludes with a description of the root-locus method. The use of this method is very much a matter of practical experience, but many readers would none the less welcome rather more discussion of its application than is actually included. These two chapters are complemented by the next on frequency response, which covers well-trodden ground in a clear style with several examples to illustrate theory. The three following chapters deal with specific types of system, namely the carrier-control, dead-time and sampled-data systems. A chapter on noise concludes the treatment of linear servomechanisms. The two chapters on the analysis of non-linear systems deal with the describing function and the phase-plane technique.

The text throughout proceeds from the general to the particular, an approach which tends in places somewhat to obscure, or postpone, full appreciation of the significance of a result or technique. The work is therefore not suitable as an introduction to the subject, but forms a useful and clearly written work at a more advanced level.

Problems are included at the end of each chapter, and a table of fifty-two Laplace transforms is included as an appendix.

E. M. Deeley

PHYSICS DEPARTMENT LIBRARY

Classification and Multiplicity of Growth Layers in the Branches of Trees at the Extreme Lower Forest Border

By Waldo S. Glock, R. A. Studhalter and Sharlene R. Agerter. (Smithsonian Miscellaneous Collections, Vol. 140, No. 1. Publication 4421.) Pp. x+294+36 plates. (Washington, D.C.: Smithsonian Institution, 1960.) n.p.

THIS very detailed study of growth rings in the branches of a number of species of angiosperms and gymnosperms emphasizes the complexity of the problem of the formation of growth rings. The investigation, which was made in Texas, was concerned mainly with trees of the forest border or beyond, where conditions may be assumed to be less favourable for tree growth than within the forest itself. Some of the trees showed multiplicity of growth layers, any one of which might extend over the tree or be confined to a larger or smaller area. One branch, known to be four years old, showed eight growth rings and reference is made to trees subjected to intermittent irrigation which had 15 sharply bordered growth layers formed in four years.

The authors found these growth layers to be extremely varied and sometimes had difficulty in distinguishing between the limits of those formed during a growing season and that which terminated the season. In a distinct seasonal layer it is to be expected that the first-formed cells will be thin walled and perhaps larger than those formed at the end of the increment, where the ring may be well marked by the radial flattening of the last-formed cells or, at least, by a distinct difference between the last-formed cells of the ring and the adjacent first-formed cells of the succeeding ring. No criteria were found by which a false (intraseasonal) ring could be certainly distinguished from one terminating the period of annual growth.

Post-seasonal growth layers were found, that is, incomplete growth-layers produced after the termination of the main seasonal growth, but probably completed before the onset of winter.

Not surprisingly, the authors conclude that ring counts may not be a reliable method of absolute dating and find that frost rings, which could also be induced to form experimentally by the application of dry ice, serve as a more dependable guide.

F. W. Jane

International Review of Neurobiology

Vol. 2. Edited by Carl C. Pfeiffer and John R. Smythies. Pp. xii+410. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 11 dollars.

THE second volume of *The International Review of Neurobiology*, like the first, is a collection of nine unrelated articles, five of which may be classified as pharmacological. J. Mardones of Chile writes on the addiction of rodents to alcohol; F. W. Schueler of Tulane, on the hemicholiniums; E. J. Walaszek of Kansas, on the cortical pressor-response to epinephrine as affected by schizophrenic serum; E. Costa of Illinois, on serotonin; and A. Herz of Munich, on drugs which influence the conditioned avoidance-response of laboratory-animals. Two articles are mainly biochemical. L. E. and M. R. Hokin of Wisconsin discuss the significance of phosphatidic acid and phospho-inositide in the transport of sodium-ions across neuronal membranes; and E. Roberts and E. Eidelberg of California, the metabolic and neurophysiological roles of γ-amino-butyric acid. The remaining two contributions are both from the United Kingdom. R. M. Gaze of Edinburgh reviews work on the regeneration of the optic nerve in amphibians; and H. J. Eysenck of London describes methods of assessing the value of drugs in cases of psychological illness. All the articles contain much interesting material, but the pharmacological five are less impressive than the other four because of a tendency to build too large a superstructure of assumption on a necessarily slight base of fact. The volume is once again well produced, with full references to the literature and a good index.

Denis Brinton

The Observer's Book of Trees

Compiled by W. J. Stokoe. Revised edition. (The Observer's Pocket Series.) Pp. 228+12 plates. (London and New York: Frederick Warne and Co., Ltd., 1960.) 5*s*.

THE Observer's books must be familiar to many. Their size and uniformity make them handy pocket books, and their clarity and arrangement enable easy reference.

This book on trees is a revised edition of that published in 1937. It describes 106 species of the trees and larger shrubs found in the British Isles. Technical terms have been avoided so far as possible, as the aim has been to assist the observer in identification.

There are more than a hundred half-tone photographs, fifty line illustrations and twelve colour plates. These cover the general appearance, bole, foliage, fruit and flower of individual trees, giving excellent impressions at a glance.

One noticeable difference from the original edition is the inclusion of an illustrated guide to 'identification by twigs and buds', which is useful for months when there are no foliage distinctions. Another difference is the newly devised section of coloured plates. These plates, though less beautiful and fewer in number than in the earlier edition, cover far more ground and are of more practical value. Each plate is divided into four or five groups, showing leaf, flower and fruit of fifty-two different trees.

The book has an introduction and two indexes: one classified by families, genera and species; the other alphabetical.

This newly planned edition will be a worth-while purchase for those whose old copies are becoming worn through well-justified use. The price is amazingly low by present-day standards.

B. J. G. Maitland

Clays and Clay Minerals

Proceedings of the Seventh National Conference on Clays and Clay Minerals, Washington, D.C., October 20–23, 1958. Edited by Ada Swineford. (International Series of Monographs on Earth Sciences, Vol. 5.) Pp. ix + 369. (London and New York: Pergamon Press, 1960.) 60*s*. net.

THIS seventh volume continues the excellent tradition of previous volumes. Most of the papers are mineralogical in nature, and constitute useful advances in our knowledge of clay deposits. There is an excellent review article by W. Parrish on the X-ray diffractometer. An article by L. G. Schutz on quantitative X-ray determination of clay minerals emphasizes the numerous difficulties that still remain in this field. There is a very full study, almost amounting to a monograph, from the Agricultural and Mechanical College of Texas, on the differential settling tendencies of clay minerals in saline waters.

Apart from this article, there are few contributions on the colloidal side of clay mineral studies. However, Dr. R. K. Schofield collaborated in an article on the swelling pressures of sodium montmorillonite pastes, and this must be one of the last contributions he made before his death.

The transference of the publishing of these proceedings from the United States to Great Britain has resulted in a definite deterioration in the standard of binding. But then, few British firms seem able to achieve the quality of binding that is almost uniform in America. They ought to give some attention to the matter. In these times it ought not to require decades for a good technique, possessed by one country, to be transmitted to another.

This volume is edited, with her usual care, by Ada Swineford. I would like to plead with her, however, to have some mercy on bibliographers. These annual proceedings ought to have some short title, by which they can be referred to—I would suggest *Proc. 7th Clay Min. Conf.* The fact that this is Monograph No. 5 of the Earth Science Series seems to be just a blind, designed to make the bibliographical position even more confusing than it already is.

D. M. C. MacEwan

Lowie's Selected Papers in Anthropology

Edited by Dr. Cora du Bois. Pp. xii+509. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1960.) 10 dollars; 80*s*.

LOWIE'S memory is well served in this selection of essays by his former pupil and friend Prof. du Bois.

Quite properly, no effort has been made to select material from his eighteen classical monographs on the Plains Indians, or from his ten major books, which ought to stand complete as they are.

Fortunately, Lowie himself had already selected a good deal of material which he regarded as reasonably original and acceptable contributions to anthropological thinking, and in an autobiographical note written in reply to questions from the National Academy of Sciences summarizes his contributions with humility and with the same critical attention that he devoted to all other work.

This selection has been amplified by the editor, who consulted, among others, Kroeber, Radin and Spier. Especially valuable are the six essays left unpublished at his death.

Lowie was completely bilingual in English and German, and fluent in other languages. Moreover, he was thoroughly conversant with the work of great philosophers and scientists, and had all the interests of a well-educated man. About a tenth of his large output was devoted to subjects outside anthropology.

When we read the section on "Theories and Theorists", and note the people whose work he esteemed and critically assessed, we get the measure of the man, and realize why the best anthropologists have come from those who entered the subject with a mind already well developed in other disciplines. In my own view, it is only such people who can give the subject the place it deserves in the universe of discourse among living and civilized minds.

T. K. Penniman

AGARD Aeronautical Multilingual Dictionary

Edited by G. H. Frénot and A. H. Holloway. Pp. xix+903. (London and New York: Pergamon Press, 1960. Published for and on behalf of Advisory Group for Aeronautical Research and Development, North Atlantic Treaty Organization.) 140*s*. net.

INTERDEPENDENCE between various peoples is particularly pronounced in the aeronautical sciences and well cared for in the Advisory Group for Aeronautical Research and Development under the chairmanship of Th. von Kármán. Thus the Documentation Committee of this Group considered it an important duty to prepare and issue a technical aeronautical dictionary in several languages: Dutch, English, French, German, Italian, Russian, Spanish and Turkish. The first issue is now available and it turns out to be rather more than a dictionary: In fact, it contains translations of the *Glossary of Aeronautical Terms and Definitions* issued by the British Standards Institution (B.S. 185). The translation has been a co-operative effort and has been carried out in each case in countries in which these languages are spoken, except for the translation into Russian, which has been attended to by the British Ministry of Aviation. The result is workmanlike and authoritative, and the *Dictionary* is easy to use. The vocabulary of a rapidly developing science develops with it and it is therefore intended to continue and to expand the dictionary so as to keep it up to date. For this purpose, it is printed on loose punched sheets and supplements can easily be obtained and inserted. In this way, revisions of the source glossary, which is now about ten years old, can also be accommodated. The *Dictionary* is a credit to the editors and their many helpers and it will serve a truly useful purpose all over the world.

D. Küchemann

Wild and Free

Stories of Canadian Animals. By H. Mortimer Batten. Pp. 192. (London and Glasgow: Blackie and Son, Ltd., 1961.) 10*s*. 6*d*. net.

THIS book is a collection of seven stories. Each is about animal adventures, covering such creatures as wolves, deer, beavers, bears, moose, and many others. The stories themselves are both interesting and thrilling, but the book offers something more than this: the reader can learn much about life in the wilds of Canada; and the stories will inculcate a deeper feeling for animals' reactions to environment and circumstance than mere facts can ever portray. It shows that zoology is not merely a text-book subject, but is vivid reality if seen with a sympathizer's eyes.

For example, there is the case of an orphan fawn that makes several attempts, before succeeding, to join a herd of deer when its mother is killed. None of the does will accept it, so from sheer need of companionship, it attaches itself to the buck. From him, by example, it learns how to cope with life at an early age, when the other fawns are still relying on their mothers. Thus it is the only fawn survivor when the herd is attacked by bears.

There are seven full-page illustrations to this, the fifth and last book by the late H. Mortimer Batten.

B. J. G. Maitland

RADIO ASTRONOMY AND COSMOLOGY*

By PROF. MARTIN RYLE, F.R.S.

ASTRONOMY differs from most sciences in that we cannot do experiments; the astronomer must build up from his existing observations a picture or 'model' of the universe, and then look for further effects which should be observable if his model were correct. Although in 700 B.C. the Babylonians were making excellent observations without attempting to interpret them, and although the Greeks proposed models without always looking too carefully into how well they fitted the facts, this approach has always been present, and in the seventeenth century led, through Kepler and Newton, to a good understanding of the solar system.

But what were the stars? Kepler had no conception of their great distance, and imagined them to be on the surface of a fixed sphere with a radius some 2,000 times that of the Earth's orbit. But it seems certain that Newton had a better idea when he wrote:

"But if the matter were evenly disposed throughout an infinite space, some of it would convene into one mass, and some into another, so as to make an infinite number of great masses scattered great distances from one another throughout all that infinite space. And thus might the Sun and fixed stars be formed, supposing the matter were of a lucid nature".

We can see here the first idea of an 'infinite' universe, in which the Sun has no more significance than any of the stars.

There now began the exploration of space on an ever-increasing scale. William Herschel, by constructing larger telescopes than had ever been built before, was able to show that the diffuse band of light of the Milky Way was in fact due to countless stars, and he thus confirmed Newton's suggestion of the insignificance of the Sun. To-day we know that our own Galaxy, the Milky Way system, is a flattened disk containing some 100,000 million stars each comparable in size and luminosity with the Sun.

But the observations of William Herschel and later his son John showed, besides the millions of star images, some thousands of diffuse sources of light or 'nebulæ'. Some of these are now known to be clouds of luminous gas in our Milky Way system, but even at that time the Herschels considered the possibility that these sources were themselves distant 'island universes' too far away for any of the stars to be resolved individually. It was not until methods of distance-measurement had been established in the present century that these suggestions could be confirmed, showing an apparently limitless universe containing, at great separations, compact islands of stars, or galaxies, of which the Milky Way is one.

Besides showing the insignificance of the Earth, these observations raised new problems. One of these, first propounded by the German astronomer Olbers, is based on an observation which any of us can make—that it gets dark at night.

If we look at the sky we see that between the stars it is dark; a larger telescope will see fainter stars here, but again it is dark between them. Now if we must suppose that the universe is infinite in extent, then in whatever direction we look we will eventually see the surface of a star. Although the intensity from each star individually will be very weak, since they must occur in every direction, the sky should have the same brightness as the surface of the Sun: but if this were the case the Earth would be heated to 6,000° and would immediately be vaporized. We cannot avoid the difficulty by supposing that the light from distant stars is absorbed; by the very fact of absorbing the light the obscuring matter will be heated until it has the same temperature as the stars—when it will radiate as much light as it absorbs.

It seems that we can only explain this paradox by supposing that the universe is not infinite. But if we ignore the local stars in our own Milky Way system, we find that the sky is uniformly dark in all directions and we should have to return to a pre-Copernican idea of a universe constructed with us at its centre, having, in the meantime, demonstrated our complete insignificance.

The discovery which eventually led to the solution of the problem was made by the American astronomer Slipher, in 1912. Slipher, who was studying the spectra of the stars in distant galaxies, found that, for a particular galaxy, the spectral lines were displaced slightly, and all occurred on a slightly longer wavelength than expected. This displacement of the lines towards the red end of the spectrum, the so-called 'red shift', is just what would be expected if the light were emitted from a moving source. His observations suggested that not only this galaxy but also a number of others were all moving away from us, and later work led, in 1928, to the conclusion that no matter in what direction one looked, the galaxies were travelling away from us; it was also found that the speed of recession was greatest for the most distant galaxies.

We can now see an explanation for the darkness of the night sky; the light we receive from a very distant galaxy will have suffered such a large red-shift that most of its energy now falls in the infra-red; any visible light received must be emitted in the ultra-violet, where the energy emitted is very small. As a result the light from galaxies beyond a certain distance will be very small, and we have in effect produced a limit to the size of the universe we can see.

Although we have found an explanation for the observations, we have had to introduce the idea of a universe changing with time, and if we relate the rates of recession of galaxies at different distances, we must conclude that all those which we now see must have been crowded close together about 10,000 million years ago. This 'age of the universe' is little more than twice the age of the Earth as indicated by the radioactivity in the oldest rocks. Although the observations cannot indicate whether this period of high density should be regarded as the 'creation' or simply a phase in a universe the density of which varies periodically, they do imply that the appearance of the surrounding universe is changing with time.

In 1948, however, Bondi, Gold and Hoyle proposed an entirely different model, based on the supposition that the universe as a whole is completely unchanging with time. In order to account for the expansion of the universe and the consequent loss to view of distant galaxies, they introduced the idea that new matter is continuously created everywhere throughout space; this matter will eventually condense to provide new galaxies to replace those lost to view through the expansion. Although many types of observation and experiment have suggested that matter and energy cannot be created or destroyed, it has never been verified with an accuracy sufficiently high to rule out the theory of 'continuous creation'.

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* Substance of a Royal Institution Discourse delivered on February 24.

If we accept this Steady-State theory, we have a model which has the philosophical attraction that the universe presents the same appearance no matter from where, or at what time, it is observed. It is a universe unlimited in space or time, and the only unfamiliar feature which we have to accept is that the law of conservation of matter and energy is not quite true.

What we must now ask is whether any of the models proposed bears any resemblance to the actual universe? Here we need to look for subsidiary consequences of the different models and see whether any form the basis for observational test. While it is likely to be very difficult to distinguish between different forms of evolving cosmology, there is a good chance that there are detectable differences between any evolving model and the Steady-State model. In the evolving case we shall expect to find a progressive ageing of the galaxies, and the average distance between galaxies will steadily increase; on the Steady-State model new galaxies are being born to replace those lost by the expansion and we shall always find a mixture of old and young galaxies in any sample we choose.

If, therefore, we could compare a photograph of the surrounding galaxies taken a thousand million years ago with one taken to-day, we should be able to make this distinction. Although this particular experiment is impossible, we can do something very like it by observing galaxies at a distance of a thousand million light years. What we see is a picture of these galaxies as they were when the light left them, and since the light has been travelling for a thousand million years, we can see what changes have occurred in this time by comparing them with nearby galaxies, from which the light has taken a much shorter time to reach us. By extending our observations to greater and greater distances we can in this way explore back to earlier and earlier periods in the history of the universe. This is the reason why astronomers want to study the faintest galaxies, and why the great 200-in. telescope on Mount Palomar was built. Unfortunately, even with this telescope, it has so far proved impossible to reach any definite conclusion, owing to the difficulties of making sufficiently accurate observations of sufficiently distant galaxies.

There seemed little hope of overcoming a barrier apparently so serious; but in 1952 a most remarkable discovery was made—that the second most intense source of radio waves which had been detected in the new science of radio astronomy was a very distant galaxy, at a distance of 500 million light years. Its intensity showed that its radio emission was about a million times more powerful than that of our own or other nearby galaxies.

The fact that a source of such power could exist was surprising enough, but almost as remarkable was the evident rarity of such sources; there are about 100 million galaxies within a distance of 500 million light years, and yet only this one produces radio emission so intense.

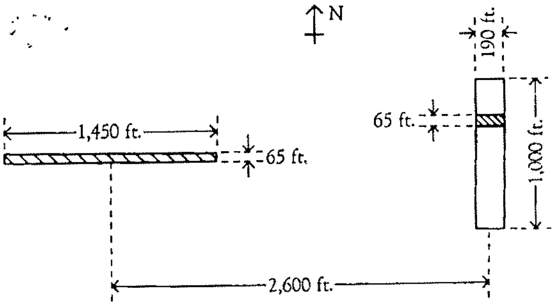

Fig. 1. Diagram of the Cambridge interferometer.

More detailed observations with the 200-in. telescope at Mount Palomar suggested that this source was a collision between two complete galaxies. This explained the rarity of such sources (for the chance of such an encounter is extremely small) and the additional energy needed to maintain the powerful emission of radio waves was supplied by the collision.

The existence of such a source suggested at once the possibility of exploring to greater distances, and thus to earlier stages in the history of the universe than would ever be possible in optical astronomy. But before we can use radio observations to test any particular cosmological theory, we must find some quantity which we can predict according to the theory, and which we can also measure. One such quantity, as we have seen, is the density of galaxies at different distances—or equally well, the density of radio galaxies. But in this case we have no way of measuring distance directly. If we knew the average power of the sources, we could use their intensities as a measure of distance.

The first problem is therefore to find the power of the sources, so that we can predict, according to a given cosmological theory, the relative number which we should expect to find in a given range of intensity. We can then compare these results with the actual numbers we find from our observations. We must endeavour to make this comparison for the weakest possible sources—for the further we can penetrate in distance, or the further back in the history of the universe we can reach, the greater will be the differences between the predictions of the different cosmological models.

It seemed impracticable to build an instrument of sufficient size by conventional means, but at Cambridge we have developed a new method of obtaining a very large resolving power, by moving a smaller aerial to different positions within a much larger effective aerial. Our new instrument consists of two elements, one of which is 1,450 ft. × 65 ft. and aligned east–west; the other is a smaller aerial 190 ft. × 65 ft. mounted on railway lines 1,000 ft. long running north–south (Fig. 1). The Earth's rotation scans the beam around a 4-deg. strip of sky each 24 hr., the strip being selected by the elevation of the two aerials.

In each set of observations, the moving aerial is driven to the north end of the rails and the observations are recorded (as holes in punched tape) for 24 hr.; we then move the aerial by about its own width and another set of observations is made. After 25 days we have all the information we need to produce a map of the whole 4-deg. strip.

In order to do this, we must make about a quarter of a million calculations, and these are made on *Edsac*, the computer at the Cambridge University Mathematical Laboratory. The output of the com-

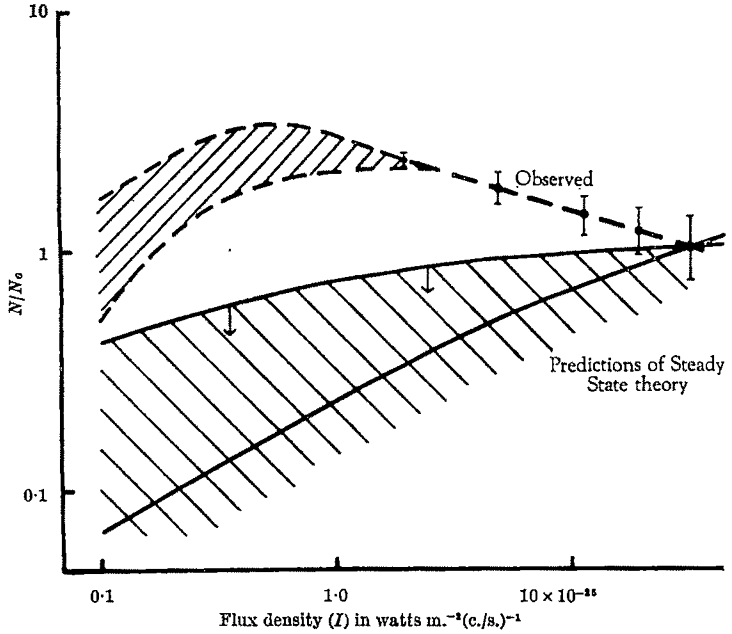

Fig. 2. Plot of relative number of sources observed (N/N_0) above given intensities I. The dotted lines represent the extension of the observations to weaker sources than can be observed individually; they are based on a statistical method which gives no information about the positions or intensities of individual sources, but allows limits to be set to the number of sources of different intensities. The full lines are the curves predicted by the Steady-State model for two values for the effective power of the sources

puter is printed on sheets which enable us to find the positions and intensities of the sources, and the detail obtained is the same as if we had built two aerials each 800 ft. × 500 ft.

We have been using this telescope continuously since it was completed in the summer of 1958, most of the time on problems relating to the cosmological question.

Our first problem was to establish the power of the sources—so that we could predict the relative number of sources we should expect to find at different intensities. At the same time we must make sure that the observations are not being diluted by large numbers of relatively weak sources at small distances —for we know that some of the sources are the remains of supernova explosions inside our own Galaxy—and others are nearby galaxies with relatively weak radio emission.

We have used three methods of finding the power of the sources, the first of which is based on radio methods alone, and involves no assumptions about the nature of the sources. This method showed that only a few per cent of the observed sources were within the Galaxy, and that most of them were external galaxies having a radio emission at least 1,000 times greater than that of our own Galaxy. If we suppose that most of the sources emit as much optical radiation as our own Galaxy, then the limiting optical brightness of the sources allows us to set an even greater limit to the radio emission. The third method was based on measurements of the angular diameters of the sources, and again indicated an average radio emission 10^4–10^5 times that of the Galaxy. From these results we can now compute the number of sources we should expect to find at different intensities; but before we can compare the results with what we observe, we must explore a number of possible difficulties which might affect the interpretation of the observations. One difficulty of this sort is the possibility that radio galaxies might occur in clusters—if they did so, then although we would be able to recognize the individual sources in a nearby cluster, a very distant cluster might be mistaken for a single nearby source. We therefore made a number of subsidiary investigations of this sort to see what corrections would be necessary.

The final, and most extensive problem was to determine the actual number of sources above given intensities, down to the smallest value we could reach. The results, plotted as the number of sources N compared with the number N_0 which would have been observed in the absence of effects of the red-shift, are shown in Fig. 2. The corresponding curves predicted by the Steady-State model on the basis of (*a*) the lowest permissible value of radio power, and (*b*) the value suggested by the optical data, are also shown in Fig. 2; the discrepancy seems too great to be explained by errors in the observations or their interpretation, and suggests that the Steady-State theory in its original form does not correspond to the actual universe.

This is only the beginning of the problem, and the simplest of the questions which one can ask. There are many versions of evolving cosmologies, and there may be special cases of the Steady-State model which fit the present results. More detailed observations may make it possible to eliminate some of these too.

CONTINENT AND OCEAN BASIN EVOLUTION BY SPREADING OF THE SEA FLOOR

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ANY concept of crustal evolution must be based on an Earth model involving assumptions not fully established regarding the nature of the Earth's outer shells and mantle processes. The concept proposed here, which can be termed the 'spreading sea-floor theory', is largely intuitive, having been derived through an attempt to interpret sea-floor bathymetry. Although no entirely new proposals need be postulated regarding crustal structure, the concept requires the acceptance of a specific crustal model, in some ways at variance with the present consensus of opinion. Since the model follows from the concept, no attempt is made to defend it. The assumed model is as follows:

(1) Large-scale thermal convection cells, fuelled by the decay of radioactive minerals, operate in the mantle. They must provide the primary diastrophic forces affecting the lithosphere.

(2) The sequence of crustal layers beneath the oceans is markedly different from that beneath the continents and is quite simple (Fig. 1). On an average 4·5 km. of water overlies 0·3 km. of unconsolidated sediments (layer 1). Underlying this is layer 2, consisting of about 2·0 km. of mixed volcanics and lithified sediments. Beneath this is the layer 3 (5 km. thick), commonly called the basalt layer and supposedly forming a world-encircling cap of effusive basic volcanics over the Earth's mantle from which it is separated by the Mohorovičić seismic discontinuity. Instead we must accept the growing opinion that the 'Moho' marks a change of phase rather than a chemical boundary, that is, layer 3 is chemically the same as the mantle rock but petrographically different with low-pressure phase minerals above the Moho and high-pressure minerals below. This change of phase may be either from eclogite to gabbro[1], or from peridotite to serpentine[2]; its exact nature is not vital to our concept, but we can tentatively accept the eclogite–gabbro transition as it has more adherents. Common usage requires that we reserve the term 'mantle' for the substance beneath the Moho, but in point of fact, the gabbro layer (as a change of phase) is also a part of the mantle—a sort of 'exo-mantle'. Except for a very thin veneer, then, the sea floor is the exposed mantle of the Earth in this larger sense.

(3) It is relevant to speak of the strength and rigidity of the Earth's outer shell. The term 'crust' has been effectively pre-empted from its classical meaning by seismological usage applying it to the layer above the Moho, that is, the sial in continental regions and the 'basaltic' layer under the oceans so that the continents have a thick crust and the ocean basins a thin crust. Used in this now accepted sense, any implications equating the crust with rigidity must be dismissed. For considerations of convective creep and tectonic yielding, we must refer to a lithosphere and an asthenosphere. Deviations from isostasy prove that approximately the outer 70 km. of the Earth (under the continents and ocean basins alike) is moderately strong and rigid even over time-spans of 100,000 years or more; this outer rind is the lithosphere. Beneath lies the asthenosphere separated from the lithosphere by the level of no strain or isopiestic level; it is a domain of rock plasticity and flowage where any stresses are quickly removed. No seismic discontinuity marks the isopiestic level and very likely it is actually a zone of uniform composition showing a gradual transition in strength as pressure and temperature rise; and in spite of the lithosphere's rigidity, to speak of it as a crust or shell greatly exaggerates its strength. Because of its grand dimensions, for model similitude we must think of it as weak[3]. If convection currents are operating 'subcrustally', as is commonly written, they would be expected to shear below the lithosphere and not beneath the 'crust' as this term is now used.

(4) As gravity data have shown, the continents are low-density tabular masses of sial—a 'basement complex' of granitic rocks about 35 km. thick with a thin sedimentary veneer. Since they are buoyant and float high hydrostatically in the sima, they are analogous to icebergs in the ocean. This analogy has additional merit in that convection of the sima cannot enter the sial. But the analogy gives the wrong impression of relative strength of sial and sima; the continental lithosphere is no stronger than the oceanic lithosphere, so it is mechanically impossible for the sial to 'sail through the sima' as Wegnerian continental drift proposes. The temperature and pressure are too high at the base of the sial to permit a gabbroic layer above the Moho; instead, there may be an abrupt transition from granite to eclogite.

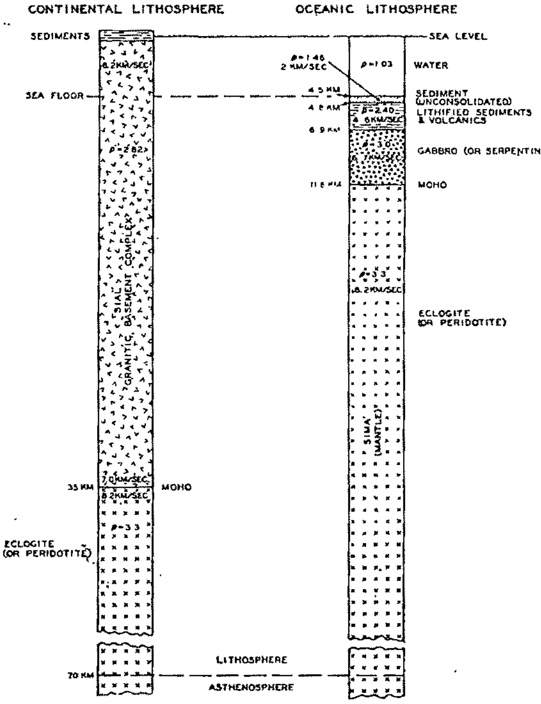

Fig. 1. Generalized crustal sections through the lithosphere beneath the continents and the ocean basins as presumed in this paper. Seismic velocities and densities are shown for the various layers

Spreading Sea Floor Theory

Owing to the small strength of the lithosphere and the gradual transition in rigidity between it and the asthenosphere, the lithosphere is not a boundary to convection circulation, and neither is the Moho beneath the oceans because this is not a density boundary but simply a change of phase. Thus the oceanic 'crust' (the gabbroic layer) is almost wholly coupled with the convective overturn of the mantle creeping at a rate of a few cm./yr. Since the sea floor is covered by only a thin veneer of sediments with some mixed-in effusives, it is essentially the outcropping mantle. So the sea floor marks the tops of the convection cells and slowly spreads from zones of divergence to those of convergence. These cells have dimensions of several thousands of kilometres; some cells are quite active now while others are dead or dormant. They have changed position with geological time causing new tectonic patterns.

The gross structures of the sea floor are direct expressions of this convection. The median rises[4,5] mark the up-welling sites or divergences; the trenches are associated with the convergences or down-welling sites; and the fracture zones[6] mark shears between regions of slow and fast creep. The high heat-flow under the rises[7] is indicative of the ascending convection currents as also are the groups of volcanic seamounts which dot the backs of these rises.

Much of the minor sea-floor topography may be even directly ascribable to spreading of the sea floor. Great expanses of rough topography skirt both sides of the Mid-Atlantic Rift; similarly there are extensive regions of abyssal hills in the Pacific. The roughness is suggestive of youth, so it has commonly been assumed to be simply volcanic topography because the larger seamounts are volcanic. But this interpretation is not at all convincing, and no one has given this view formality by publishing a definitive study. Actually, the topography resembles neither volcanic flows nor incipient volcanoes. Can it not be that these expanses of abyssal hills are a 'chaos topography' developed as strips of juvenile sea-floor (by a process which can be visualized only as mixed intrusion and extrusion) and then placed under rupturing stresses as the sea floor moves outward?

The median position of the rises cannot be a matter of chance, so it might be supposed that the continents in some manner control the convection pattern. But the reverse is considered true: conditions deep within the mantle control the convective pattern without regard for continent positions. By viscous drag, the continents initially are moved along with the sima until they attain a position of dynamic balance overlying a convergence. There the continents come to rest, but the sima continues to shear under and descend beneath them; so the continents generally cover the down-welling sites. If new upwells do happen to rise under a continental mass, it tends to be rifted. Thus, the entire North and South Atlantic Ocean marks an ancient rift which separated North and South America from Europe and Africa. Another such rift has opened up the Mediterranean. The axis of the East Pacific Rise now seems to be invading the North American continent, underlying the Gulf of California and California[8]. Similarly, the Indian Ocean Rise may extend into the African Rift Valleys, tending to fragment that continent.

The sialic continents, floating on the sima, provide a density barrier to convection circulation—unlike the Moho, which involves merely a change of phase. The convection circulation thus shears beneath the continents so that the sial is only partially coupled through drag forces. Since the continents are normally resting over convergences, so that convective spreading is moving toward them from opposite sides, the continents are placed consequently under compression. They tend to buckle, which accounts for alpine folding, thrust faulting, and similar compressional effects so characteristic of the continents. In contrast, the ocean basins are simultaneously domains of tension. If the continental block is drifted along with the sima, the margin is tectonically stable (Atlantic type). But if the sima is slipping under the sialic block, marginal mountains tend to form (Pacific type) owing to drag forces.

Implications of the Concept

Ad hoc hypotheses are likely to be wrong. On the other hand, one which is consonant with our broader understanding of the history of the Earth may have merit. While the thought of a highly mobile sea floor may seem alarming at first, it does little violence to geological history.

Volumetric changes of the Earth. Geologists have traditionally recognized that compression of the continents (and they assumed of the ocean floors as well) was the principal tectonic problem. It was supposed that the Earth was cooling and shrinking. But recently, geologists have been impressed by tensional structures, especially on the ocean floor. To account for sea floor rifting, Heezen[10], for example, has advocated an expanding Earth, a doubling of the diameter. Carey's[11] tectonic analysis has resulted in the need for a twenty-fold increase in volume of the Earth. Spreading of the sea floor offers the less-radical answer that the Earth's volume has remained constant. By creep from median upwellings, the ocean basins are mostly under tension, while the continents, normally balanced against sima creepage from opposite sides, are under compression.

The geological record is replete with transgressions and regressions of the sea, but these have been shallow and not catastrophic; fluctuations in sea-level as severe as those of the Pleistocene are abnormal. The spreading concept does no violence to this order of things, unlike dilation or contraction of the Earth. The volumetric capacity of the oceans is fully conserved.

Continental Drift. The spreading concept envisages limited continental drifting, with the sial blocks initially being rafted to down-welling sites and then being stablized in a balanced field of opposing drag forces. The sea floor is held to be more mobile and to migrate freely even after the continents come to rest. The sial moves largely *en bloc*, but the sea floor spreads more differentially.

Former scepticism about continental drift is rapidly vanishing, especially due to the palæomagnetic findings and new tectonic analyses. A principal objection to Wegener's continental drift hypothesis was that it was physically impossible for a continent to 'sail like a ship' through the sima; and nowhere is there any sea floor deformation ascribable to an on-coming continent. Sea floor spreading obviates this difficulty: continents never move through the sima—they either move along with it or stand still while the sima shears beneath them. The buoyancy of the continents, rather than their being stronger than the sima, accounts for this. Drag associated with the shearing could account for alpine folding and related compressional tectonic structures on the continents.

Persistent freeboard of the continents. A satisfactory theory of crustal evolution must explain why the continents have stood high throughout geological time in spite of constant erosional de-levelling. Many geologists believe that new buoyancy is added to continents through the gravitative differentiation from the mantle. Spreading of the sea floor provides a mechanism whereby the continents are placed over the down-wells where new sial would tend to collect, even though the convection is entirely a mantle process and the role of the continents is passive. It also follows that the clastic detritus swept into the deep sea from the continents is not permanently lost. Rather, it is carried slowly towards, and then beneath, the continents, where it is granitized and added anew to the sialic blocks.

Youth of the ocean floor. It follows paradoxically from the spreading concept that, although the ocean basins are old, the sea floor is young—much younger than the rocks of the continents. Marine sediments, seamounts, and other structures slowly impinge against the sialic blocks and are destroyed by underriding them. Pre-Cambrian and perhaps even most Palæozoic rocks should prove absent from the ocean floors; and Mohole drilling should not reveal the great missing sequence of the Lipalian interval

(Pre-Cambrian to Cambrian) as hoped for by some. All this may seem surprising, but marine geological evidence supports the concept.

On his discovery of the guyots of the Pacific, Hess[12] supposed these were Pre-Cambrian features protected from erosion by the cover of the sea. But Hamilton[13] proved the guyots of the Mid-Pacific Mountains were Cretaceous, and these seem to be among the oldest of the seamount groups. In an analysis of the various seamount groups of the western Pacific, I was forced to conclude that none of them was older than mid-Mesozoic. The young age of the seamounts has been puzzling; certainly they can neither erode away nor subside completely. Also, there seem to be too few volcanic seamounts, if the present population represents the entire number built over the past hundred million years or more. The puzzle dissolves if sea floor spreading has operated. Modern examples of impinging groups of seamounts may be the western end of the Caroline Islands, the Wake–Marcus Seamounts, and the Magellan Seamounts[14]. All may be moving into the western Pacific trenches. Seamount *GA*-1 south of Alaska may be moving into the Aleutian Trench[15].

The sedimentary layers under the sea also appear to be young. No fossiliferous rocks older than Cretaceous have yet been dredged from any ocean basin. Radioactive dating of a basalt from the Mid-Atlantic ridge gave a Tertiary age[16]. Kuenen[17] estimated that the ocean basins should contain on an average about 3·0 km. of sedimentary rocks assuming the basins are 200 million years old. But seismic reflexions indicate an average of only 0·3 km. of the unconsolidated sediments. Hamilton[18], however, believes that much of layer 2 may be lithified sediments. If *all* layer 2 is lithified sediments, Hamilton finds that the ocean basins may be Palæozoic or late Pre-Cambrian in age—but not Archæan. But very likely layer 2 includes much effusive material and sedimentary products of sea floor weathering. In summing up, the evidence from the sediments, although still fragmentary, suggests that the sea floors may be not older than Palæozoic or even Mesozoic.

Spreading and magnetic anomalies. Vacquier, V., *et al.* (in the press) recently have completed excellent sea-floor magnetic surveys off the west coast of North America. A striking north–south lineation shows up which seems to reveal a stress pattern (Mason, R. G., and Raff, A. D., in the press). Such interpretation would fit into spreading concept with the lineations being developed normal to the direction of convection creep. The lineation is interrupted by Menard's[6] three fracture-zones, and anomalies indicate shearing offsets of as much as 640 nautical miles in the case of the remarkable Mendocinco Escarpment[19]. Great mobility of the sea floor is thus suggested. The offsets have no significant expression after they strike the continental block; so apparently they may slip under the continent without any strong coupling. Another aspect is that the anomalies smooth out and virtually disappear under the continental shelf; so the sea floor may dive under the sial and lose magnetism by being heated above the Curie point.

By considering an Earth crustal model only slightly at variance with that commonly accepted, a novel concept of the evolution of continents and ocean basins has been suggested which seems to fit the 'facts' of marine geology. If this concept were correct, it would be most useful to apply the term 'crust', which now has a confusion of meanings, only to any layer which overlies and caps the convective circulation of the mantle. The sialic continental blocks do this, so they form the true crust. The ocean floor seemingly does not, so the ocean basin is 'crustless'.

I wish to express my appreciation to E. L. Hamilton, F. P. Shepard, H. W. Menard, V. Vacquier, R. Von Herzen and A. D. Raff for critical discussions.

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MOUSE CHIMÆRAS DEVELOPED FROM FUSED EGGS

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THE regulative capacities of an egg can be defined by following the development of either its separated parts (or blastomeres) or of united whole ones. The mammalian egg as an object of experimental embryology has been studied more extensively along the first line of approach[1–6]. It has been shown that the isolated blastomeres of 2-cell and even 4-cell eggs can effect regulation. Normal offspring from 'half' blastomeres were reported in the mouse[2,3] and the rabbit[4,6]. 'Quarter' blastomeres of the mouse egg can develop at least into blastocysts[2,3] and in the rabbit can give rise at least to 9½-day-old embryos[5,6].

The only attempt, so far, to fuse mammalian eggs was reported by Nicholas and Hall[1]. These authors transferred pairs of naked, slightly adhering 1-cell rat eggs. A single embryo developed until term, but as the effectiveness of the union achieved had not been checked, the result was not quite conclusive.

In undertaking the work to be described here, it was decided that the *conditio sine qua non* of experiments

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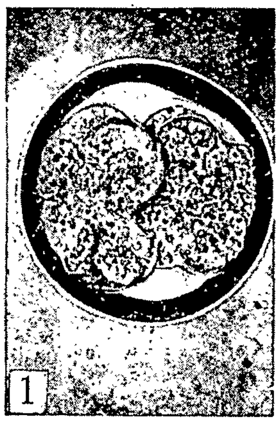

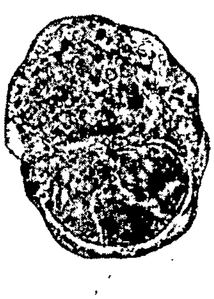

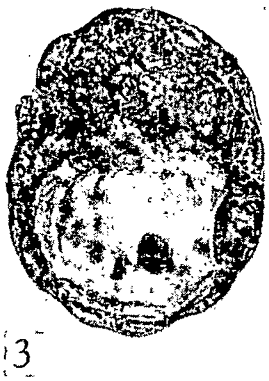

Fig. 1. Two 8-cell eggs compressed in a small drop of medium suspended in liquid paraffin. (× 300)

Fig. 2. Single naked blastocyst developed from 8-cell egg cultured for 24 hr. (× 400)

Fig. 3. Double blastocyst developed from two fused 8-cell eggs after 24 hr. in culture. (× 400)

of this kind was to keep the fused eggs *in vitro* up to the formation of the blastocyst. Moreover, the conditions of culture must be such as to allow the eggs to be observed during development, thus ensuring that all the blastomeres had been incorporated into the one blastocyst.

Work of this type may contribute, as does the other line of approach, to the understanding of the mechanism of normal early development. It should offer, in addition, some new opportunities for research into genetics, developmental genetics and the factors controlling sexual differentiation. Unless eggs from an inbred strain are used, the united components are not genetically identical. In addition, in 50 per cent of cases, sex chimæras would be expected.

The eggs used throughout these experiments were in late phase of the 8-cell stage. The donors were from A_2G albino and $C57$ black inbred strains and from a *LAB* Grey random-breed colony. The eggs of the first two strains were from spontaneous ovulations and the animals were killed in the afternoon of the third day (day when vaginal plug is found is denoted as first day of pregnancy or pseudopregnancy). All the eggs of *LAB* Grey origin were from hormonally induced ovulation in mature animals[7]. Intraperitoneal injection of 3–4 I.U. of pregnant mare serum ('Gestyl') were given, followed after 37–42 hr. by 3–4 I.U. human chorionic gonadotropin ('Pregnyl', Organon Laboratories), and the animals were paired with either *LAB* Grey or A_2G males. Autopsy was performed 78–83 hr. after the second injection. Combinations of eggs united were as follows: (1) $(C57)^2 + (A_2G)^2$, (2) $(LAB\ \mathrm{Grey})^2 + (LAB\ \mathrm{Grey})^2$, (3) $(LAB\ \mathrm{Grey} \times A_2G) + (LAB\ \mathrm{Grey} \times A_2G)$, (4) $(LAB\ \mathrm{Grey})^2 + (LAB\ \mathrm{Grey} \times A_2G)$. It must be added that the offpsring from *LAB* Grey × A_2G cross is dark agouti with black eyes, and so in combination (3), both components had the factor for dark pigmentation and in the combination (4) only one of them. As recipients, *LAB* Grey females mated with vasectomized males were used.

The technical procedure comprised: (*a*) removing the zona pellucida, (*b*) joining the eggs, (*c*) culturing, (*d*) transplanting.

(*a*) It was previously reported[3] that the zona pellucida can be removed by mechanical means. The technique was improved for this purpose. A micropipette, with an internal diameter of 65μ, attached by rubber tubing to a compressible rubber bulb was used. The egg was rapidly sucked into the pipette, causing rupture of the zona and allowing the naked egg to escape. The size of the pipette and the suction pressure applied are critical for the success of this operation. All manipulations were carried out under a dissecting microscope. The eggs were under constant observation, and only those with all blastomeres undamaged were used for further work.

(*b*) Two naked eggs were sucked into a pipette and placed with a small drop of medium in liquid paraffin. With another pipette (internal diameter not more than 40μ) attached to a compressed rubber bulb, most of the medium was withdrawn from this drop, thus squeezing the eggs together (Fig. 1). Such pressure was applied for a period of 5–30 min. Several such drops were placed on the bottom of a shallow glass dish treated previously with silicone fluid ('Repelcote', Hopkin and Williams). Up to this moment all manipulations had been carried out in Tyrode's solution with 0·1 per cent bovine plasma albumin, fraction V (Armour).

(*c*) The culture medium was based on that of Whitten (refs. 8, 9 and personal communication). The medium finally adopted consisted of Krebs–Ringer bicarbonate containing per 130 ml. of solution, 2·6 mgm. of phenol red, 4·0 mgm. of both penicillin salt and streptomycin, 400 mgm. of bovine plasma albumin fraction V, and 130 mgm. of glucose. The medium was sterilized by filtration through 'Sterimat' filters (T. B. Fords, Ltd.), in Hemmings bottles, then covered with liquid paraffin and stored at − 20° C. With a sterile pipette, medium was taken through a layer of liquid paraffin and a drop of about 2–4 mm.^{3} (though the amount of medium is not critical) placed over each of the small drops containing eggs. The small drop was immediately incorporated into the big one and thus each pair of eggs was cultured separately. More liquid paraffin was added to fill up the dish, which was then covered with a piece of glass. The eggs were cultured at 38° C. Liquid paraffin was gassed before use with 5 per cent carbon dioxide in air, and the *p*H of the medium in the drops immediately adjusted itself to 7·4.

(*d*) The eggs were cultured for 24 or 36–40 hr. and transplanted only after reaching the blastocyst stage. The eggs from the first group (24 hr.) were transferred either to the oviduct on the first day (using the method previously described[10]) or to the uterus on the third day according to the method of McLaren and Michie[11], slightly modified. The eggs kept in culture for 36–40 hr. were transplanted to the uterus only on the third or fourth day of pseudopregnancy. It has been shown previously by McLaren and Biggers[12] that normal 8-cell mouse eggs kept *in vitro* for two days and transferred as blastocysts, develop normally to term, the rate of survival being the same as with untreated controls.

After a few hours in culture, the boundary between the eggs was less clearly defined and in 12–16 hr. each pair had completely rounded up into a single morula, leaving no trace of dual origin. In some of these eggs a small blastocœl had appeared by this time and after 24 hr. nearly all had developed into early blastocysts. In general, the rate of development was not very uniform and depended on the phase of 8-cell stage used and on the time spent by the eggs at room temperature during the manipulations. After 36–40 hr. the blastocysts were swollen and very large. No strict measurements of the size of 'double' blastocysts nor of single control ones have been made, because of their quick shrinkage at room temperature. The difference in volume of double and single blastocyst was, however, unmistakable, the difference in the size of the inner masses being very conspicuous (Figs. 2 and 3). The lack of the zona pellucida does not affect the size of the naked, single blastocysts, which compare well with their littermates cultured with the zona pellucida intact.

Usually, only a single blastocœlic cavity was formed from the very beginning. Sometimes, however, two distinct cavities appeared, separated either by trophoblastic cells or by the whole bulk of the fused inner masses. With advancing development the two cavities invariably joined together and a perfectly normal blastocyst was formed. In some cases the fusion was not complete, some 1/8 blastomeres or their derivatives being left outside. It was decided, therefore, not to use for transplantation those blastocysts in which more than one 1/8 blastomere (or its equivalent in 1/16 or 1/32 blastomeres) were not completely incorporated.

The general results of the development after transplantation are presented in Table 1. The results concern only those experiments in which eggs of *LAB* Grey origin were used, as no embryo has developed after transferring 19 fused $(C57)^2 + (A_2G)^2$ eggs. Among four different combinations of time and type of transfer, only one (line 3) proved to be completely unsuitable. The lack of success was certainly due to the unprepared state of the uterus, which does not allow retention of the eggs.

The first autopsies were performed on the tenth day of pregnancy. At this time, all 6 embryos available were already within the range of normal size variation. A pair of identical twins was also found, the embryos being of different size and retarded approximately 24 and 12 hr. Recipients killed on the twelfth and the fourteenth day yielded 4 (+ pair of identical twins) and 5 embryos respectively. Apart from the twins, all the others fall within the range of normal variation in size.

Of the females left until term, 4 were allowed to litter naturally, the other 4 were killed in the evening on the nineteenth day, the young were removed by Cæsarean section and placed with foster mothers which had littered on the same day, or one or two days before. Although the young seen just after birth and all those seen at the time of Cæsarean section appeared morphologically completely normal and alive, the subsequent mortality-rate was very high. Out of 10 young littered naturally, none survived. Some of them were partially eaten by their mothers immediately after birth, the others were found dead, though intact, during the first 24 hr. Among 8 young delivered by Cæsarean section, 3 died immediately, 1 survived for 20 hr., 2 for 3 days, and 2 are still alive at the time of writing, being 2 and 5 weeks old respectively. The factors causing this high post-natal mortality are obscure at the moment.

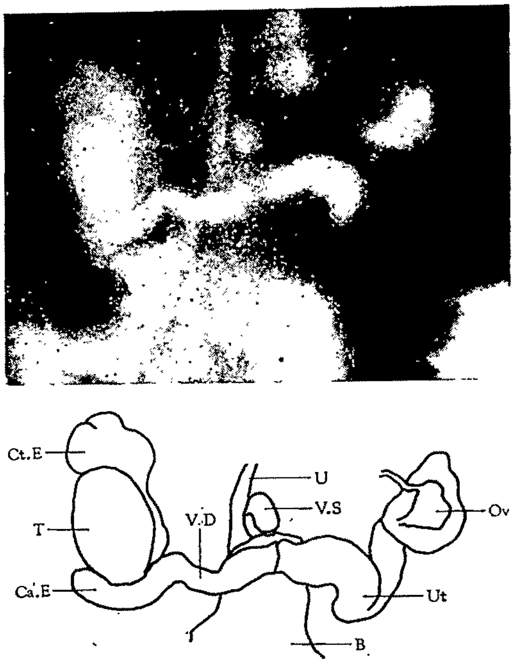

Fig. 4. Photograph (× about 16) of genital apparatus with what appears to be male organs on the left side and female on the right side. The interpretation is given in the key diagram. *B*, bladder; *Ca.E*, cauda epidydymis; *Ct.E*, caput epidydymis; *Ov*, ovary; *T*, testis; *U*, ureter; *Ut*, uterus; V.D., vas deferens; V.S., vesicula seminalis

Theoretically, 50 per cent of experimental embryos should be female/male genetical chimæras, 25 per cent males and 25 per cent females. 14 young were available for examination, but only macroscopical details have, so far, been studied. The results of dissection are as follows: 2 females, 9 males and 3 animals with genital apparatus of a clearly mixed type, with a varying degree of intersexuality (Fig. 4). Although the genital apparatus of some other animals classified as males seems to exhibit some deviation from the normal type, nothing more can be said until histological analysis is made. The ratio—2 females, 3 female/male and 9 males—suggests that some of the 'males' are in fact also sex chimæras in which the male component dominates. In the mouse (as in other mammals) the male gonads begin to differentiate earlier than the female gonads[13]. It is possible to

Table 1. DEVELOPMENT OF DOUBLE BLASTOCYSTS AFTER TRANSPLANTATION

Duration of culturing	Time and type of transplantation	No. of recipients	Total No. of transplanted blastocysts	Implantations	Embryos or young	Successful transplantations
24 hr.	Evening 1st day oviduct	13	63	22	16	8
	Evening 3rd day uterus	7	32	18	13	6
36–40 hr.	Morning 3rd day uterus	7	21	1	1	1
	Morning 4th day uterus	12	37	10	6*	6
	Total	39	153	51 (33 per cent)	36 (38*) (21 per cent)	21 (54 per cent)

* 2 pairs of identical twins.

assume that in the sex chimæras the hormones from the male part of the gonads suppress, to some extent, the development of female characters. On the other hand, the 3 clear cases of intersexes, mentioned above, exclude the possibility of complete dominance being an absolute rule. However, nothing is known at present about the distribution in the embryo of the cells derived from the two-component eggs. It seems more likely that the contribution from these two sources to different regions of the body, or even of the organs, is not uniform and varies from individual to individual. The genital apparatus can provide a clear indication of irregularities of this kind. The inconstant genetical constitution of the tissues contributing to this apparatus, when influenced by secondary stimuli, could produce a variety of abnormalities.

The striking ability of mouse eggs to unite and develop into one blastocyst, at so late a stage as the 8-cell, can be accounted for on the basis that the position of the cells is not yet determined. It is during the succeeding mitotic cycle that the trophoblastic cells (or strictly speaking enveloping cells) move and overgrow the cells of the inner mass[14,15]. The movements of enveloping cells must be caused by differing surface activities of the two kinds of cells. In the case of fused eggs, these movements are influenced by the presence of two groups of cells of the inner mass. Consequently, the enveloping cells tend to overgrow the two inner masses together, leaving free from the beginning, or retreating afterwards from, the surface between them. The observations made just after joining and during the first few hours of culturing support this hypothesis. The mechanical squeezing is applied for a short time and results only in approximation of the eggs. Though sticking together, the eggs represent at this time two distinct entities. It is during culturing, when no mechanical squeezing is applied, that the boundary gradually vanishes and real union takes place. The 8-cell stage is the latest convenient stage for this purpose. Eggs joined at the 12-cell stage do adhere together, but later almost invariably separate completely to form two blastocysts.

The perfect morphology of 'double' blastocysts would seem to preclude the possibility that the descendant cells of only one egg contribute to the developing embryo. It will be possible, perhaps, to obtain in future additional proof on purely genetical grounds. In the present work one combination of fused eggs—$(LAB\ \mathrm{Grey})^2 + (LAB\ \mathrm{Grey} \times A_2G)$—offers a possibility of tracing the existence of one component due to its factor for pigmentation. It can be assumed that if the pigment were revealed in some form in all or at least in the majority of cases, this could be considered as indirect evidence that the non-pigmented component is also present. As all but one of the young died before the time when skin pigmentation appears, the only criterion available was the eye colour. Among 15 embryos and young, 14 had black eyes, including both embryos in one pair of identical twins. The one additional animal which has survived has black eyes and a uniform coat of the type of the hybrid component.

Briefly, the evidence for real chimærism in the experiments described is three-fold: (1) complete union of two eggs into one blastocyst as seen in culture *in vitro*; (2) the presence of intersexes; (3) the presence of black pigment in the majority of embryos developed from pigmented + non-pigmented fused eggs.

Experiments are still in progress and a detailed account will be published elsewhere.

This work was done during the tenure of a Rockefeller Foundation fellowship. I wish to thank Prof. F. W. Rogers Brambell for allowing me to use the facilities of this laboratory and for his interest and encouragement.

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OBITUARIES

Maurice, Duc de Broglie, For.Mem.R.S.

MAURICE DE BROGLIE, who died on July 14, 1960, was one of that small band of scientists who pursue their researches privately with their own means. In some respects he was unique. Not only did he establish a laboratory for his own research in his Paris home, but he also collected around him a group of younger men who worked under his guidance. His brother, Louis de Broglie, joined him shortly before the First World War, and much of the initial inspiration of Louis's work came from Maurice's influence. Louis Leprince-Ringuet, Jean Trillat, Jean Thibaud, Alexandre Dauvillier, Bruno Rossi, were among those in his research school. It was his achievement to establish a private research centre of world-wide reputation in his mansion in the rue Chateaubriand.

Maurice de Broglie was born on April 27, 1875, in Paris. He entered the Ecole Navale in 1893 and served for ten years in the Navy. When he left it, in 1902, he established a small laboratory in his house. He was, at that time, interested in the electrical charges on fine particles suspended in an ionized gas, and studied these movements in an electric field, the method afterwards used by Millikan to measure the electronic charge. In 1913 he was one of the first to study X-ray spectra and X-ray absorption. He developed a spectrometer in which he registered, on a photographic plate, the reflected rays from a lattice plane of a slowly rotating crystal. I remember that time well, because Maurice de Broglie and my father corresponded so regularly and compared their results.

During the First World War, he had a commission in the French Navy and made a considerable contribution to the underwater detection of submarines and the development of radio.

After the War, de Broglie continued his researches on X-ray absorption discontinuities, and published, with his brother Louis, *La Physique des Rayons X*, which was a well-known standard work at that time. The laboratory was greatly enlarged and the number of research workers increased. De Broglie's interests turned towards nuclear physics, and some of the devices for observations of high-energy particles which have now become classical were first developed by de Broglie's team. He was elected to the Académie Française in 1934. For ten years he was president of the Conseil Scientifique de l'Energie Atomique.

De Broglie won the warm affection of his students and his many friends all over the world, who will retain pleasant memories of their visits to his home and laboratory, where he was the kindest and most genial of hosts.

W. L. BRAGG

Prof. E. Henriot

WITH the death on February 1 of Emile Henriot, Belgian science lost one of its foremost representatives. Although of French origin (he was born in Besançon in 1885), he spent practically his entire scientific life in Belgium as professor of physics in the Université Libre de Bruxelles and was made emeritus professor in 1956. He suffered in the last few years from ill-health, which fortunately did not diminish in any manner his lucidity or his mental powers.

Among his many contributions to physics, two stand out by which his name will be remembered. The first was the subject of his doctor's thesis. By a masterly series of investigations, he was the first to show definitely that potassium and rubidium are naturally radioactive. This work, done before the First World War, was an investigation in the best tradition of the great French school of radioactivity measurements.

In more mature years came his justly famous investigations of extremely high angular velocities. He found that suitably placed air-jets can be used to spin tops at very high speeds, and he developed this technique to the point where it could be used for certain physical measurements. Although he originally wanted to use it for a precision determination of the speed of light, in the hands of Beams these air-driven tops became the mechanism of the then most successful ultracentrifuges.

Henriot was attracted time and again to questions of optics, where he was able to use his great theoretical skill. Problems of birefringence and molecular vibrations have been treated very successfully in several of his papers.

His contributions to physics went considerably beyond his own research. He was an outstanding teacher, and several of his former pupils are now professors of physics in different places. To the physics community at large, he was known as the organizer of the physics conferences of the Solvay Institute. For more than thirty years, he was a member of its administrative committee and for about twenty years he was its secretary. To the physicists going to these conferences he was a familiar figure, and he succeeded in maintaining these conferences at a very high level.

Physics was not his only love. Everything human attracted him, and his friends could, and did, learn much from him about art or archæology. He had a fine collection of Greek marbles and a beautiful library adorned by many valuable books. Moreover, he was an extremely warm human being, and those who had the good fortune of being his friends deplore his death.

The many distinctions which came to him were never mentioned by him. He was a very modest person, modest almost to shyness. He married one of his former students, who became his collaborator for very many years. During the Second World War, Henriot was professor at the University of Algiers, when evacuated from Belgium and France. By coincidence, his daughter still lives there. In the opinion of some of his colleagues, the good physics tradition in Algiers really started with his teaching there.

All his friends and the world of physics will miss him.

L. MARTON

Mr. G. W. G. Briggs

GUY BRIGGS, assistant director of the National Institute of Agricultural Botany, died in hospital at Cambridge on March 7. His death at fifty-seven brings a deep sense of loss to his colleagues in many countries who shared his interest in problems relating to grassland and to seeds.

His early interest in agriculture developed as he lived during school vacations on his grandfather's farm at Melbourne in Derbyshire. From King's College, Wimbledon, he went to Wye College, where he gained distinction in botany and in agriculture in the diploma examinations.

Entering the Colonial Agriculture Service in 1926, he was in close touch with the experimental farms and with advisory work among the growers. Those who knew him at that time remember the keen personal interest he took in all who sought his assistance, both Africans and Europeans. He played a leading part in the development of the rice crop and he had the satisfaction of seeing Nigeria become self-supporting in this cereal.

Retiring on medical advice from the Colonial Service in 1945, he was appointed as a technical officer at the National Institute of Agricultural Botany, becoming head of the Seed Production Branch in 1948 and assistant director of the Institute during January 1960.

Briggs's great organizing ability was admirably combined with a deep appreciation of technical problems on seed production. The researches of Stapledon and his colleagues at Aberystwyth had shown the value of leafiness and persistence in grasses and clovers, and had led to the development of a series of varieties which possessed these qualities. Briggs played an important part in bringing these to the notice of growers and in helping to develop methods of seed production which enabled the new varieties to be grown commercially on a large scale without losing their distinctive features.

The agricultural industry was beginning to appreciate the value of seed produced under technical supervision, and it was Briggs who was mainly responsible for organizing, on behalf of the National Institute of Agricultural Botany, the national certifying authority for herbage seeds. In this he had the strong support of the Welsh Plant Breeding Station, the seed trade and the National Farmers' Union.

Briggs took an active interest in developments in seed production abroad, and when, in 1958, the Organization for European Economic Co-operation adopted throughout the eighteen member countries an international scheme for herbage seed certification, experience gained in working the British scheme proved most valuable.

The comprehensive survey Briggs gave in his paper to the International Grassland Congress at Reading in 1960 has led to a better appreciation of the part seeds of high quality can play in the improvement of crop production.

At Cambridge, Guy Briggs was particularly valued for the soundness of his advice, which was always given in the most kindly way. He was able to make friends of those with whom he worked and he has made a big contribution to the good relations which exist between research workers, seedsmen and farmers.

His influence will continue to be felt, both through the work he did and through the help and training he gave to so many others.

Guy Briggs leaves a widow and a son of his first wife, who died in 1945. F. R. HORNE

NEWS and VIEWS

New Scientific Attachés : Dr. C. R. S. Manders and Mr. W. F. G. Drury

DR. CHARLES MANDERS has been appointed to the new post of scientific attaché to the British Embassy, Tokyo. He will hold the rank of senior principal scientific officer and joins the Department of Scientific and Industrial Research from the Ministry of Defence. Dr. Manders was educated at King's College, University of London, where he obtained a double first (general and physics). He served in the army during 1941–47, including a two-year spell as a lecturer at the Military College of Science. In 1947 he joined the Scientific Civil Service and for the past ten years has been with the Ministry of Defence on scientific duties. He is interested in education and has a translation of a collection of Soviet technological curricula, and also a translation of a French book on thermodynamics, in the course of publication.

Mr. W. F. G. Drury, scientific assistant to Sir John Carroll, deputy controller (research and development), Royal Naval Scientific Service, has been appointed scientific attaché to the British Embassy in Stockholm, in succession to Mr. G. H. Greenhalgh. He was educated at University College School, Hampstead, and on leaving school at 18, joined the Electrical Research Association as a technical assistant. He entered the Civil Service two years later, and during 1938–53 he was at the Admiralty Signal School, working mainly on radar and radio research and development projects. In 1953 he was appointed to the British Joint Service Mission in Washington. He returned to the Admiralty Signal Research Establishment in 1955 and was appointed to his present post in 1956.

Pharmaceutical Chemistry in London: Prof. W. H. Linnell

PROF. W. H. LINNELL, who is retiring from the chair of pharmaceutical chemistry in the School of Pharmacy, University of London, was appointed to the staff as reader in 1926 and to the chair of pharmaceutical chemistry in 1949. He had previously studied at the University of Durham, 1919–23, and, following the award of the Earl Grey Memorial Fellowship, at Lincoln College, Oxford. He afterwards had experience as a member of the research staff of H.M. Fuel Research Station. In 1956, while retaining the chair of pharmaceutical chemistry, he was appointed dean of the School of Pharmacy in succession to Prof. H. Berry. He is a member of the British Pharmacopœia Commission and the Revision Committee of the British Pharmaceutical Codex. In 1960 he was chairman of the British Pharmaceutical Conference at Newcastle upon Tyne. Prof. Linnell has been responsible for the development of the new subject of pharmaceutical chemistry in the School, and his own research work has been mainly in the field of chemotherapy.

Prof. W. B. Whalley

DR. W. B. WHALLEY has been appointed to the chair of chemistry created on Prof. Linnell's retirement from the chair of pharmaceutical chemistry at the School of Pharmacy, University of London. Throughout his academic career, Dr. Whalley has been associated with the University of Liverpool. After taking the Ph.D. degree in 1940 he worked in the Research Department of the General Chemicals Division of Imperial Chemical Industries, Ltd., at Widnes. A few years later he returned to Liverpool and began a long and productive collaboration with Prof. Alexander Robertson. Together they investigated successfully the structures of many natural products, especially complex heterocyclic compounds of oxygen. However, the most notable development was the study of fungal metabolites with varied structures, many of which were elucidated. Citrinin, rosenonolactone, and the novel class of pigments typified by sclerotiorin are good examples. Since Prof. Robertson's retirement in 1957, Dr. Whalley has continued energetic work with fungal metabolites, partly in collaboration with Dr. J. S. E. Holker, and he has turned his attention to their biosynthesis. Dr. Whalley's research interests have extended to other fields, and his numerous publications include several valuable review articles. In 1952 he spent six months at Smith, Kline and French Laboratories, Philadelphia. At Liverpool, where he has been reader since 1957, Dr. Whalley's work has not been confined to the Organic Chemistry Department. He is at present senior tutor of the Faculty of Science, and for some years he has been prominent in educational circles in Merseyside as a school governor, extra-mural lecturer, etc. Dr. Whalley's new post will give scope to his talents as an administrator, research worker and teacher, and his numerous friends look forward to his success in it.

The Royal Aeronautical Society : Elections and Awards.

AIR MARSHAL SIR OWEN JONES has been elected president of the Society. He has served on the Council since 1955 and was a vice-president of the Society during 1957–60. *Honorary Fellowship* of the Society has been conferred on Sir Sydney Camm, director and chief engineer, Hawker Aircraft, Ltd., and J. D. North, chairman and managing director, Boulton Paul Aircraft, Ltd. *Honorary Companionship* of the Society has been awarded to J. N. Toothill, director, Ferranti, Ltd., Hollingwood, Lancashire, and general manager, Ferranti, Ltd., Edinburgh.

At the annual general meeting of the Society held on May 4 the following awards were announced: *Simms Gold Medal*, to Prof. D. B. Spalding for his paper, "Heat and Mass Transfer in Aeronautical Engineering"; *George Taylor (Australia) Gold Medal*, to A. J. Troughton for his paper, "Relationship between Theory and Practice in Aircraft Structural Problems"; *Edward Busk Memorial Prize*, to C. Bourque and Prof. B. G. Newman for their paper, "Reattachment of a Two-dimensional, Incompressible Jet to an Adjacent Flat Plate"; *Orville Wright Prize*, to Prof. W. H. Wittrick for his paper, "Analysis of Stress Concentrations at Reinforced Holes in Infinite Sheets"; *Herbert Akroyd Stuart Memorial Prize*, to J. S. Alford, for his paper, "Power Plants for Supersonic Transports"; *Branch Prize*, to Lieut. Commdr. D. J. Whitehead for his paper, "The Low Speed Approach and Catapult Launch Problems in High Performance Naval Aircraft"; *Usborne Memorial Prize*, to T. W. F. Moore for his paper, "Some Experiments on the Reattachment of a Laminar Boundary Layer separating from a Rearward Facing Step on a Flat Plate Aerofoil".

The Society has also announced the following awards: *Silver Medal*, to R. Hafner, technical director (research), Westland Aircraft, Ltd., for outstanding work in the design and development of rotary-wing aircraft; *Bronze Medal*, to L. Haworth, assistant chief engineer, Propeller Turbines, Rolls-Royce, Ltd., for his work leading to advances in aircraft gas-turbine design; *British Gold Medal for Aeronautics*, to Dr. S. G. Hooker, technical director (Aero), Bristol Siddeley Engines, Ltd., for outstanding achievement in aero-engine design and development; *R. P. Alston Medal*, to R. P. Dickinson, superintendent, Aeroplane and Armament Experimental Establishment, Boscombe Down, for his achievement in the execution and direction of flight test observation work; *Wakefield Gold Medal*, to Dr. E. Eastwood, director of research, Marconi's Wireless Telegraph Co., Ltd., for his contributions to the development of air traffic control and navigational aids; *British Silver Medal for Aeronautics*, to Pierre Satre, technical director, Sud-Aviation, Paris, for his work on aircraft design, with special reference to his development of the rear-engined configuration. *N. E. Rowe Medals*, for younger members of the Society, and awarded for the best lecture given to any Branch of the Society by a member of a Branch: 21–26 years age-group, to J. Wilding, of the Chester Branch, for his paper "Bird Flight and the Aeroplane"; under-21 years age-group, to W. Bishop, of the Brough Branch, for his paper "The Development of Tailless Aircraft and Flying Wings".

The Queen Elizabeth II Canadian Medical Fund

DR. M. W. PARTINGTON has been appointed the first "Queen Elizabeth II Scientist" by the Queen Elizabeth II Canadian Fund to Aid in Research on the Diseases of Children; the Fund was established by the Federal Government of Canada to mark the occasion of Her Majesty's visit to Canada in 1959. Dr. Partington, a Research Fellow in neurology at the Research Institute of the Hospital for Sick Children, Toronto, will take up his new post on July 1 in the Department of Pædiatrics at Queen's University, Kingston, where a permanent research unit will be set up to initiate, foster and carry out research on pædiatric problems, such as mental deficiency and epilepsy in children. Three "Queen Elizabeth II Fellowships", totalling 13,500 dollars, were also awarded to: Dr. John Alexander Lowden, to continue studies towards a Ph.D. degree in biochemistry at McGill University, under Drs. K. A. C. Elliott and L. S. Wolfe; Dr. George Greenough Hinton, research fellow in neurology at the National Hospital for Nervous Diseases and Hospital for Sick Children, London (England), to work at the War Memorial Children's Hospital and the Psychiatric Research Centre, London (Ontario), under Drs. J. C. Rathbun and D. Zarfas; Dr. Hugh Taylor, chief resident of the Children's Hospital, Winnipeg, to work in the Department of Pediatrics, New York University, under Drs. J. Dancis and S. Krugman.

Science in Society

THE impact of scientific and technological progress on society is being examined in more than 200 research projects in the colleges, universities, and other institutions in the United States. These studies are described in a newly released issue of the National Science Foundation publication, *Current Projects on the Economic and Social Implications of Scientific Research and Development*, 1960 (Pp. v+124. Washington, D.C.: Government Printing Office, 1961. 40 cents). The effects of science and technological progress are examined in relation to such subjects as agriculture, impact on selected industries, administration and management, and labour. Other factors being studied are patents, scientific man-power and creativity, and science policy. Each project is summarized and listed with the names of the investigators, address of institution, and the anticipated form of publication of results. The first issue of this publication covered the year 1959. During 1959-60 the number of projects reported to the Foundation more than doubled.

Bibliography of Influenza

THE American Institute of Biological Sciences has issued the first of a new quarterly publication containing a bibliographical record of articles published on influenza and related subjects in the United States and elsewhere (*An Annotated Bibliography of Influenza*, Vol. 1, No. 1; September 1960. Pp. 20. Published quarterly. Washington, D.C.: American Institute of Biological Sciences, 2000 P Street, N.W., 1960). Approximately 2,600 journals are being examined for information. Each issue of this bibliography is divided into the following major categories: general and review; epidemiology; clinical; pathology; prophylaxis; immunology; virology; and animal studies.

The Smithsonian Institution

BULLETIN No. 220 of the United States National Museum is a list of type specimens of reptiles and amphibians in the Museum (*Type Specimens of Reptiles and Amphibians in the U.S. National Museum*. By Doris M. Cochran. Pp. xv+291. Washington, D.C.: Government Printing Office, 1961. 1.25 dollars). It has been compiled by Miss Doris M. Cochran, curator, and contains 1,742 names. An interesting introduction reviews the history of the collections and is followed by the list giving the original genetic, specific and sub-specific names. The name of the author is added and also the name of the publication where the name and description first appeared. Afterwards, if the original name of the genus or species is no longer accepted, the present synonym is added in italics. A comprehensive index completes the monograph.

Eye Examinations of Asian School Children

THE eyes of 359 children attending the Indian public primary school in Mwanza have been examined by members of the Biochemistry Nutrition Division of the East African Institute for Medical Research (Report, 1959–60. Pp. ii+36. Mwanza: East African Institute for Medical Research, 1960. Sh. 3). Apart from refractive errors there was almost no eye disease. Heights and weights were also obtained and all the data have been prepared so that comparison can be made with a similar study made by Pendse (1954) in Poona. This is the first time that refractive errors and growth data have been compared for two closely related communities in different parts of the world. In both height and weight the Gujerat children of Mwanza are superior to the Maratas of Poona. It is postulated that the greater growth of the Mwanza children is due to the improved conditions under which they now live in East Africa. The Mwanza refraction data showed a marked excess of emmetropic and near-emmetropic refractions compared with the normal distribution and also a myopic excess and a prolongation of the myopic tail. When a comparison is made of the percentage distribution of refraction in the Mwanza and the Poona children, close similarity is seen. Mixed astigmatism and anisometropia were present in 6·6 per cent and 8·6 per cent respectively of these children. These figures occupy a position intermediate between those of the Mwanza African children and those for Mvuni African children. About 1 per cent of the children had squint and less than 1 per cent were wearing glasses at the time of the examination. These Asian children were also examined for the presence and degree of water cleft opacities in the periphery of the lens. Comparison of the details shows that the incidence was slightly lower than in the Mwanza African children.

Journal of the Association for Computing Machinery

THE January 1961 issue of the *Journal of the Association for Computing Machinery* contains six major papers, at least three of which will be of general interest. The first of these, by Herbert Keller, is an analysis of finite automata with special reference to pattern recognition. The main theme is a description of various 'perceptrons', a mysterious term which for some time has puzzled British users of computers, being claimed by some to hide a concept of deep significance, and by others to be a triviality. Keller takes as his definition something which is usually regarded in Britain as a variety of synthetic neurone, and his analysis of the potentialities of this class of device will, no doubt, stimulate discussion. Ivan Flores contributes a paper which analyses the use of computers as sorting machines. This particular class of operation is one which does not show the machines at their best so that analyses which estimate efficiencies are always useful. Probably the most generally interesting contribution is that by Leiner and Youden on generating pronounceable names on a computer. Their particular names have four letters and an amusing touch is their lament: " . . . some of the computer-generated words, although highly efficient phonetically, were semantically unsuited for permanent inclusion in the records". It seems that some humans had to draw up a proscribed list before the records were fit for publication.

The Pontide Paradox

ACCORDING to a number of prominent geologists, the region occupied by the present Black Sea ('Pontus Euxinus' of the ancients), even as late as the second half of the Tertiary era, was occupied by a land mass called Pontide. Furthermore, according to this hypothesis the present Crimean mountains represent the northern fringe of this land mass untouched by a widespread subsidence affecting the greater part of this continent. On the other hand, some geologists query the idea of the existence of Pontide in the late Tertiary era, and their views are supported by M. V. Muratov (*Priroda*, 6, 57; 1960), who, in a series of palæogeographical maps, illustrates the evolution of the future Black Sea from its initial stages to the northern geosyncline of the Tethys. Such a hypothesis also finds its support in the observed peculiarities of the present-day flora and fauna of the Crimea. These biogeographical data, on the other hand, provide N. I. Rubtzov (*Priroda*, 8, 83; 1960) with an additional argument in the support of the hypothesis of the existence of Pontide.

Cultural Sequences in Hokkaido, Japan

QUITE a lot is known about the prehistory of China. Pekin man and the stone-age industry found in the cave with his remains have at least given a start for the study of the earliest cultures of that vast region. Again, not a few of the museums in Britain display painted pots of Neolithic date—the earliest of a long line of painted pottery from China. Much less is known about the earliest history of Japan. The article by Lieut.-Col. H. A. MacCord in a recent paper (*Proc. U.S. Nat. Mus.* (No. 3443, Vol. 112): Pp. 481–503 + 14 plates. Washington, D.C.: Government Printing Office, 1960) does not profess to fill this gap, but does describe some research work undertaken during 1953–54 in the island of Hokkaido, in the region of the Ishikari Plain. Pottery, most of it decorated, and stone tools were found, and many of these are illustrated in 14 full pages of half-tones. The stone tools include polished 'celts', knives and arrow heads, some of obsidian. Some day we shall hope to see a volume on Japanese prehistory; the results published here will certainly merit inclusion in all libraries of archæology and ethnology.

The Advisory Group for Aeronautical Research and Development

THE Advisory Group for Aeronautical Research and Development announces the following schedule of meetings: June 5–9, Documentation Committee on "Exchange of Information in the Nato Countries" (Paris); July 12–19, Seminar on Astronautics, jointly sponsored by the University of Southampton and AGARD (Southampton); July 18–21, Fluid Dynamics Panel on "The Use of Rocket Vehicles in Flight Research" (The Hague); July 24–26, Avionics Panel Technical Meeting on "Microminiaturization" (Oslo); Structures and Materials Panel Specialists' Meeting of the Material Group (Oslo); July 27–28, 11th AGARD General Assembly, "Scientific Goals of Space Exploration and Research" (Oslo); July 31–August 2, Avionics Panel Technical Meeting on "Low-noise Electronics" (Oslo).

Field Studies Council: Summer and Autumn Courses

THE Field Studies Council has announced the programmes of special courses to be held during

1961 at its various Field Centres. *Malham Tarn Field Centre* (near Settle, Yorkshire): grasses, sedges and rushes (July 5–12); mosses and liverworts (August 23–30); identification and ecology of lichens (August 30–September 6); ecology of freshwater animals (August 30–September 6); meteorology, organized in conjunction with the Royal Meteorological Society (September 6–13); fungi (September 6–13); Pennine fossils (September 13–20); rivers and streams (September 20–27); karstic land-forms (September 20–27); spider ecology (September 20–27). *Flatford Mill Field Centre* (East Berghold, near Colchester, Essex): art of botanical illustration (July 19–26); landscape painting courses (August 9–23); animal life in the soil (August 9–16); insect ecology (August 16–23); field archæology (August 23–September 6); small mammals (September 16–23); autumn art week (October 4–11). *Slapton Ley Field Centre* (Slapton, Kingsbridge, Devonshire): outdoor painting course (August 9–16); natural history fortnight (August 9–23); introduction to the study of lichens (August 16–23); introduction to the study of seaweeds (August 16–23); natural history of south-western woodland (August 30–September 6); geology and scenery for beginners (August 30–September 6); introduction to the study of lower plants (September 20–27). *Dale Fort Field Centre* (Haverfordwest, Pembs.): weather and bird movements, organized under the auspices of the Royal Meteorological Society and the British Trust for Ornithology (September 6–13). Further information on each of the courses can be obtained from the Warden of the Field Centre concerned.

Keele Foundation Year Course

The Nuffield Foundation has made a grant of £5,000 to the University College of North Staffordshire to pay for an investigation into the effects of the Keele Foundation Year course. This course, unique to Keele, is taken by all students in the first year of the four-year degree course and is designed to introduce the student to some of the methods and information necessary to an estimate of the inheritance, the problems and the achievements of modern western European man. The grant will pay the salary of a Research Fellow and an assistant who will attempt to establish any relationship there may be between performance in the Foundation Year course and such factors as previous educational record, subsequent performance in the degree course, career after graduation, etc. It is hoped that the information obtained may give valuable indications of ways in which the Foundation Year course can be further developed or modified and that this may be the first stage of a continuing inquiry.

Announcements

The Meldola Medal for 1960 has been awarded to Dr. J. N. Bradley, for his work in the field of physical chemistry, with special reference to the kinetics of reactions involving free radicals and reaction in shock waves studied by mass spectrometry. The award is made by the Royal Institute of Chemistry with the concurrence of the Society of Maccabaeans.

Dr. R. Conrad, assistant director of the Medical Research Council's Applied Psychology Research Unit at Cambridge, will act as consultant in applied psychology to the General Post Office for an initial period of two years.

At a meeting held on May 11, the Council of the Association of British Chemical Manufacturers appointed Mr. H. W. Vallender as deputy director of the Association.

The following appointments at the U.N. Food and Agriculture Organization have been announced: Dr. Hans Dietrich Cremer, to be chief of the Applied Nutrition Branch; Dr. Arthur H. Dolff, to be assistant chief of the Research Branch of the Radiological Health Division.

The sixteenth annual electronics, instruments and components exhibition and convention of the Institution of Electronics will be held at the College of Science and Technology, Manchester 1, during the periods July 6–8 and 10–12 inclusive. This programme will incorporate a scientific and industrial research section, a manufacturers' section and a number of lectures and films. Further information can be obtained from the general secretary, W. Birtwistle, Institution of Electronics, 78 Shaw Road, Rochdale, Lancs.

The Department of Mathematics of the Hatfield College of Technology is organizing a summer school on "Mathematics of Automatic Control" during June 26–30, the object being to enable mathematicians and engineers working on problems of automatic control to study some of the relevant mathematical techniques developed in recent years in Britain, America and the U.S.S.R. Further details can be obtained from the Academic Registrar, Hatfield College of Technology, Hatfield, Herts.

The annual meeting of the American Society of Pharmacognosy will be held at the University of Houston during June 19–21. Among the subjects to be considered are: opportunities for Ph.D. pharmacognosists; specific techniques of thin-film chromatography; uridine nucleotides in glycoside biosynthesis; chemo-taxonomy of selected groups within the Coniferae; research on south-western drug plants; the pharmacognosy of the anti-cancer plants, the periwinkles. Further information can be obtained from Melvin R. Gibson, School of Pharmacy, Washington State University, Pullman, Washington.

Elliott Brothers (London), Ltd., a member of the Elliott-Automation Group, is organizing a private symposium and exhibition on "Analytical Instrumentation of Industrial Process Control" at the Connaught Rooms, London, during June 13–14. Included will be papers on: the application of analysis instruments in the United States; the continuous measurement of moisture; analysis instruments from the point of view of the user; experience with plant stream analysers at Imperial Chemical Industries, Ltd., Billingham; trends in continuous process analysis. The exhibition will include a selection of 'Quality Control' instruments supplied by the Analytical Instruments Division of Elliott Brothers (London), Ltd., Further information can be obtained from John Geddes, Elliott-Automation, Ltd., 34 Portland Place, London, W.1.

Errata. The following corrections are necessary in the communication entitled "Minimum Range to Artificial Earth Satellites" in *Nature* of April 22, p. 333. In the first line of the paragraph containing equation (5), for "$\rho_s = \rho_s = \infty$" read "$\rho_s = \rho_p = \infty$". Further, the numerator on the left-hand side of equation (5) should read "$\lambda(\dot{f}')_{f'=f}$"; in some copies the differentiation sign has not registered.

ELECTRICAL CONTACTS

A SYMPOSIUM on "Electrical Contacts", organized by the Institute of Physics and the Physical Society, in collaboration with the Institution of Electrical Engineers, was held during April 5–7, at Brunel College of Technology. The membership was limited to some 260 by the accommodation only, and interest in the subject was such that acceptances were closed some weeks before the date of the symposium, which, although not meant to be international in character, was, nevertheless, attended by representatives from a dozen different countries. The subject under consideration, being of fundamental scientific interest as well as of obvious practical significance, proved itself to be of concern to physicists, engineers, metallurgists and chemists from both industrial and Government research establishments as well as universities. The symposium was so arranged that the main aspects of electrical contact phenomena could be discussed in some detail in separate sessions.

The opening session consisted of an address by Prof. F. Llewellyn Jones (University College of Swansea) on "The Physics of Electrical Contact Phenomena", in which the main aspects of contact operation were surveyed, and the important problems of fundamental significance indicated, as well as the methods by which they are now being attacked in the various laboratories. The sessions which followed each consisted of between four and six short papers grouped under the headings: principles, fundamental investigations and techniques, contact surfaces, methods and design, non-metallic contacts, and miscellaneous subjects. Each session included a discussion. In this brief communication it is not possible to give an account of all twenty-five papers in detail; a fuller report is being published in the *British Journal of Applied Physics*. This notice will be confined to comments on the scope of the discussion and the general conclusions reached.

The opening survey address considered the whole contact operation from the initial approach to the final separation of the electrodes, in the order in which the events usually take place. The aspects concerning fundamental physics and some of the problems still unsolved were pointed out. For example, the behaviour of a contact just before the electrodes actually touch ($\sim 10^{-4}$ cm. apart) involves interesting problems of surface physics relating to the electrical activity of surfaces due to the presence of thin films. Attention was also directed to the interesting and varied phenomena associated with molten, and possibly boiling, metal in the microscopic region of contact. In this connexion, contact physics can make a contribution to our knowledge of the properties of metals at high temperatures in the molten state. A ciné-film was exhibited, showing the quasi-static molten metal bridge passing through a series of mathematically predictable shapes before sudden breaking. The establishment of a micro-arc immediately on the breaking of the bridge was then considered, and the possibility of ionic transfer in the high-temperature plasma pointed out. Finally, the importance of surface films was referred to in the operation of very light-duty contacts with negligible currents—the electro-static contacts.

In the second session dealing with principles, the theme of the properties of metals at high temperatures was further developed by Dr. M. R. Hopkins (University College of Swansea). Thermal instability in a contact was discussed by Dr. J. B. P. Williamson (Cavendish Laboratory and Tube Investments, Ltd., Cambridge). Because of the importance of the nature and behaviour of surface films on contact electrodes (continually emphasized during this conference), the mechanism of formation of oxide films on metals was considered by Dr. O. Kubaschewski (National Physical Laboratory). Interest in non-metals was illustrated by a paper on the friction and wear of graphitic and non-graphitic carbon in which the important rule of adsorbed layers was stressed by Dr. J. W. Midgley (English Electric, Ltd., Leicester).

In the third session, the different techniques of investigations of the various aspects of contact operation were described. P. E. Watts (Associated Electrical Industries, Aldermaston) showed how the effect of temperature asymmetry on matter transfer was being investigated, while Dr. C. H. Jones (Royal Marsden Hospital) described the work done at the Department of Physics, University College of Swansea, using radioactive tracer techniques for the measurement of metal transfer. This sensitive method enables the transfer in an opening contact to be measured with very low series inductances, and it was concluded that transfer in platinum, palladium and silver was not independent of the micro-arc discharge. Dr. A. Fairweather (Post Office Research Station) illustrated the properties of the mechanical deformation of contact electrodes at closure by means of models in two-colour laminated 'Plasticene'. He showed that different forces produce geometrically similar indentations, and that the flow-patterns depend on frictional constraints.

Most of the remainder of the session was devoted to the properties of contact surfaces. The practical aspects of very light-duty electrical contacts were assessed in a review paper by Dr. J. C. Chaston (Johnson Matthey, Ltd., London). From the practical point of view of design and manufacture important factors were electrode shape and material, surface finish, contact-loading force, and in particular the separating force and freedom from bounce.

The cleanliness of precious metal surfaces was discussed by H. C. Angus, while Dr. W. Betteridge (both of International Nickel Co. (Mond), London) considered the operation of extremely light-duty contacts at high temperatures. For example, at temperatures of 500° C. contact, operation can become difficult because of oxidation, welding and thermoelectric effects. So far as noble metals are concerned, investigations show that gold and platinum are film-free, but that ruthenium has a highly conducting oxide. Electro-deposition of metals was described in some detail by F. H. Reed (International Nickel Co. (Mond), London), and other papers provoked much further discussion on this topic. Quantitative assessment of electrode activity due to surface films was described by E. B. Pattinson (Department of Physics, University College of Swansea) and the influence of dust on contacts was considered by Dr. J. A. Greenwood (Tube Investments, Ltd., Cambridge). Dr. Fairweather discussed the effects of plastic lubricants and the behaviour of granular materials. With these materials, energy losses depend not only on the properties of the material but also on granule dimensions and the structure of the granular boundaries. A description of recent contact research in Japan was given by Prof. G. Matsumoto (Tohoku University). The properties of electrodes of silver–nickel and silver–metallic oxides, produced by powder metallurgy, were described, as well as work on arc suppression by electro-chemical treatment of the electrode surfaces. The selection of electrical contact materials for light-duty applications was surveyed by Dr. V. G. Morradian (Engelhard Industries Inc., New Jersey). The proceedings closed with a summary by Dr. M. R. Hopkins.

The last conference on electrical contacts in Britain was held in 1952 (see *Nature*, 169, 960; 1952), and it is interesting to consider the developments which have occurred since then. Despite hopes that recent developments in electronics, particularly in the field of semi-conductors, might avoid difficulties associated with conventional metal-to-metal contacts, it is clear that basic problems in electrical contacts are still with us. Some practical problems have been solved, or circumvented, but others have appeared in new forms. However, considerable advances have been made in our knowledge of the physics of the molten metal bridge, its formation, growth and final destruction, and of the micro-arc to which it appears to give rise in certain conditions. At the same time, work in this field is enlarging our knowledge of the properties of metals at very high temperatures.

Another important conclusion can be drawn from this most interesting and worth-while symposium. In the conference of 1952 it was not clear that the engineer concerned with the operation of contacts, and the physicist concerned with fundamental processes, were attacking the problem together; at the present conference there appeared to be far closer liaison between the physicist and engineer, and, further, it was apparent that the designer now fully appreciates methods of approach depending on investigation in controlled conditions of, for example, ambient atmosphere and surface state.

Dr. J. Topping, principal of the Brunel College of Technology, who welcomed and opened the conference, is to be congratulated on the excellent arrangements.

GAS CHROMATOGRAPHY

THE Gas Chromatography Discussion Group held its third annual general meeting on April 21 at the University of Birmingham. Membership had risen by 75 during the preceding year to 346, including 94 from 18 countries other than Great Britain.

Highlights for the year had been the publication of the first two volumes of *Gas Chromatography Abstracts*, and the very successful third international symposium at Edinburgh the previous June which had attracted more than five hundred delegates from twenty countries. The *Proceedings* had been published in a 466-page volume before the end of the year.

The group is planning another informal symposium in the autumn, and arrangements for the fourth international symposium, this time in Hamburg during June 13–16, 1962, are already well advanced.

The Retention Data Sub-Committee reported that very little data had been submitted to them, and after considerable discussion agreed to consider the usefulness of relative retention data, which require less rigorous control of conditions and would probably be more readily available than the absolute data they had been seeking.

The scientific section of the meeting opened with a welcome by Prof. R. C. Robb.

The first paper was presented by Mr. M. B. Evans on the relation between the retention volumes of unsymmetrical compounds and cognate symmetrical ones. The work has since been published in the *Journal of Chromatography*[1].

Mr. C. E. Roland Jones discussed technical details in the examination of pyrolysis products, describing a micro-apparatus for use with the Pye argon chromatograph. He illustrated its use in the identification of plastics, especially those which were not amenable to identification by infra-red spectroscopy.

Dr. R. P. W. Scott discussed the effect of temperature on resolution both theoretically and in the light of experimental results. He showed that for any required separation there was an optimum temperature for a given film thickness and this was more marked with thick films of stationary phase than with thin ones; conversely there was an optimum thickness for a given temperature and an optimum combination of both for minimum analysis time.

Dr. I. Halasz read a paper entitled "Quantitative Analysis of Hydrocarbons with Capillary Columns and Flame Ionization Detectors". He had investigated thoroughly a large number of factors affecting quantitative accuracy, and designed a stream splitter which apparently eliminated the uncertainties of previous models since the scores of tabulated results which he produced showed a consistent accuracy of about 1 per cent.

In the discussion which followed, Dr. Kovats produced some quantitative results from 2 μl. of rose oil on an unusually wide capillary column used in conjunction with a tungsten filament katharometer. Efficiencies of 40,000–100,000 theoretical plates had been obtained from such columns.

The final paper by J. G. A. Hölscher of the University of Eindhoven was presented by Dr. M. Van Swaay. He was able to show that ionization in argon detectors could be caused by photons from a pulse discharge. Such a discharge caused a current to flow between two electrodes in a mixture of argon and a hydrocarbon, with a time lag characteristic of the velocity of photons rather than metastables, ions from the discharge being screened off by a series of grids.

S. J. HAWKES

[1] *J. Chromatog.*, 5, 300 (1961).

TELEVISION AND FILM TECHNIQUES

A CONVENTION on television and film techniques, sponsored jointly by the British Kinematograph Society and the Television Society, was held at the Institution of Electrical Engineers during April 20–21.

Sir Harold Bishop, director of engineering of the British Broadcasting Corporation and president of the Television Society, opened the Convention with an address on the topical problems of television in the United Kingdom. He said that the B.B.C. supported the report of the Television Advisory Committee and that it was convinced of the long-term advantages which would result from the use of 625-line standards in the ultra-high-frequency bands IV and V (470 Mc./s.–960 Mc./s.). In particular, line visibility was notably less than with our present 405-line standards and the introduction of larger screens would aggravate the 'lininess' of the present images. Programme interchange with European countries, all of which except France had adopted the 625-line standard, would also be facilitated. Sir Harold also spoke of the B.B.C.'s wish to start a limited service of colour television compatible with the present 405-line service in band I.

Mr. J. Roizen gave a lecture on "The Use of Videotape for Colour Television Recording". He described the various methods that the Ampex Co. in California had investigated with the object of achieving satisfactory magnetic-tape recording of colour television signals. The composite colour video signal contains a subcarrier which is, in the American National Television System Committee system, amplitude-modulated with chrominance signals in phase quadrature. The frequency of this subcarrier is 3·59 Mc./s. and 1 cycle, or 360° of it, occupies a time duration of 0·28 μsec. The phase of the vector representing the chrominance signal carries the information from which the colour being transmitted is obtained and it has been found by experiment that errors of the order of 5° can be detected by a skilled viewer. A 5° error phase can therefore arise from a time variation or error of 4 nanosec. (10^{-9} sec.). In the recording of television signals on film (telerecording), the complete television image is exposed to the film, but in magnetic-tape recording the signal wave-form is recorded as a function of time or distance across, and along, the tape, the synchronizing pulses also being included. It is apparent that any non-uniformity of relative velocity between tape and 'head wheel', in the periphery of which four recording heads are embedded, will give rise to a timing error which will result in a change of the resulting colour. One line of investigation consisted, therefore, of an attempt to stabilize the relative velocity between tape and 'head wheel' to a much greater precision than had been required for the recording of monochrome (black-and-white) television. The servo-mechanism normally used employs a 240 c./s. electric signal derived optically from black-and-white patches on the 'head wheel', but a more accurate feedback signal was needed so the frequency was raised to more than a hundred kilocycles per sec. Even this was scarcely adequate, and another line of investigation led to the correction of signal-timing errors by means of a delay line having a controlled variation of delay. The difference in phase between the synchronizing signal derived from the video wave-form of the outgoing 'playback' television signal and a source of stable synchronizing signals is used to control the capacitance of a silicon diode forming part of the capacitance of the delay line. The outgoing video signal is passed through the delay line, and the delay is varied in such a manner as to compensate for the timing errors in the signal. The compensation is such that quite satisfactory results are now obtained: the timing error accumulated during the interval between successive colour synchronizing signals (a line-scan period) presumably being not greater than a few nanosec. The scheme described is known as 'Super-Amtec'.

Mr. B. J. Davies described a rapid-processing machine for 16-mm. film. The increasing use of magnetic-tape recording for television, or 'videotaping' as it is now termed, has caused the technical side of the film industry to consider means of accelerating the developing of film to the point at which it offers an attractive alternative method of making permanent records of television programmes, despite the fact that the advent of 'videotape' has resulted in an increase in the use of film in the television industry. The Kodak Co. has developed this machine around a very rapid processing cycle, the duration being about 60 sec. and the film velocity 38 ft./min. The method is called viscous-layer processing and is a departure from the liquid-bath methods previously used. The developer and fixer are applied to the photographic emulsion by means of special rollers over which the film is passed.

Messrs. Polonsky, Cassagne and Sauvanet described "Recent Progress in the SECAM Frequency Modulation Colour Television System". This system of colour television, the idea of M. Henri de France, has been developed by the Compagnie Française de Télévision, and has been improved over the years by the authors and M. Chaste who, with M. Cassagne, delivered a paper on the subject before the Institution of Electrical Engineers in April 1960. As in the American National Television System Committee's method, the colour information is transmitted by means of a modulated subcarrier but, instead of amplitude modulation being used, the subcarrier is frequency-modulated. Furthermore, unlike the Committee system wherein both the amplitude and the phase of the amplitude-modulated subcarrier are used simultaneously, the one representing saturation and the other hue, in the SECAM system the frequency-modulated subcarrier represents only one quantity at a time (television line by television line). This subcarrier is thus frequency-modulated alternately, line by line, by one of two colour-difference signals (for example, red intensity minus luminance and, afterwards, blue intensity minus luminance). This method of transmitted colour information renders the transmitted signal more 'rugged' and resistant to the various electrical and propagation hazards likely to be encountered. It is claimed that colour receivers based on this system are simpler to align in the factory, easier to service and more stable, as well as being more suitable for an unskilled viewer to operate. The receiver requires an expensive delay line in order to compensate for the non-simultaneous transmission of colour signals, and there is a theoretical loss of vertical colour resolution of two to one as compared with National Television System Committee, but the authors state that the Committee's system contains several times more vertical colour resolution than is required, and it must be said that the excellent demonstration given by the authors, with assistance from the Hirst Research Centre of the General Electric Co., the General Post Office and the B.B.C., showed no apparent lack of vertical resolution. It was suggested that, from the technical and picture quality point of view, there was little to choose between the SECAM and the National Television System Committee systems.

Dr. W. E. Glenn described a thermoplastic recorder for television and similar signals. The device, developed in the General Electric Research Laboratories in Schenectady, bears a fundamental resemblance to the Eidophor, which will be mentioned later. Brighter pictures than those from a cathode-ray tube could be obtained if the image were stored for display and then illuminated by a separate source of light, such as an arc. Photographic recording (telerecording) is a good example since, in reproduction, a film projector may be used, although many applications require re-televising of the storage medium. In Dr. Glenn's apparatus, which consists of an evacuated enclosure, an electron beam, appropriately modulated with a television signal and deflected in the usual manner of scanning impinges on a thin film of low-melting-point thermoplastic on which a charge image is thus placed. Beneath the top film is another of a transparent conducting material and beneath this is a transparent high-melting-point tape. The conducting layer acts as one plate of a condenser, the other being the charge image itself. By melting and then freezing the top layer, the charge image is replaced by the same image impressed into the thermoplastic by electrostatic force in the form of hill-and-dale deformations. The film or tape now has a permanent record on it and may be stored for subsequent play-back. For this purpose a modified schlieren optical system and a light source are required, although, for editing purposes, the actual image can be made visible on the film. The entire process of melting and freezing the thermoplastic takes a few milliseconds, and the ultimate resolution is limited by the wave-length of the light used for projecting (200 television lines per mm. is a practical figure). For colour images, a phase-diffraction-grating method may be used and a special optical system is required for projection. The author projected some thermoplastic film through a suitably modified projector.

Prof. E. Baumann described "Recent Developments on the Eidophor Large Screen Projector". The idea, conceived by Prof. Fischer in 1939 and afterwards developed with Dr. Thiemann, Prof. Baumann and others at the Zurich Technische Hochschule and, later, by Eidophor, Ltd., relies on the light-valve principle described earlier. In an evacuated enclosure, an electron beam, suitably modulated with a television signal and scanned in the usual manner, 'writes' a charge image on a thin film of oil on the conducting surface of a concave mirror. The viscosity of the oil is sufficiently low to allow the electrostatic forces between the charge image and the mirror surface to deform the oil into hill-and-dale irregularities. The mirror is in the light path of a schlieren optical system, and the deformations of the oil, acting as the reflecting surface, are revealed as optical images on a large projection screen. Prof. Baumann demonstrated the Eidophor by receiving, from a B.B.C. studio, a 405-line film transmission which, after passage through the Eidophor, was seen on the large screen in the lecture theatre. Although the film was not particularly bright, the possibilities of the system were quite apparent.

Mr. Grimshaw of the Kodak Co. read a paper by Messrs. Veal and Richey of Eastman Kodak Research Laboratories, Rochester, U.S.A. The paper, "Technical Requirements of Systems and Film for Colour Television", dealt with the difficulties of reproducing colour film on colour television. In particular, the contrast-range normally encountered in colour film is too high for colour television, resulting in loss of luminance and colour information in dark parts of the viewed image. Whereas a contrast-range of 100 : 1 is not rare in film, colour television is at its best within a range of about 20 : 1. This is due to many cases, but it is not difficult to see that, since the optical projection of film in the cinema (or home) relies on the light-valve principle, in which a source of light illuminates a screen after multiplication of the light intensity by the transmission factor of the film, the contrast-range of projected film may be limited only by flare and diffusion in the projector and on the screen, provided that the ambient illumination of the screen is zero. The fact that the reproduction of colour by photographic film is a negative-colour process, whereas its reproduction by television is a positive-colour process, also makes the colour televising of colour film rather more difficult. The paper presented graphs which revealed that greater horizontal resolution could be obtained from a three-Vidicon film scanner than from a flying-spot film scanner employing three photomultiplier cells. This came as a surprise to some members of the audience, who wondered whether American experience with flying-spot scanners had, perhaps, been less extensive than European.

Messrs. A. R. Stanley and J. Treays read a paper entitled "Colour Television Engineering: Studio Problems and Techniques". They gave a short historical account of the experimental colour transmissions radiated by the B.B.C. and went on to discuss a number of matters connected with studio programmes in colour. Experience showed that it is preferable for each picture-originating source to have its own coder so that the primary-colour signals are not sent round the studios. They found that the composite signal, that is, the luminance signal added to the modulated subcarrier, was more 'rugged' and suitable for the various processes which occur in a television studio, such as cutting, mixing and fading. It was interesting to note that during a slow mix from one composite colour signal to another, during which the two signals are added, the resulting colours were quite satisfactory and gave the impression of two colour pictures being superimposed. Presumably because a colour picture contains more information, it was found that individual shots could last longer than in the case of a monochrome programme, although the compatible black-and-white programme viewer must not be forgotten. The performers' make-up presented some novel problems: it seems that the chemical composition of perspiration varies from individual to individual and the colour of make-up can vary throughout a day's rehearsals and performance. Coloured performers require less make-up and are, therefore, easier to deal with than white people. In order to align and set up the cameras, these were presented with a set of Munsell colour chips illuminated by the normal studio lighting and the images on the picture monitors in the control room were compared with a similar set of Munsell chips illuminated with illuminant *C*, which has the chromaticity of the 'white' standard for the National Television System Committee colour television. When a programme involves film inserts, great care has to be taken to ensure a colour match between similar scenes which may be viewed in succession from a colour-television camera and from a colour-film scanner, or telecine machine. Although the necessarily low contrast required for the colour transmission seemed to be quite satisfactory for the compatible monochrome viewer there was, at times, a subtle change of mood in that the dark colours which were required for the colour viewer made the compatible black-and-white picture rather sombre. During the lecture, some very fine colour slides were projected, showing studio scenes for colour programmes.

Messrs. A. R. Stanley and S. N. Watson introduced "Colour Television Engineering: Equipment Problems and Techniques". The authors discussed five characteristics of colour television which gave least satisfaction to viewers during the B.B.C. colour field-trials mentioned in the historical survey of the previous paper. It had been clearly demonstrated that the colour cameras were the weakest link in the chain. The paper dealt with a number of matters which affect the correct reproduction of colour scenes. For example, no effort is made to ensure negative lobes in the spectral response characteristics of the colour analysis filters in the cameras, although it is possible to achieve an approximation to negative-lobed characteristics despite the impossibility of using 'negative light'. The 'taking gamma' of the cameras is important, as are the errors due to shading (or spurious) signals in the image orthicon tubes used in the cameras. The latter may cause errors of hue, particularly in low-luminance patches of the scene. Since the phase of the modulated colour subcarrier determines the reproduced hue, any departure from linearity of the phase/frequency characteristic of any part of a transmission network in the region close to the frequency of the subcarrier (2·66 Mc./s. in the case of the National Television System Committee system adapted to 405-line standards) causes errors of hue. In particular, if the transmission system suffers from a non-linear effect which causes a change of phase to occur in sympathy with a change of signal-level (in the composite video signal the luminance signal and the chrominance or modulated subcarrier signal are added) a change of hue and brightness will result. The possible existence of this phenomenon places a rather stringent requirement on the linearity of television equipment such as studio broadcast apparatus, and radio links. An excellent demonstration followed the lecture. Colour film and slides originating in a B.B.C. studio were transmitted by Post Office equipment to the lecture theatre of the Institution of Electrical Engineers and the received images were projected on to the large screen by a Marconi's Wireless Telegraph Co.'s three-tube Schmidt projector. The registration of the three primary-colour images was very good indeed. The effect on hue of a 10° change of subcarrier phase was shown and also the deterioration in picture quality to be expected from the use of two transmission links in series. Both deteriorations were slight. The technique of using a low-pass filter to attenuate all Fourier components (and noise) of the luminance signal, which would otherwise occupy the frequency band set aside for the chrominance signal (which shares an upper part of the full luminance channel) and the subsequent employment of a non-linear device ('crispener') to reconstitute artificially an impression of sharpness was also successfully demonstrated. An electronic masking unit showed how an improvement can be made to the properties of colour film for colour-television purposes.

R. D. A. MAURICE

STUDY OF DEFECTS IN PORPHYRIN METABOLISM BY MEANS OF ISOTOPES

THE Biology Division of the French Atomic Energy Commission held, during September 1960, a colloquium at Saclay on the use of tracers in the study of diseases of porphyrin metabolism. Participants from Europe and the United States enjoyed three days of intense discussion as guests of the Atomic Energy Commission and the Ministry of National Education. The topics debated ranged over a wide field; full proceedings of the conference will be published in due course.

In the opening address, Prof. A. Bénard reviewed our present knowledge of porphyrin biosynthesis and of the disturbances giving rise to the different clinical forms of porphyria. It was his view that such diseases were variants of a single fundamental abnormality. Symptoms were due not to the porphyrins themselves but to associated metabolic disturbances such as interference with the synthesis of purines. He concluded an impressive survey by reference to recent therapeutic measures which have shown some promise in the treatment of acute porphyria, namely, administration of 2 : 3-dimercapto-propanol, ethylenediaminetetraacetic acid or adenosine monophosphate.

The genetic aspect of the porphyrias was then dealt with. It was pointed out that the several hundred South African cases of 'porphyria variegata', all being descended from a single pair of Dutch settlers, necessarily represent manifestations of the same genetic abnormality or group of abnormalities found in the progenitors and might well, therefore, display a characteristic pattern. A fascinating account of the recently discovered 'Turkish porphyria' was given by Dr. R. Schmid. It is an acquired porphyria due to consumption of wheat containing hexachlorobenzene. Several thousand human cases have occurred and the disease has also been reproduced experimentally in animals. Topics further discussed during the first day's sessions were the symptomatology of acute intermittent porphyria and its causation, and also the difficulties attending the interpretation of investigations made with isotopic tracers on such patients.

While it is generally felt that the excessive excretion of porphobilinogen and δ-aminolævulic acid in acute hepatic porphyria is due to over-production of these materials, the exact biochemical mechanism leading to such over-stimulation of the porphyrin pathway is still in doubt. Experiments designed to test the possibility of restricted conversion of glycine into serine were inconclusive. A hint as to the means by which δ-aminolævulic acid production is normally regulated may be forthcoming from the experiments described by Shemin who showed, in one system studied, that δ-aminolævulic acid is itself an inhibitor of the enzyme producing it. This points to the possibility of a negative feed-back control operating in the normal cell. The combination of administration of porphyrinogenic drugs with pantothenate deficiency promises to throw further light on the development of the nervous symptoms in acute porphyria. It has been generally assumed, since the self-experiment of Meyer-Betz, that photosensitization in the cutaneous porphyrias is actually caused by the porphyrins themselves. Direct evidence was lacking, however, until quantitative measurements were made by Magnus and collaborators on patients with the use of a powerful monochromator. This technique and the results it afforded were described and exemplified by the case of a man with sun-burn type photosensitivity who, nevertheless, showed highest cutaneous sensitivity in the Soret band region of the spectrum. Further investigation showed that his red cells and fæces did, in fact, contain abnormally large quantities of porphyrins.

The increase in urinary excretion of porphyrin which accompanies lead intoxication has aroused the interest of biochemists for many years, but is as yet little understood. New light may be thrown on this problem by the finding of Gajdos and his collaborators that lead ions interfere with the passage of iron through the membrane of the developing red cell, the stroma, under these conditions, retaining iron rather firmly.

The biochemical mechanism of incorporation of iron into protoporphyrin and the part played by 'bound' forms of these reactants, the biosynthesis of porphyrins by human leucocytes and the metabolism of hæmoglobin within the red cell were next considered. It would appear from the work of Schapira and his co-workers that the hæmoglobin molecule within the erythrocyte is not metabolically inert, but that progressive structural alterations occur with the course of time. The effects of radiation on the synthesis of hæmoglobin in the bone marrow, and the results of a great deal of work on the relation of porphyrin structure to X-ray sensitivity, were also reported.

At the close of the meeting, Prof. C. Rimington endeavoured to lay before participants an appraisal of the most significant conclusions and to point to the fields from which the next major advances towards an understanding of normal and pathological porphyrin metabolism might be expected to come. He took the opportunity of thanking the organizers and the two bodies sponsoring the colloquium for their excellent work on behalf of porphyrin biochemistry and for their generous hospitality. This meeting will be long remembered with gratitude and pleasure by those who were gathered together in the stimulating atmosphere of Saclay.

C. Rimington

CONSERVATION OF BRITISH NATIONAL PARKS

In its recent eleventh annual report the National Parks Commission referred briefly to the question of afforestation in the National Parks, especially on Dartmoor and Exmoor, and also to its disappointment at the delay in amending the Act, particularly with regard to financial resources. Both these aspects have since been the subject of short adjournment debates in the House of Commons. In the first of these, on March 29, Mr. E. du Cann raised the question of afforestation, particularly in the Dartmoor and Exmoor National Parks. While supporting the voluntary agreement recently reached between the Timber Growers' Organization, the Country Landowners' Association, the Forestry Commission and the National Parks Commission regarding afforestation in the National Parks, Mr. du Cann questioned whether a voluntary agreement would be sufficiently effective, as it did not include all those concerned with afforestation in such areas.

In his reply, the Parliamentary Secretary to the Ministry of Housing and Local Government, Sir Keith Joseph, agreed that it was important to give the new scheme a fair trial. He thought that there was no reason to fear that landowners in the areas concerned, who were not members of the Association, would not, as individuals, comply with such agreements and, in reply to a specific question about trees on High House Moor, Dartmoor, said that discussions were still proceeding between the proposed developers and the authorities. On this specific issue it should be noted that considerable uneasiness still exists that the National Parks Commission will not be supported. The parties to the agreement were not despondent as to the prospects that the agreement would succeed, and Sir Keith Joseph appeared to have substantial grounds for his confidence that the spirit of co-operation already demonstrated by the production of this voluntary agreement, and the way it had been received and implemented indicated that, with common sense, it should be possible to reconcile the claims of forestry and the National Parks and the rights of landowners.

Nevertheless, Sir Keith recognized that failure of the scheme would create a new situation and this would have to be faced. Meanwhile, he said, the Minister wished to give the scheme a fair trial and he made it clear that he also wished that all concerned, whether members of the particular organizations or not, should participate in this voluntary consultation at all times. The Minister and the National Parks Commission would keep progress under careful review.

On the wider issue raised in the debate on March 30, progress also may be regarded as largely a matter of co-operation and common sense, but on the part of the Government common sense implies providing the financial means to the end enacted. The root of the difficulties encountered in the development of the National Parks lies in the failure of the Government to provide, under the National Parks and Access to the Countryside Act of 1947, the finance required to make the provisions of the Act effective. Under that Act the responsibility for the National Parks was placed within the planning system set up by the Town and Country Planning Act of 1947 instead of the national system envisaged by the Hobhouse Committee. The counties provide two-thirds of the Committee members and almost all the funds, although some of these counties are among the poorest in the country.

Nevertheless, the system has worked surprisingly well, though it is obviously unfair to expect local authorities to take a national point of view, and invariably set the national interest before local interests, particularly at heavy cost to the ratepayers who elect them. The advantages of close association with local interests are such, however, that in view of this experience, it seems highly probable that, given quite moderate Government support, sufficient, for example, to ensure the provision of adequate planning staff and a reasonable contribution to the capital costs of positive projects of development, the existing system could well be continued with success. Much could be done if the National Parks Commission had an annual sum of even as little as £100,000, in place of the present £20,000 (with some £5,000 in Exchequer grants to the National Parks), which it could use at its discretion, either in helping the weaker authorities to strengthen their planning staffs, or in assisting projects which, at present, the park planning authorities hesitate to undertake because of their fear to place excessive burdens on local ratepayers for the benefit of outsiders.

Such a system admittedly is pragmatic rather than the ideal national system, in which national purposes and projects are financed directly from national funds, as Mr. F. H. Hayman advocated on March 30. Admittedly, the development and preservation of the National Parks is often too dependent on the finance committee of the county councils concerned, as Mr. Hayman pointed out ; but to transfer the whole cost of the National Parks to national funds is not the only way of overcoming the difficulty, and if such a transfer lost local interest in, and support for, the National Parks the price might well lead to local misunderstanding and opposition. Mr. Hayman did not, however, appear to advocate quite such a drastic step though he made it clear enough that local resources and prejudices should not determine the objectives and purposes of a national undertaking.

In replying on this debate, Sir Keith Joseph asked for specific examples of projects which were now being frustrated by lack of funds, but claimed that direct Parliamentary grant, and not discretionary payment, was the appropriate way to finance public schemes. He confirmed that the Minister would look at all proposals made to him for amending the Act and said that he was at present seeking more information from the National Parks and the National Parks Commission to assist in this review. Sir Keith was emphatic that there was much more which could be done by the individual Park Committees which would receive Exchequer aid up to 75 per cent, but he thought the time might well have come to extend the legislative framework in some ways. Nevertheless, he did not think that finance was the only answer. Vigilance, effective administration and an imaginative approach must also play an important part, and probably the main value of both debates lies in the extent to which they demonstrate the need for public co-operation and understanding, and for such unceasing vigilance if the purposes of other Parks or Nature Reserves are to be served.

The whole subject was further discussed in the House of Lords on April 19 on a motion of Lord Silkin, who again stressed the importance of adequate financial provision. Lord Feversham also pointed out that existing provisions were completely inadequate, either to prevent or to control fires on heaths and moors, and Lord Dalton, who agreed as to the need for adequate financial provision, also pressed the claims of the long-distance footpaths. Lord Chorley specifically suggested that the National Parks Commission should have an annual sum of £150,000 at its disposition, and like most other speakers was critical both of the Electricity Authorities and of afforestation, though tribute was freely paid to the sympathetic attitude of the Forestry Commission and its present chairman. Earl Jellicoe, who replied on the debate for the Government, added little to what had been said in the Commons by Sir Keith Joseph, but referred particularly to Lord Feversham's suggestion of a direct Treasury grant to the National Parks Commission.

THE RESEARCH COUNCIL OF ALBERTA

THE forty-first annual report of the Research Council of Alberta covers the year 1960*, and includes a list of publications for 1956–60, and lists of members of Advisory and Technical Advisory Committees and of the staff of the Research Council. The Petroleum Division of the Fuels Branch has largely completed its work on the movement of oil in the presence of water in a pipe-line, and studies on capsule pipe-line transportation indicate that the presence of capsules in a flowing stream does not significantly alter pressure gradients, nor does the moving core appreciably distort the flow in the annulus. The capsule velocity appears to depend directly on capsule length and inversely on capsule diameter. The Division has also completed the first major phase in developing a generalized mechanism for the control of composition and accumulation of crude oils and natural gases in sedimentary basins, and the light paraffinic oils, which are usually found in positions corresponding to the areas of maximum depth of sedimentary basins, appear to owe their paraffinic constitution to the extensive action of oxidation and microbiological attack, both of which tend to destroy the less-resistant components of the developing crude oil. With the Coal Division the Petroleum Division is studying the transport of mixtures of pulverized coal and oil, while the Coal Division has also studied the combustion of pulverized fuel, the crystal structure and magnetic properties of coal chars, the plastic properties of Alberta coking coals and coal blends. Work on the mechanisms of coal pyrolysis and carbon formation is expected to bear directly on carbon production from low- and medium-rank coals, and physico-chemical investigations on humic acids from coal have shown that simple coupling reactions can convert humic acids into water-soluble materials without otherwise altering their chemical properties, and that humic acids can be made to yield five-membered cyclic anhydrides.

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* Research Council of Alberta. Forty-first Annual Report, 1960. Pp. v+55. (Report No. 80.) (Edmonton: Research Council of Alberta, 1961).

The Natural Gas Division has been engaged largely in the separation of gases by selective adsorption on porous solids and with catalytic reactions in the adsorbed state, particularly the catalytic conversion of *cyclo*propane to propylene on crystalline alumino-silicates. The Groundwater Division has provided information which assisted in meeting several serious water-shortages, while the programme of the Geology Division included a study of the fossil content, distribution and physical make-up of Cretaceous rocks, the study of iron deposits and examination of a gypsum deposit at Mount Heed. The reconnaissance soil survey of the Hines Creek map sheet was completed, as well as an exploratory soil-survey by helicopter of some 10 million acres in the north-western part of the Province, while the Soils Division has also co-operated in work on the classification of forest sites and the irrigation of Solonetzic soils. The basic cloud-physics programme, with special reference to hail, included establishment of an enlarged meso-barograph network covering 10,000 sq. miles, upper wind observations stations at Penhold, Rimbey and Rocky Mountain House, and an expanded hailstone collection network. Synoptic investigations on hail now permit some differentiation of storms as related to certain areas to low-level convergence and moving cold-front activity.

ERADICATION OF MALARIA

ALTHOUGH progress towards world-wide malaria eradication has, in general, been satisfactory, there are some malaria eradication programmes that have failed to develop as speedily as they could. A report of the World Health Organization's Expert Committee on Malaria is mainly concerned with the reasons for these failures, and with methods of increasing the efficiency of eradication programmes*.

The technical, operational and administrative causes of failure are analysed in detail, and it is emphasized that success depends not only on careful planning and meticulous execution of the programme but also on the full support of the national authorities at every stage. This must be ensured at the outset by a signed undertaking from the Government.

The criteria given in the Committee's seventh report for discontinuing spraying operations and starting the consolidation phase are amplified and a fresh criterion added, namely, that surveillance should be fully established and adequate. The measures undertaken should include the passive as well as the active detection of cases, this being the only way to ensure that all areas are reached and that observations are continuous. With the view of reducing the costs of the consolidation phase without sacrificing efficiency, the report recommends that the World Health Organization should study the costs by heads of expenditure in as many countries as possible, and should encourage the detailed analysis of costs both by activity and by heads of expenditure in selected representative countries.

The report reaffirms that eradication may be considered to have been achieved "when an adequate surveillance mechanism has not discovered any evidence of transmission or residual endemicity, despite careful search, during three consecutive years". The criteria of eradication given in the sixth report of the Expert Committee on Malaria are re-examined; while the basic principles remain unchanged, the criteria are re-defined and expanded in order to cover the needs of special situations that have been met in practice. With the aim of establishing an official register of areas where eradication has been achieved, recommendations are given on the methodology of inspection and certification.

In countries where the administrative structure and the health services are still in the developmental stage, a "pre-eradication programme" may be necessary to create suitable conditions for the success of malaria eradication. An outline of such a programme is given, and it is suggested that an international team should be provided to advise the Government and, if necessary, to carry out the initial stages of the programme.

Health education forms an essential part of a malaria eradication programme; it must reach all levels of the population from the outset of the campaign and be continued throughout the consolidation and maintenance phases, in order to ensure continued co-operation of the public at a time when interest in malaria will have begun to wane. The report advocates that provision should be made for a special staff to run health education programmes under the malaria eradication programme.

The establishment of a standard terminology for use in evaluation and surveillance was considered by the Committee to be highly desirable, and it was recommended that the World Health Organization set up a drafting Committee to undertake this task.

The report directs attention to the value of chemotherapy in the attack phase of eradication programmes as well as in the later stages. The 4-aminoquinolines remain the most effective drugs and no significant changes are recommended in the regimen for radical cure. The use of salt medicated with chloroquine shows promise. Research is needed on the improved use of available drugs and to discover compounds with an improved action against malaria parasites.

It is believed that, in some countries, a reduction in the standard dosage of DDT (1 gm./sq. m.) used in spraying might be possible, but no such reduction should be made until appropriate field trials have provided unequivocal evidence that this would not jeopardize the success of the eradication programme.

Finally, the report considers the relation of simian to human malaria, particularly the recent experimental transmission of malaria from the lower monkeys to man. It is concluded that the practical importance of a simian reservoir of malaria is likely to be small.

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* World Health Organization. Technical Report Series, No. 205. Expert Committee on Malaria—Eighth Report. Pp. 50. 2 Swiss francs; 3*s.* 6*d.*; 0.60 dollar. (Geneva: World Health Organization; London: H.M.S.O., 1961.)

A READING MACHINE FOR THE BLIND

By M. P. BEDDOES, E. BELYEA and PROF. W. C. GIBSON

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WE have investigated possible methods by which a machine can help blind persons to read from the printed page. This article describes work done on the problems of recognizing the alphabet letters, coding the output, and placing a hand-held pick-up probe accurately on the page.

The first notable reading machine was called the optophone by its inventor Dr. E. E. Fournier d'Albe[1,2], and in the early 'twenties a speed of 25 words a minute was claimed.

A devoted but small band of people have laboured with it since then with results, quoted recently in the Press[3], of up to 60 words a minute. This is not impressive considering the vast amount of experience which has been gained over the years, and it indicates two defects of the optophone principle: one is that the code sounds are difficult to learn; the other is that the fastest rate is well below that of normal reading speeds.

An American version[4] of the optophone which employs up-to-date engineering techniques has been disappointing also. Tests in progress since 1957 have established reading rates, when tape-recordings of the code-sounds are presented to subjects, of about 30 words a minute; but when a hand-held pick-up probe is used, 8 words a minute seems to be the fastest rate for experienced operators. The differences in the rates of the two versions (60 down to 8 words a minute) lie chiefly in the fact that the English machine uses a mechanical device scanning along the line to be read whereas the American one uses a manually held pick-up probe. The latter has advantages as regards cost, reliability and convenience to the user which are, at present, outweighed by its poor performance.

The optophone converts material from the page, a letter at a time, almost directly into electrical signals. For this reason it has been called[5] a 'direct translation' machine. Our work is with a 'letter-recognition' machine, so called because it first identifies (or 'recognizes') a letter and then triggers the appropriate code sound. With this type of machine there is a wide choice of audible codes making possible psychological advantages in learning and increased speed. Many workers in recent years have favoured the 'letter recognition' approach[7-11] because it permits the problems of identifying letters and of designing a suitable output code to be dealt with independently of one another.

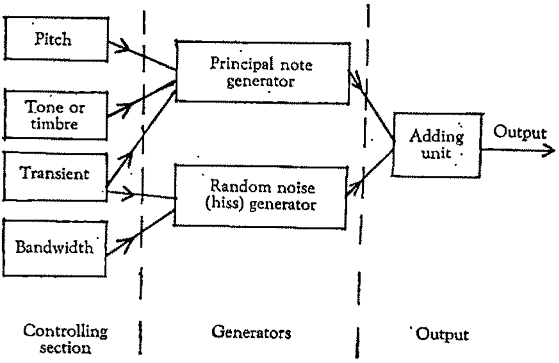

Fig. 2. Block diagram illustrating the generation of the sounds for the blind readers' code

The problem of recognizing standard typewritten print was investigated. Transparencies were made from impressions on ordinary bond paper of upper-case and lower-case alphabet letters from a portable typewriter. These slides were scanned by a flying-spot cathode-ray tube (Fig. 1). Sawtooth scanning was employed: vertical rate = 3,300 c.p.s.; horizontal rate = 80 c.p.s. Output from a photo-multiplier was processed by an R–C integrator with a time-constant of 11 m.sec. The scanning rates are not critical provided that this time constant is short compared with the period of the horizontal scanning (= 125 m.sec.) and long compared with that of the vertical scanning (= 0·3 m.sec.).

The operation of the apparatus is as follows. The output voltage, V_0, was large when scanning a long vertical line of appreciable width; such a line, for example, is the principal part of the letter I and is known as a 'riser'. Risers in letters b, d, f, h, etc., were easily spotted from the wave-form of V_0. A single horizontal line in letters such as n and u, etc., gave rise to a small value of V_0.

In identifying a letter, the apparatus of Fig. 1 may be used. The following sequence of tests on the wave-form V_0 was found necessary: (*a*) measurement of the height of the first maximum (results in Table 1: accuracy of ± 0·05 in 3 or ± 1·7 per cent is necessary); (*b*) measurement of the minimum following the above maximum; (*c*) a count of the number of maxima (used with letters such as m, w, M and W), with the scanning raster rotated through right angles; (*d*) measurement of the maximum; (*e*) measurement of the minimum between any adjacent maxima. Analogue and digital techniques are both used, and all letters can be decoded. The number of tests required to identify a letter varies, but the greatest number is four.

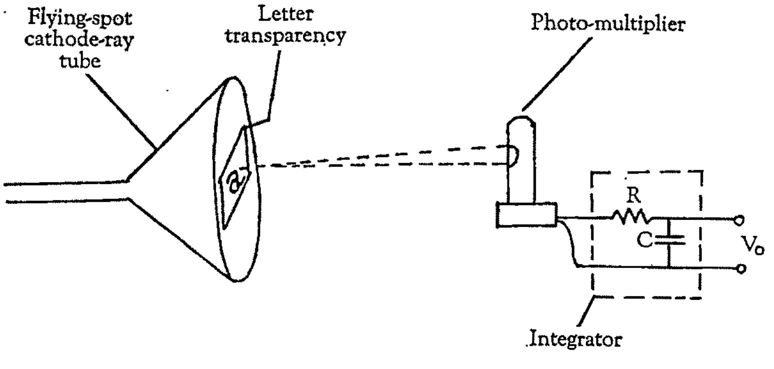

Fig. 1. Apparatus for investigating properties of letter recognition

Table 1. Classification of Letters according to Height of First Maximum of V_0

Height	1·0	1·1	1·2	1·3	1·4	1·5	1·6	1·7	1·8	1·9	2·0	2·1	2·2	2·3	2·4	2·5	2·6	2·7	2·8	2·9	3·0
Letter			dj			xy	cs	oa	euw	gmz	v	n	k	f	rbth	i	p			q	
Letter									CGO	SX	V	UZ	AMN		RY	HJTW	BDK	FQ	E	LP	I

In order to arrive at the best coding for the alphabet letter detected by the above procedure, we conducted experiments with various proposed audible codes of a semi-musical nature. Each alphabet letter is represented by a single sound (as opposed to the many in Morse). Such a sound is the result of combining the components listed below.

(*a*) 'Pitch'—four frequencies (corresponding to doh, ray, fah and sol of the sol-fah notation). (*b*) 'Timbre'—two wave-forms (sine-wave or rectangular wave). The above are produced in the 'principal note generator' (Fig. 2). (*c*) 'Hiss'—broadband noise from a separate generator is filtered through one of three bands in the audio spectrum. (*d*) 'Transient'—with or without a click at the commencement of the sound. Sixty-three distinct sounds can be made. The above list represents the best compromise obtained so far as judged by the following experiments.

Experiments were made to determine whether or not the components could be perceived either singly or in the presence of others. A pair of sounds each 0·1 sec. long and separated by 0·1 sec. silence was conveyed by a loudspeaker to a subject who was asked to judge if the pair was made up of the same sounds or different ones. About two hundred such tests, each separated by 3 sec., were presented to up to seventy-six subjects at a time. Errors with the final code (above) were less than 10 per cent.

The choice of components was made so that clear patterns were perceptible when the sounds were presented rapidly. The following word experiments indicate that such is the case.

Eleven three-letter words were chosen. Each was coded to occupy 0·7 sec., and they were presented in six random sequences to seven blind persons. Ages varied from 15 to 52 years; average age was 33. The code version of the word was followed by a 10-sec. silence; this was followed by the spoken word, and, finally, by the code-version again. Words were always presented in this order. In the first sequence, words were heard for the first time, but nothing was required from the subjects. In the second sequence and those following, the subjects were asked to write in Braille the appropriate word in the 10-sec. pause. Their 'guess' was confirmed or corrected by the subsequent voice-code cycle. Results are given in Table 2: no difficulty was found in decoding meaningful sentences made of strings of these words.

Further word experiments using the same material with some additions were made with thirty-seven sighted university undergraduates in psychology. Results are not as good as those for the blind group (cf. Table 2 with Table 3), but are still considered to be acceptable. Poor motivation probably accounts for the slower learning-rate.

Table 2

Sequence	2	3	4	5	6	7
Correct (per cent)	65	60	82	82	80	80

Table 3

Sequence	2	3	4	5	6	7	8	9	10	11	12
Correct (per cent)	17	23	33	42	44	48	53	54	59	62	63

A machine has been constructed with a hand-held probe for reading a very special type of print—printed Braille. This machine is being used to investigate the problem of placing the probe accurately on the page. A feature is its simplicity. Six miniature photocells are used to read from the Braille character; three cells are used to identify position; eighteen transistors and nine relays are required for reading purposes; only six transistors are required to provide the coded output. If printed Braille could be made popular, such a machine would provide a very economical link between the blind and the printers' art.

Acknowledgment is made to the National Research Council of Canada (Ottawa) for financial support of part of the work.

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THERMAL SHOCK AND ITS EFFECT ON THERMISTOR DRIFT

By J. H. SWARTZ and R. RASPET

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IN 1948, one of us (J. H. S.) undertook an investigation of sub-surface temperatures in drill-holes in Navy Petroleum Reserve No. 4 as a part of a study of permafrost problems in arctic Alaska. Early measurements were made with copper-coil resistance thermometers. Beginning in 1949, however, thermistors were used as the temperature-sensing element because of their high thermal sensitivity. These were mounted in a multiconductor cable employing a circuit designed to permit the use of a minimum number of conductors for a given number of thermistors and to allow a correction for temperature distribution along the wires[1]. In construction the cable jacket was slit to permit the insertion of the thermistors in the appropriate conductors. Originally the slits were closed by wrappings of pressure-sealing vinylite tape. Later the cable slits were sealed by special vulcanizing techniques.

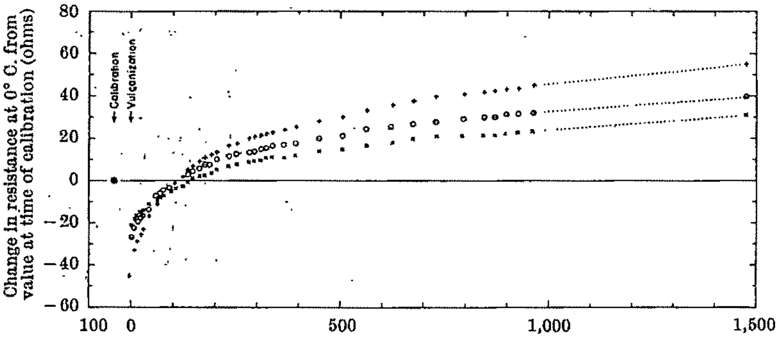

Fig. 1. Change in resistance at 0° C. with time after vulcanization (at 120°–130° C.) for 56 thermistors vulcanized in cables 199 and 200. ⊙, Mean of all 56 thermistors, cables 199 and 200; ×, thermistor 3786 (minimum change); +, thermistor 3834 (maximum change)

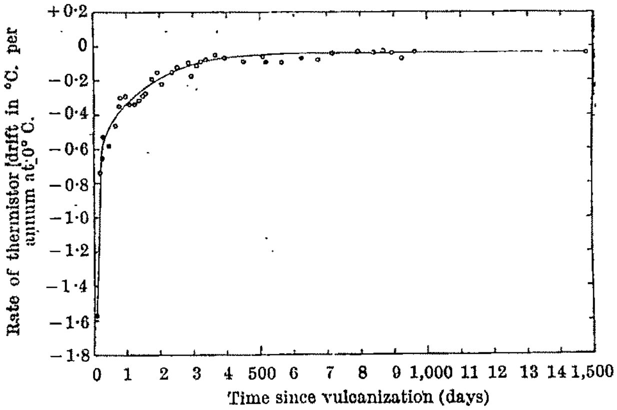

Fig. 2. Change in the rate of temperature drift with time for the mean of the 56 thermistors in cables 199 and 200

It was soon found that thermistor calibrations were disturbed by the vulcanization process. It was thought that this might be due to either of two factors: (1) chemical effects caused by free sulphur during vulcanization; (2) thermal shock caused by the heat of vulcanization. Experiments with litharge rubber, furnished through the kindness of the Rubber Section of the National Bureau of Standards, to eliminate most of the free sulphur during vulcanization, showed no apparent reduction of the effect. Nor was the effect either eliminated or reduced when a plastic-insulated cable was employed. This supported the idea that the effect was due largely, if not entirely, to thermal shock.

To test this hypothesis 12 thermistors were placed in a clean mould and raised to vulcanizing temperatures (120–130° C.) for 1 hr. All twelve showed the same typical calibration disturbance as those vulcanized in a cable.

Since that time some 80 thermistors have been investigated for the effects of thermal shock, and their subsequent calibration drift has been studied by recalibrations at approximately regular intervals in either a high-precision temperature-controlled bath or in an ice-bath. In particular, the resistances of 56 thermistors in two vulcanized cables have been rechecked in carefully prepared ice-baths, first at weekly, later at two-, three- and four-weekly intervals over a period of 961 days (2·6 years) with a later follow-up check at 1,475 days (4·04 years). This work has indicated certain generalizations to hold for the thermistors investigated (mostly type 17*A*, material No. 1 composition, disk-shaped). These were mostly a commercially available type composed of manganese and nickel oxides, disk-shaped, approximately 0·2 in. in diameter and 0·04 in. thick, with axial leads attached by silver-eutectic solder to a ceramic silver button attached to the face of the disk; at room temperatures its resistance is approximately 1,000 ohms and its rate of change of resistance with temperature approximately 4·4 per cent per deg. C.

(1) Of 70 thermistors heated to vulcanization temperatures the calibrations of all but one were offset in the heating direction, that is, after vulcanization their resistance at a given temperature was found to be lower than before vulcanization so that they registered too high an apparent temperature. For 55 of the 56 thermistors in the two vulcanized cables, for example, there was a decrease in resistance after vulcanization of 18–45 ohms, resulting in an increase in the apparent temperature of the ice-bath of 0·11–0·28° C. with an average of 0·20° C. as measured by the two cables. The one aberrant thermistor, No. 3,800, showed an increase in resistance after vulcanization of 1·9 ohms corresponding to a decrease of − 0·01° C. in the apparent temperature of the ice-bath.

Following the initial offset, all the heated thermistors began to increase in resistance (Fig. 1), returning exponentially towards their pre-vulcanization resistance. However, instead of approaching this value asymptotically, as had been expected, they reached this value after approximately 120 days, crossed over it, and continued to increase in resistance, but at a gradually diminishing rate. After four years they are still continuing to change, but at a slow and apparently nearly uniform mean rate of approximately 6 ohms per annum, equivalent to a decrease in the apparent temperature of the ice-bath of approximately 0·036° C. per annum (Fig. 2). The aberrant thermistor, No. 3,800, after the initial abnormal offset has shown a normally increasing resistance (decreasing apparent temperature) and has in fact closely paralleled the drift of the normal thermistors, as seen in Fig. 3.

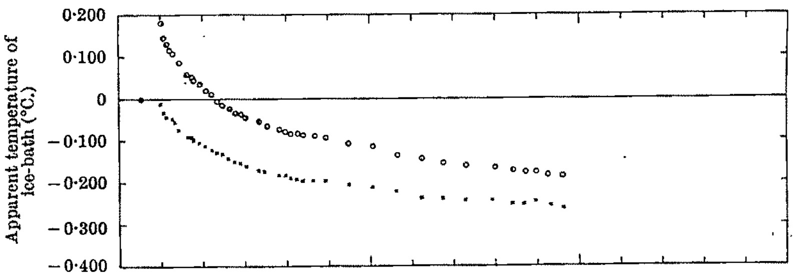

Fig. 3. Change of the apparent temperature of the ice-bath with time after vulcanization (at 120°–130° C.) as measured by the mean of 56 thermistors in cables 199 and 200 (⊙), and by thermistor No. 3800 (×)

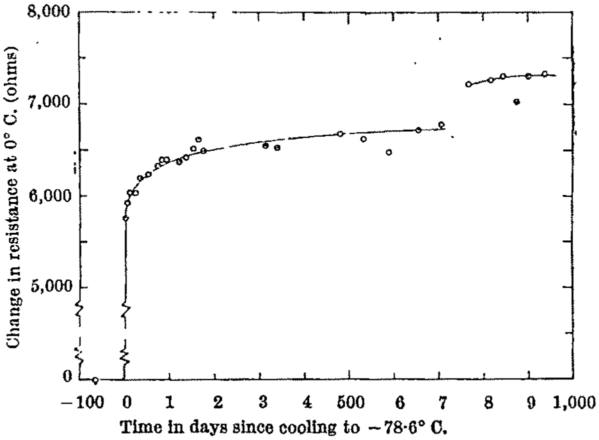

Fig. 4. Change with time in the mean resistance at 0° C. of 12 thermistors which were cooled to −78·6° C. at t = 0 days. The offset at 766 days was caused by a measurement at −30° C. made at t = 715 days following a long period at temperatures not lower than 0° C. Note that the vertical scale in this figure is 25 times that of Fig. 1

(2) Because heat shock offset the calibration in the 'hot' direction it was thought that cold-shock might produce an offset in the 'cold' direction, that is in the direction of increased resistance. Accordingly 12 thermistors were wrapped in paper and placed next to a block of dry ice (− 78·6° C.) for 15 min., then unwrapped and placed in contact with the dry ice for 1 hr. They were then allowed to return slowly to the temperature of the room and recalibrated. As was anticipated, all were found to have been offset to a higher resistance when rechecked in the ice-bath. The effect was, however, much more severe than in the case of the heated thermistors, the mean resistance of the 12 cooled thermistors at 0° C. having increased 5,754 ohms (Fig. 4). Of the 12 thermistors tested two were not greatly affected. The remaining 10, however, were seriously disturbed, one thermistor, No. 3,538, having its resistance increased nearly 500 per cent. The corresponding offsets in the apparent temperature of the ice-bath ranged from − 0·10° to − 29·73° C., with a mean offset for all 12 thermistors of − 18·91° C. This may be compared with a mean offset of only + 0·20° C. both for the 12 heated thermistors and for the 56 thermistors vulcanized in cables 199 and 200.

Following this initial 'cold-shock' offset the resistances of the 'frozen' thermistors did not, however, return towards their pre-shock values. Instead they too continued to increase exponentially at a gradually diminishing rate which, however, exceeded that of either the normal or the heat-shocked thermistors, as seen in Fig. 4. It may also be noted that the 'frozen' thermistors tended to become somewhat erratic, so that it was difficult at times to obtain a satisfactory bridge balance.

Because of the greatly increased resistance, as well as the erratic character, of some of the readings it was thought possible that the rather rapid chilling might have caused actual physical damage to the 'frozen' thermistors. A careful examination of all 12 thermistors was made under a microscope at magnifications up to × 100. Three of the twelve showed very fine, hair-line cracks in the side of the disk extending for short distances more or less parallel to the faces of the disk. These three were the most seriously disturbed of the 12, and thermistor No. 3,858, the most seriously disturbed of the three, was also the one with the most pronounced crack. The remaining 9 thermistors showed no evidence of any physical damage. This does not, of course, preclude the possibility of internal strains or lattice dislocations not externally visible.

(3) The thermistor drift after the initial thermal-shock offset, in all 80 thermistors investigated, was always an increase in resistance with time, although at a gradually diminishing rate of change, whether the thermal shock was due to heat or cold, and whether the initial shock-offset was plus or minus.

(4) Evidence from arctic measurements had indicated that thermistors permanently installed at points of constant temperature had a much smaller calibration drift than those exposed to variable temperatures. Accordingly a thermistor, No. 3,538, which had been subjected to more thermal shocks than usual, was placed in an ice-bath and kept continuously at 0° for 2·6 years. During the 269 days prior to this it had been vulcanized at 125° C., calibrated three times in a hypsometer at 100° C., and used for temperature measurements in a drill hole at 95° C. It would therefore have been expected to be more disturbed than the usual thermistor and subject to a faster rate of drift. Despite these facts, as seen (Fig. 5), its drift at a constant temperature of 0° C. was only 0·4 that of the normal vulcanized thermistor exposed to a variable temperature.

That this was not due to a naturally low drift-rate is shown by the two ice-bath determinations at − 47 and − 5 days in Fig. 5. The last previous heat shock was at − 242 days. Despite recovery periods, therefore, of 195 and 237 days respectively, these readings show a rate of change slightly greater than the mean after that recovery time for the 56 thermistors in cables 199 and 200.

(5) Because of the characteristic drift of the thermistors investigated it was found possible to use the drift curve to make corrections to an accuracy of the order of one or two hundredths of a degree Celsius over a 2·6-year period within which the calibration changed as much as 0·6° C.

In the light of these facts, it is evident that thermistors should be protected from large and sudden changes in temperature, whether hot or cold. Serious cold-shock is especially to be avoided. Room-temperature vulcanization is possible with special rubber compounds and accelerators, as well as with certain plastics. Pressure-sealing tapes may be used where they give adequate protection against leakage or the entry of water into the cable.

Calibrations should be made at the approximate temperature to be measured and should be restricted

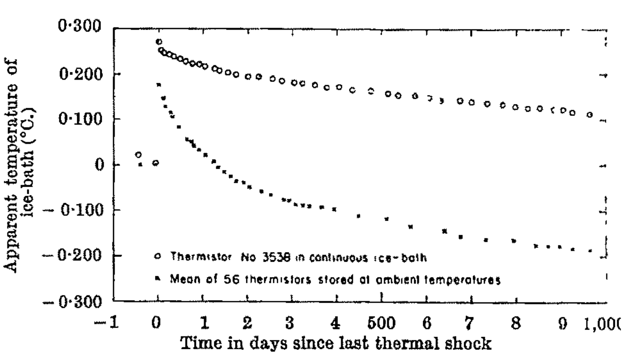

Fig. 5. Changes in the apparent temperature of the ice-bath with time (after hypsometer test at 100° C.) as measured by thermistor No. 3538 kept continuously at 0° C., compared with those measured by 56 thermistors vulcanized in cables 199 and 200 and stored at ambient temperatures between ice-point measurements

to as narrow a range on either side of that value as is feasible.

Thermistors which have been subjected to thermal shock should be allowed a sufficient subsequent rest period to allow their return to a normal drift-rate. They should be recalibrated just prior to, or just after, subsequent measurements, or both, depending on the precision desired and the length of the measurement period involved. If possible the thermistors used should be kept continuously at the temperature being measured for the entire measurement period, especially during a long-continued measurement series, in order to reduce thermistor drift to a minimum. With these precautions drift can be reduced to very small values. These values may often be still further reduced by properly applied corrections.

Publication of this investigation is authorized by the Director of the U.S. Geological Survey.

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TWILIGHT AND THE 'CREP' UNIT

By Dr. ERIK TETENS NIELSEN

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FOR a very long time many kinds of animals have been known to be most active around sunset. A relationship between the changing light intensity and activity is now usually presumed, and it has been shown[1] that the duration of the swarming of mosquitoes depends on the duration of twilight.

Civil twilight is the period from sunset to the time when darkness compels man to suspend labour in the open air. Similarly, the beginning of dawn in the morning is the moment when the light becomes sufficient for the resumption of normal human activity. In this article the discussion will be confined to conditions at sunset with the tacit understanding that corresponding conditions prevail at sunrise.

Even if the end of civil twilight is rather loosely defined it has long been known[2] that several natural phenomena occur at the very end of man's day: (1) stars and planets of the first magnitude are just becoming visible; (2) the purple light above the sunset disappears; (3) the anticrepuscular arch passes zenith (this is the borderline between the reddish tinged and the ashy or deep blue part of the sky); (4) the centre of the Sun is about 6° below the horizon. The last point permits an unequivocal definition of the end of twilight: it is the moment when the Sun's altitude is − 6° 00′.

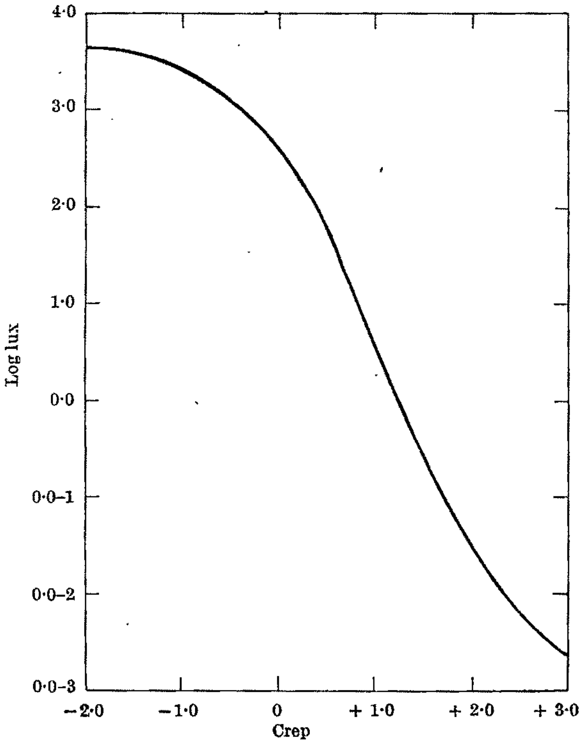

Fig. 1

The definition of the beginning of twilight depends on the various definitions of sunset. In most of the tables now in use[3–5] sunset is defined as the moment when the uppermost part of the Sun appears to coincide with the horizon. Due to the refraction of the atmosphere the Sun will at that moment be about 34′ below the horizon, and as the half diameter of the disk of the Sun is about 16′, the Sun's altitude at the moment of sunset is actually about − 50′. The refraction changes with the atmospheric conditions, and the sunset may be observed to occur one or even a couple of minutes in variance with the moment to be expected from astronomical tables. The duration of civil twilight given in such tables is the number of minutes required for the position of the Sun to change from − 50′ to − 6° 00′. The duration varies according to geographical latitude and season from 21 min. to several hours.

The effect of twilight on the habits of animals is caused by the change in illumination. The unit of illumination is the lux. It is convenient, and, according to Weber–Fechner's law, also the most correct, to operate with the logarithms of lux. The illumination is best measured as the diffuse light from the sky incidental to a horizontal plane. If the sky is clear the illumination depends almost exclusively on the altitude of the Sun. At sunset it is thus 2·60 log lux (400 lux), and at the end of twilight it is 0·55 log lux (3·5 lux). Biological phenomena depending on the illumination should therefore be related to the Sun's altitude rather than to the hour angle. Instead of using hours and minutes to describe the time-sequence of such habits of animals it is more convenient and logical to use a time unit based on the duration of twilight, especially when observations made at different latitudes and in different seasons have to be compared. In work now in progress on the comparative ethology of swarming of mosquitoes it has been found very useful to transform all time indications into such twilight values, called 'crep' (from *crepusculum*). One crep is defined as the number of minutes which, under the given conditions, correspond to the duration of twilight. Hours and minutes are changed to crep values by means of the expression:

$$\frac{\text{time of day} - \text{time of sunset}}{\text{duration of twilight}}$$

and correspondingly:

$$\frac{\text{time of sunrise} - \text{time of day}}{\text{duration of twilight}}$$

If sunset is at 18 h. 00′, and the twilight lasts 30 min., the hour 19 h. 00′ will be + 2·00 crep, and 17 h. 15′ will be − 1·50 crep. The values of crep are positive when they refer to the period during which the Sun is below the horizon, negative for periods after sunrise to before sunset. Sunrise and sunset have the crep value 0, while the end of twilight in the evening and the beginning of dawn have the values + 1·00 crep.

When the sky is clear a certain illumination corresponds to any given value of crep. Using the data of Kimball[2], Provost[6] and my own measurements from Florida, Iraq and Denmark, a table of corresponding values has been prepared; it will be published later[7], and is represented here as Fig. 1.

White clouds reflecting sunlight may give higher illuminations than those of the table, and lower values may be found when the sky is covered by dark clouds. Especially after sunset the differences are usually small compared with the rapid change of illumination. During a recent field investigation in Denmark the sunset illumination (2·60 log lux) occurred at the calculated minute on 21 out of 49 evenings; on 22 evenings this illumination occurred at an average of 0·1 crep (3½–6 min.) before sunset. On 6 evenings it became dark so quickly that the illumination was reduced to 2·60 log lux at 0·20–0·84 crep before sunset.

Fig. 1 shows that at least the middle part of the curve approximates a symmetrical S-shape with the turning point at + 1·00 crep. We have thus one more characteristic of the end of twilight: it is the moment when the illumination changes from an accelerating rate of change to a decelerating rate.

At places where animals are resting the illumination is often lower than in the open; however, there is usually a simple relationship between the illumination at such places and that measured in the open.

The calibration of light-meters often meets with difficulty because the lux is not defined purely as a physical unit. It is based on the curve of photonic relative luminous efficiency[8], which is an expression for the relative sensitivity of the human eye to light of different wave-lengths. A light-meter calibrated by means of a standard light source can only be used in the field by means of special filters. It is easier to calibrate the instrument by measuring the illumination in arbitrary units on a few clear evenings, and by means of the crep/(log lux) function to find the relationship between the arbitrary units and the illumination.

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FOOD SELECTION BY SILKWORM LARVÆ, *BOMBYX MORI*

Citral, Linalyl Acetate, Linalol, and Terpinyl Acetate as Attractants of Larvæ

ONE of us (Y. H.)[1] has reported on the mechanism of food selection of silkworm larvæ, and has pointed out with regard to a principle of food selection that silkworms are attracted by three stimulants in mulberry leaves, namely, attractant, biting factor and swallowing factor. The attractant only induces the larvæ to the food and the biting factor raises the action of biting and the third, swallowing factor, helps the larvæ to swallow the food continuously. Agar jelly containing these three stimulants is eaten by the larvæ as from the mulberry itself.

This communication is concerned with the attractiveness of some terpenes for silkworms and the existence of citral, linalyl acetate, linalol, and terpinyl acetate in mulberry leaves.

As already described[1], the ether-soluble fraction of methanol-soluble matter from fresh mulberry leaves attracts silkworms. When a small amount of active carbon is added to this ether fraction the yellowish ether solution is obtained as a result of the absorption of green substances. This yellowish solution powerfully attracts the larvæ (Table 1). The neutral fraction of the ether solution, which is fractionated by washing with a 1 per cent solution of sulphuric acid and a 2 per cent solution of sodium carbonate in this order, and the steam distillate of this neutral fraction are also powerful attractants (Tables 2 and 3). The yield of an active fraction in this distillate is about 0·2 gm. per 4 kgm. fresh leaves. The ultra-violet absorption curve of this distillate resembles that of terpene alcohols, and the infra-red absorption spectra also closely resembles that of terpinyl acetate, and further detection by gas chromatography suggests the existence of citral, linalyl acetate, linalol and terpinyl acetate in this fraction. The attractive force was tested on the pure chemicals, citral, terpinyl acetate and some terpene alcohols (Table 3).

Six small circles along the circumference of a round filter paper are drawn in equal intervals and the paper is set in a shale (diameter: 8·5 cm.). Samples absorbed on cellulose powders are prepared as follows. A certain amount of the solution of the samples is absorbed on 0·25 gm. cellulose powder, and solvent is evaporated in the air, and then water is sprayed on it: on the other hand, the same amount of cellulose powders containing a small amount of water is prepared as control. These cellulose powders are divided into three portions respectively and put on six circles reciprocally. When the test on the difference of activities between *A* and *B* is taken in a set, the powders are put on in the order *A*, *B*, control, *A*, *B*, and control. Thirty hatched larvæ are placed on the centre of the filter paper. After leaving the set at 24° C. for a certain time, the number of larvæ gathered on each powder and wandering on the intermediate

Table 1. NUMBER OF HATCHED SILKWORMS GATHERED ON THE CELLULOSE POWDER

Amounts of sample sprayed on cellulose powder correspond to that in 2 gm. of powdered mulberry leaves

	30 min.	75 min.	120 min.
Ether solution	15	23	23
Hexenol	8	6	6
Control	0	0	1
Intermediate space	7	1	0
Total	30	30	30

Table 2

	30 min.	75 min.	120 min.
Neutral fraction	17	21	22
Acidic fraction	2	3	3
Basic fraction	0	0	0
Intermediate space	11	6	5
Total	30	30	30

Table 3

	10 min.	30 min.	60 min.
Steam distillate	13	23	25
Non-distillable fraction	7	3	1
Control	5	1	0
Intermediate space	5	3	4
Total	30	30	30

Table 4

Conc. of substrate	1/5			1/2			1			3			5		
Position (substrate)	*S*	*C*	*I*	*S*	*C*	*I*	*S*	*C*	*I*	*S*	*C*	*I*	*S*	*C*	*I*
Terpinyl acetate*	10	6	14	9	3	18	13	6	11	10	8	12	*16*	10	4
Linalyl acetate*	19	7	4	*25*	1	4	17	12	1	17	11	2	19	3	8
Linalol*	14	4	12	11	2	17	20	0	10	*22*	2	6	21	8	1
Citral*	*20*	7	3	19	6	5	13	13	4	6	13	11	10	15	5
Terpineol							2	15	13						
Geraniol							0	12	18						
Geranyl acetate							3	15	12						
Oxycitronellal							0	17	13						
Hexenol*	11	2	17	12	3	15	13	4	13	11	2	17	6	8	16

Concentration 1 means that 1 c.c. of 10 mgm. per cent ethereal solution of substances was absorbed by 0·25 gm. cellulose powder and the powder was divided into three portions as described above, 1/2 means 1/2 c.c. of the solution and so on. *S*, *C* and *I* mean sample, control and intermediate space, respectively. Numbers of the larvæ are counted after 1 hr. at 24° C. Substances marked with an asterisk are those presumed to exist in mulberry leaves by gas-chromatographic techniques ; others do not exist in the leaves.

space are counted. The results of the test are given in Tables 1–4.

Thus, from these experiments, citral, linalyl acetate, and linalol, which are seen to exist in mulberry leaves, are very attractive to silkworm larvæ, and terpinyl acetate and hexenol[2] are less attractive. Other terpenes shown in Table 4 which have not been detected in mulberry leaves do not attract. These terpenes attract silkworm larvæ but do not stimulate biting.

We wish to thank Dr. Teruhiko Hosoi for his advice and Dr. H. Okamura of the Eikodo Co., and Dr. M. Ono of the Institute of Chemistry, Kyoto University, for the chemically pure perfumes and β-γ-hexenol, respectively.

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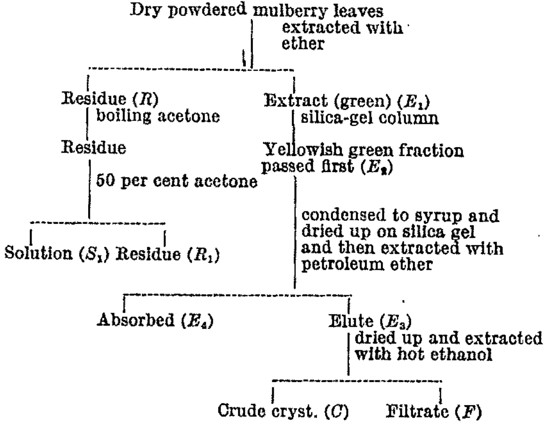
β-Sitosterol as one of the Biting Factors

DRY powdered mulberry leaves were extracted with ether and the extract was fractionated as in Scheme 1.

E_1 markedly attracts and stimulates the biting action of silkworm larvæ as described[1]; E_2 and E_3 are also positive in both respects. Crude crystal (*C*) from E_3 strongly stimulates biting but is not an attractant. On the contrary, filtrate (*F*) scarcely stimulates biting but is a strong attractant (Table 1). Therefore, it is possible to distinguish the biting factor from the attractant in the mechanism of food selection by silkworms.

Scheme 1



The crude crystal (*C*) from E_3 shows a strong Liebermann's sterol reaction. Thus the active principle to stimulate the biting action seems to be a sterol-like substance. Two of us (Y. H. and K. N.)[2] isolated β-sitosterol in a chemically pure state from mulberry leaves. As Table 2 shows, β-sitosterol showed strong activity on the biting test on agar thread which was softened with a small amount of water and sprayed with an ethereal solution of β-sitosterol. Other sterols, cholesterol and ergosterol, gave little activities on this test (Table 2).

Hobson reported[3] that a fat-soluble accessory growth factor required by all insects is cholesterol ; Fraenkel and Blewett have stated that sitosterol is utilized in place of cholesterol by phytophagous insects. But we have found that β-sitosterol is required as a biting factor in the mechanism of their food selection, since β-sitosterol, not only on agar thread but also on glass, stimulates biting, and although cholesterol and ergosterol probably have higher nutrition value than β-sitosterol, they give lower activities on the tests of both biting and swallowing actions as shown in Tables 2 and 3.

In Table 3, a set of test dishes requires a much larger amount of β-sitosterol (20 mgm.) in comparison with the sterol content in mulberry leaves (about 0·2 per

Table 1

	Test of attraction No. of larvæ gathered on. Total larvæ, 30				Test of biting action
	I	II	III	IV	
E_2	28				+ + + + +
E_3		28			+ + + + +
C			4		+ + + + +
F				28	+ − − − −
Control	0	0	12	0	− − − − −
Intermediate	2	2	14	2	
Total	30	30	30	30	

Test of attraction was done by the method described in the preceding communication. In the test of biting action, + + + + + denotes that all larvæ bite the agar thread and − − − − − denotes none of larvæ does.

Table 2

	Test of biting action
β-Sitosterol (conc.)	+ + + + +
β-Sitosterol (dil.)	+ + + + −
Cholesterol (conc.)	+ + + − −
Ergosterol (conc.)	+ + − − −
Control	− − − − −

Table 3

	Test of swallowing (number of fæces)
Basal diet (B.d.) only	26
B.d. + leaf powder (0·3 gm.)	262
B.d. + β-sitosterol (20 mgm.)	223
B.d. + β-sitosterol (4 mgm.)	116
B.d. + β-sitosterol (20 mgm.) (R_1 absent)	47
B.d. + cholesterol (20 mgm.)	16
B.d. + ergosterol (20 mgm.)	44

cent). Doubts may be expressed concerning the relation between the concentration and the activity unless it is supposed that there is an accumulation of β-sitosterol on the surface of mulberry leaves. It has been proved that when the leaves are wiped with an absorbent cotton containing a small amount of ether, the cotton strongly stimulates biting, and as a matter of course indicates a positive reaction for Liebermann's sterol test.

The swallowing test by newly hatched larvæ was made using pure β-sitosterol in place of the crude one (E_1 or E_2) by the modified method already described[1]. One basal diet consists of the following: 3 c.c. 2 per cent agar solution containing 1 per cent sucrose, 0·2 gm. cellulose powder and 0·3 gm. of R_1 which seems to have a swallowing factor (diameter of a Petri dish: 4·5 cm.). Ten newly hatched larvæ were put on an agar diet for 18 hr. at 24° C. and the fæces were counted (Table 3).

From Table 2 it is concluded that β-sitosterol is the biting factor and some sterols may be an accessory in mulberry leaves. From Table 3 it is clear that the larvæ require an unknown swallowing factor together with the biting factor, β-sitosterol, for their complete food taking, and the amount of food eaten is controlled by the concentration of β-sitosterol.

Further experiments suggested the existence of another water-soluble biting factor in the S_1 fraction.

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CYCLICAL FEEDING-ACTIVITY IN COLONIZED MOSQUITOES

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EXTENSIVE field-studies on African mosquitoes made over the past 18 years by Haddow and others[1] have demonstrated the cyclical nature of biting activity. These investigations show that a species usually exhibits a characteristic biting pattern, although this is not always constant throughout its range; they do not, however, show to what extent such patterns are a direct response to environmental stimuli, or to what extent they represent an expression of inborn rhythms. Such work has been based on the catching, and removal from the population, of female mosquitoes that alight on the bait, the mosquitoes so removed in unit time being sorted and counted at leisure. Clearly such a method depends on the presence of what, for practical purposes, can be regarded as an infinite population of mosquitoes, and this automatically precludes its use if biting activity is to be studied under controlled laboratory conditions. Yet it is precisely this approach that is now urgently needed[2]. The problem can, however, be studied in the laboratory if, instead of counting the number of mosquitoes that alight on the bait, we measure the amount of food they remove from it. The present article describes an experiment to test this method, using sugar solution as a source of food.

An apparatus has been devised which provides a continuous supply of food from a graduated tube. The apparatus was developed independently from, and it differs in principle from, the feeding apparatus described by Rodhain *et al.*[3], which allowed tsetse flies, *Glossina*, to suck blood through a mouse- or rat-skin membrane. They used an open graduated pipette in order to record the hydrostatic pressure of the blood, but mentioned that this would also allow measurement of the amount of blood ingested by each fly. The principle of the present apparatus is to record the amount of fluid removed from the bait in a given period of time as a measure of feeding activity. To do this it was essential that the apparatus be capable of feeding a large number of insects over a continuous period of several days. Changes in attractiveness were reduced by the use of perforated polyethylene or of fine-mesh metal gauze in place of animal skin. The alternative choice of metal gauze as a feeding surface was partly influenced by the description of an ingenious feeding device given by Dr. P. A. Woke at the eleventh International Congress of Entomology held in Vienna during August 1960.

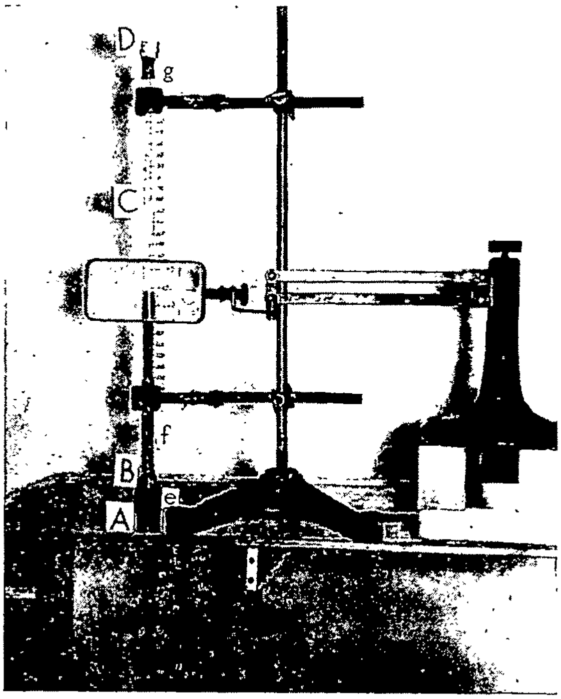

Fig. 1. Apparatus for recording the amount of fluid removed from the bait as a measure of cyclical activity in fluid-feeding insects

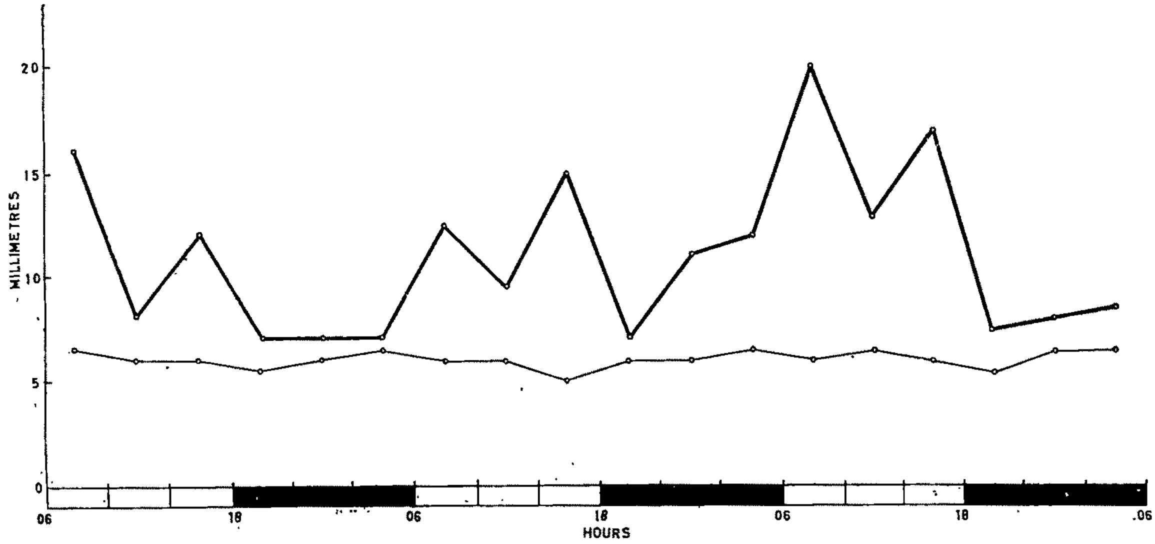

Fig. 2. Sugar feeding-cycle in the mosquito *A. aegypti*. Thick line: loss of fluid each 4 hr. through feeding plus evaporation (test series); thin line: loss of fluid each 4 hr. through evaporation only (control series). Light switched on 06–18 hr. (East African Standard Time) each day

The apparatus (Fig. 1) as used here consists of a wide feeding-tube, *A*, closed at the lower end by a 0·5-mm. mesh, non-corrosive, non-absorbent perforated cap, and connected above by a tapering middle-piece, *B*, to a narrow, transparent measuring-tube, *C*, graduated in mm. (7·0-mm. internal diameter and 300 mm. in length). These three parts are joined together by two pieces of flexible tubing (*e* and *f*), the latter being long enough (about 60 mm.) to allow flexure through 90° without occlusion. The open upper end of the measuring tube is attached to a short piece of flexible tubing, *g*, which can be closed by means of a screw-clip, *D*.

To prepare the apparatus for use, screw-clip *D* is left open, and the whole apparatus is bent at *f* to form a U-tube. Feeding-fluid is introduced, until the whole apparatus is nearly full. The two limbs of the U-tube are then adjusted until the fluid reaches the perforated end of the feeding-tube in one limb, and nearly to the top of the measuring-tube in the other. Screw-clip *D* is then closed and the apparatus is straightened out to a vertical position, with the feeding-tube at the lower end. It is placed on the gauze top of a cage containing the mosquitoes, the perforated end of the feeding-tube being in direct contact with the gauze top of the cage.

Fluid will be lost from the apparatus by two means: evaporation and mosquito-feeding. Allowance for the amount of fluid lost through evaporation can be made by using a duplicate apparatus over an empty cage as a control; the difference in loss of feeding-fluid in the two tubes can then be taken as a measure of feeding activity in a given time. With the dimensions given above, a drop of 0·5 mm. on the scale records a volume of $19 \cdot 24 \times 10^{-6}$ litre (19·24 µl.). For practical purposes this can be taken as roughly equivalent to five female-mosquito meals[4]. The estimate would, of course, vary according to species and strain used and will probably be different if both sexes were feeding on the fluid.

Two series of observations were run concurrently: a control series using the apparatus with 10 per cent w/v sugar solution (5 per cent glucose, 5 per cent sucrose) over an empty cage, and a test series using a similar arrangement over a cage of mosquitoes. These were *Aedes* (*Stegomyia*) *aegypti* (L.) from Ilobi, Nigeria, and had been colonized in Entebbe for two years. Until the day before the experiment was started, the mosquitoes had had access to raisins (continuously) and to blood (infant mice for 1 hr. a day). Throughout the experiment an egg-laying funnel was present[5]. The experiment was carried out under controlled conditions of temperature (28° C.) and humidity (70 per cent relative humidity) with an artificial light regimen of 12 hr. light each 24 hr. Observations were made every 4 hr. for 3 consecutive days and nights.

Table 1. Feeding-cycle in the Mosquito *A. aegypti* under Conditions of 12 hr. Light each 24 hr., expressed as the Amount of Fluid removed from the Bait each 4 hr.

Date	Time (E.A.S.T.)	Fluid loss in mm.		
		(*a*) Test	(*b*) Control	(*a*-*b*) Difference
30.10.60	0600			
		16·0	6·5	9·5
	1000			
		8·0	6·0	2·0
	1400			
		12·0	6·0	6·0
	1800			
		7·0	5·5	1·5
	2200			
		7·0	6·0	1·0
31.10.60	0200			
		7·0	6·5	0·5
	0600			
		12·5	6·0	6·5
	1000			
		9·5	6·0	3·5
	1400			
		15·0	5·0	10·0
	1800			
		7·0	6·0	1·0
	2200			
		11·0	6·0	5·0
1.11.60	0200			
		12·0	6·5	5·5
	0600			
		20·0	6·0	14·0
	1000			
		13·0	6·5	6·5
	1400			
		17·0	6·0	11·0
	1800			
		7·5	5·5	2·0
	2200			
		8·0	6·5	1·5
2.11.60	0200			
		8·5	6·5	2·0
	0600			

The results are shown in Table 1 and in Fig. 2. The difference between the two series is very marked: whereas in the control the rate of loss of fluid is almost steady at 6 mm. each 4 hr., in the test series the rate fluctuates, showing two peaks in each light-'stretch', one in the first 4 hr. light-'period', another in the last (the terms 'stretch' and 'period' have been defined previously for use in this sort of work[6], and are used in the same sense here).

During the final 12 hr. light-stretch, observations were made hourly, in order to obtain a preliminary analysis of the two peaks. The results for this single 12-hr. stretch indicate that the two peaks are different in form—that in the first 4-hr. light-period being confined to the single hour following onset of light, that in the last 4-hr. light period being less concentrated.

It is not claimed that the feeding-cycle obtained in the present experiment represents the definitive cycle for the species, or even for the strain used. This particular cage of mosquitoes had previously been supplied with a blood-meal at a set time each day, and an acquired blood-feeding rhythm may have influenced the sugar-feeding rhythm. Moreover, it is not known whether both sexes contributed equally to the two diurnal peaks of feeding activity. The results do, however, indicate that, with this apparatus as a tool, or with other similar apparatus employing the same principle, the way is now open for a detailed study of feeding-cycles along the lines already used in the study of mosquito oviposition-cycles[2,5,6]. It is planned to use the apparatus first with sugar solution or fruit juice, using male and female mosquitoes both separately and together, and later with vertebrate blood. Initially, observations will be made on an hourly basis, but when the cycles have been 'established', a study of the mechanisms involved may then be made on a 4-hr. basis. Improvements and modifications can doubtless be made on the prototype described here; the apparatus is, for example, particularly suitable for use with photographic recording.

Certain inherent difficulties have to be overcome before this method can be adapted for the study of blood-feeding cycles. While clotting can easily be prevented by one of the standard methods, sedimentation of the cells, or denaturing and precipitation of the blood proteins, presents a more difficult problem. Sedimentation can be retarded considerably by agitation, and in preliminary trials this has been achieved by convection set up by a heat exchanger coil wrapped around the feeding-tube. If laked blood is employed, it is necessary to use perforated polyethylene or to coat the metal gauze of the feeding-tube with a fine film of paraffin wax. The most promising results so far have come from the use of laked blood with a heat-exchanger coil. Details about dilutions and preservation will be given in a later communication.

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LEAKAGE CURRENT RECTIFICATION IN THE SQUID GIANT AXON

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THE total current which flows across the membrane of the giant axon of the squid in response to stepwise changes in membrane potential in a voltage-clamp arrangement was shown[1] to consist mainly of sodium and potassium ions plus a small unidentified component, termed 'leakage'. This component was approximated by an equivalent conductance and apparent equilibrium potential, both constant with time and membrane potential.

We intended, in this work, to test these approximations and to determine, if possible, what ions might be involved in the leakage.

We used the standard, point-control voltage-clamp arrangement[2] and attempted to eliminate the currents due to sodium and potassium by means of the following procedure: (1) the membrane potential was controlled at a value close to the resting potential, during periods of measurement; (2) at intervals of about 1 sec. a 55 mV. hyperpolarizing pre-pulse of 7 msec. duration was followed immediately by the test pulse, either to a further hyperpolarized value or to the sodium equilibrium potential; (3) the membrane current at 0·4 msec. following the application of the test pulse was measured and taken to be the leakage current; (4) the sodium equilibrium potential was varied by substitution of choline ions for sodium ions in the external medium; (5) temperature was maintained at 10 ± 1° C.

For hyperpolarizing pulses sodium and potassium contributions to the leakage current are expected to be negligible, because the total current does not depend on the external sodium or potassium concentration (ref. 1 and this article). At the sodium equilibrium potential there is no net sodium current. If the depolarizing steps follow a hyperpolarizing pre-pulse, the onset of the potassium current is delayed[3] to such an extent that a negligible potassium current is expected during the first half millisecond.

We must distinguish between components of the total current, which are carried by sodium and potassium ions and are subject to the delayed rectification processes, and the possibility of an additional rectification with a very short delay. For depolarizations, a short time-constant increase in the potassium conductance might occur and not be observed as such.

In Fig. 1*A*, the leakage current is plotted as a function of membrane potential, for three typical

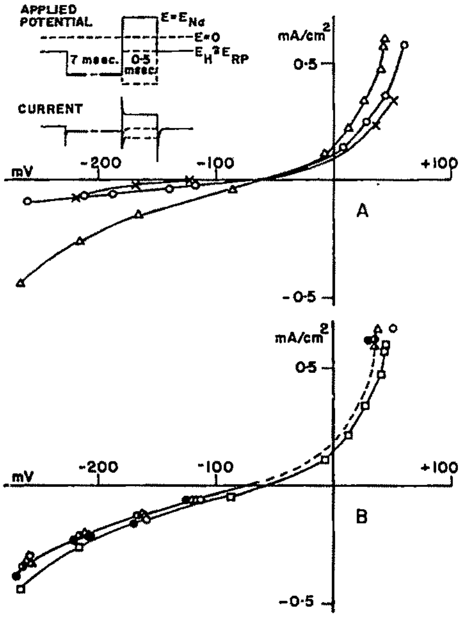

Fig. 1. *A*, leakage current versus membrane potential for three axons in varying concentrations of external sodium ion. Currents plotted were measured as indicated in the insert. E_H, holding potential just before the pre-pulse; E_{RP}, resting membrane potential; E_{Na}, sodium equilibrium potential. Zero current was taken as the current at 0·4 msec. after the application of test-pulse to the holding potential. Temperature, 10° C. ×, axon 60–47 with E_{RP} 67 mV.; ○, axon 60–61 with E_{RP} 66 mV.; △, axon 60–60 with E_{RP} 54 mV. *B*, leakage current versus membrane potential with 21 and 61 per cent of the normal external chloride. Curve for axon 60–60 replotted from Fig. 1 for reference. Temperature, 10° C. ○, artificial sea-water 1; ▲, 61 per cent chloride; ◑, artificial sea-water 2; △, 21 per cent chloride; ●, artificial sea-water 3; □, axon 60–60

axons. The insert in Fig. 1 indicates the method of measurement. The leakage current is not constant but exhibits marked rectification. The time-constant of delay was not obtainable with our system but is probably less than 100 µsec. In the hyperpolarized (more negative than the resting potential) region the curves showed no change on varying external sodium concentration from 430 to 43 m*M*/litre. The points associated with depolarizations to the sodium equilibrium potentials fall along a smooth curve. Axon 60–60 is typical of axons exhibiting low membrane resting and action potentials.

In an effort to identify the leakage-current charge-carriers we altered external chloride, potassium, calcium and magnesium. External chloride was altered by substitution with methyl sulphate.

Fig. 1*B* illustrates the results of measurements on axon 60–69 with one-half, and all, of the sodium chloride replaced by sodium methyl sulphate, giving a concentration of chloride of 61 and 21 per cent of the normal. The points in the depolarized region were obtained at the sodium equilibrium potential in normal sodium. For hyperpolarization there is clearly only negligible effect of chloride reduction. It is likely that this is true for the entire curve. The leakage curve for axon 60–60 is replotted from Fig. 1 for reference as being similar to axon 60–69. The steepness of the curve near the normal sodium equilibrium potential reduces sensitivity, but it would seem that here not more than 10 per cent of the leakage is accounted for by chloride ions.

In Fig. 2 the resting potential (*A*), and leakage current for a hyperpolarizing pulse to − 180 mV. following the standard pre-pulse (*B*), are plotted against time, while the external potassium, chloride and calcium and magnesium concentrations were varied. Since the resting potential, and probably the holding current, varied with time, there was some difficulty in comparing complete leakage curves. During periods of measurement, the membrane potential was held at a constant value regardless of the resting potential, introducing an uncertainty in the baseline to which leakage current is referred. However, the currents at a pulse potential of − 180 mV., measured in the usual way, are plotted as the circles in Fig. 2, giving the smooth curve through the four control values obtained in artificial sea-water. The current at 0·4 msec. following the pre-pulse relative to the current at the end of the pre-pulse is also plotted in Fig. 2*B* (triangles). For both cases the points obtained in 1/3 potassium and 1/5 chloride do not deviate from the line through the artificial sea-water points, while the points for 1/10 calcium and magnesium do. It is well known that lowering external calcium concentration tends to increase resting membrane conductance. It is important to distinguish, however, that here these currents do not significantly involve sodium or potassium. Further experiments are required to determine whether or not the increased inward current in low divalent ions is due to chloride.

For outward current 1/3 external potassium did not alter the leakage while low external divalent ions increased it. However, a fast time-constant potassium component might not be sensitive to external potassium changes.

Our conclusions are: (1) the leakage current, as defined, cannot be represented as a constant conductance and an apparent, constant, equilibrium potential; (2) the leakage current curve is not symmetrical about the resting (or zero) potential ; (3) the time-constant of the leakage rectification is shorter than 100 µsec.; (4) only a small fraction of the leakage current can be accounted for by the inward movement of potassium, chloride or sodium ions;

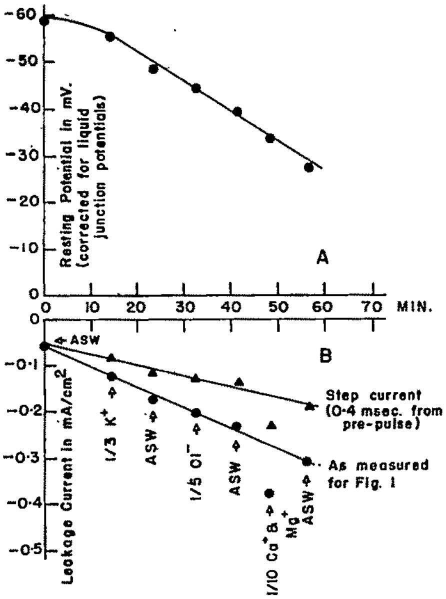

Fig. 2. *A*, Resting membrane potential versus time. *B*, Leakage current at −180 mV. (lower curve) and this current minus that at the end of the pre-pulse (upper curve) for variations in external potassium (1/3 *N*), external chloride (1/5 *N*) and external calcium and magnesium (1/10 *N*). Temperature, 10°C.

(5) lowering external divalent ions increases the leakage; (6) the chloride conductance of the resting membrane is probably negligible; and (7) it is necessary to suppose that the leakage is mainly the outward movement of so far unidentified internal ions—cations for outward and anions for inward current components. If the properties of the axon membrane were altered so that a large fraction of membrane current had properties of the leakage current discussed here, empirical utilization of conductances and equilibrium potentials, which has been so successful for the normal squid axon, might prove impracticable.

We wish to thank Dr. K. S. Cole, Dr. U. Kishimoto, Dr. W. K. Chandler and Mr. L. Binstock for helping us.

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REPLACEMENT OF THE PROTOPLASM OF A GIANT NERVE FIBRE WITH ARTIFICIAL SOLUTIONS

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SINCE the action potential of a nerve fibre almost certainly arises at the surface membrane, it should, in principle, be possible to replace the axoplasm by an aqueous solution of appropriate composition. The difficulty in carrying out this experiment is that the axoplasm of squid nerve fibres is a gel and is not easily washed out of an intact fibre. The method described here depends on the observation that the giant nerve fibres of *Loligo forbesi* retain their ability to propagate action potentials after the axoplasm has been squeezed out and replaced either with natural axoplasm or with an artificial solution[1]. Fig. 1 illustrates the replacement with natural axoplasm. Record *A* shows the action potential recorded with external electrodes from the central half of an intact axon. In *B*, the axoplasm from the central half had been extruded; the action potential was still present, but as a result of the increase in the internal electrical resistance, it was propagated more slowly and the component recorded with external electrodes was greatly reduced. On moving axoplasm from the distal to the central part of the fibre, the external action potential was restored to its original amplitude (*C*) and was still the same size 2 hr. later (*D*). Such experiments showed that the excitable properties of the membrane are not destroyed by extrusion and encouraged us to attempt the replacement of the axoplasm with artificial solutions.

The procedure for perfusing an axon consisted in: (1) dissecting, but not cleaning, 6–10 cm. of a fibre 500–900μ in diameter; (2) cannulating the axon at its distal end; (3) squeezing out axoplasm[2] by stroking the fibre in a series of sweeps, the first sweep starting at a distance of 2 cm. from the cut end, the second at 4 cm. and so on; (4) re-inflating the fibre at a steady rate by driving in an appropriate perfusion fluid at about 6 μl./min. After re-inflation, the electrical properties of the fibre were studied with external electrodes or, in the later experiments, with an internal electrode, using the method illustrated in Fig. 2.

For the normal perfusion fluid, isotonic solutions containing salts of potassium were employed: for example, isethionate (which is present in high concentrations in the axoplasm of squid nerve[3]), sulphate, methylsulphate or chloride. As a rule the solutions contained 30 m*M* phosphate buffer, normally at *p*H 7·7, and the tonicity was adjusted after the vapour pressure had been compared with that of the sea-water used as an external medium. Short-term experiments indicated that the action potential was little changed over a range of tonicity of ± 10 per cent and a range in *p*H of 7–8·5. A hydrostatic pressure of 2–3 cm. of water was required to keep the fibre inflated. If the pressure was released, the axon shrank to about a quarter of its original volume. In the shrunken condition the velocity of conduction was reduced but the action potential recorded with an internal electrode was little changed, as would be expected from the local circuit theory. Hydrostatic pressures of 10–20 cm. of water abolished the action potential if applied for more than a few minutes, but there was often a slow recovery after the pressure had been removed.

Potassium sulphate or isethionate media were used extensively, and with these solutions there was good evidence that the electrical properties of perfused axons were similar to those of intact axons. The action potential (100–110 mV.) and resting potential

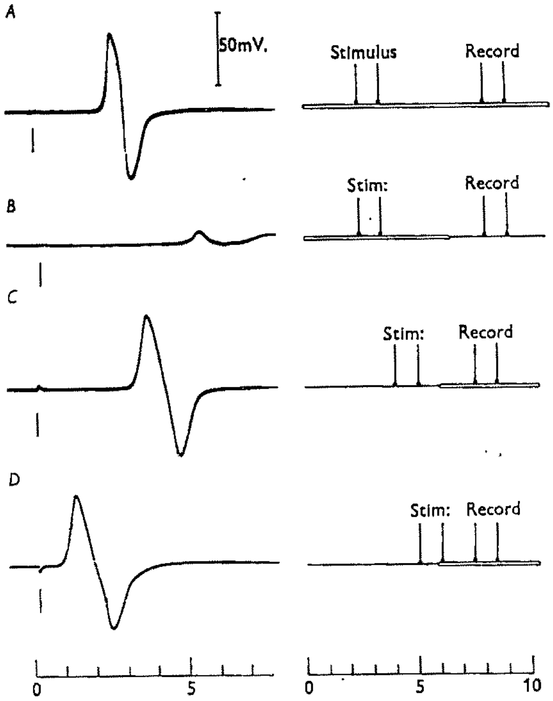

Fig. 1. Restoration of externally recorded action potential by filling 'sheath' with axoplasm. *A*, intact axon; *B*, right-hand half extruded; *C*, right-hand half filled by moving in axoplasm from left-hand half; *D*, as in *C* but 2 hr. later

A diphasic response was often observed at stage *B*, but in this experiment there was a local block between the recording electrodes which is attributed either to minor injury or to irregular flattening during extrusion

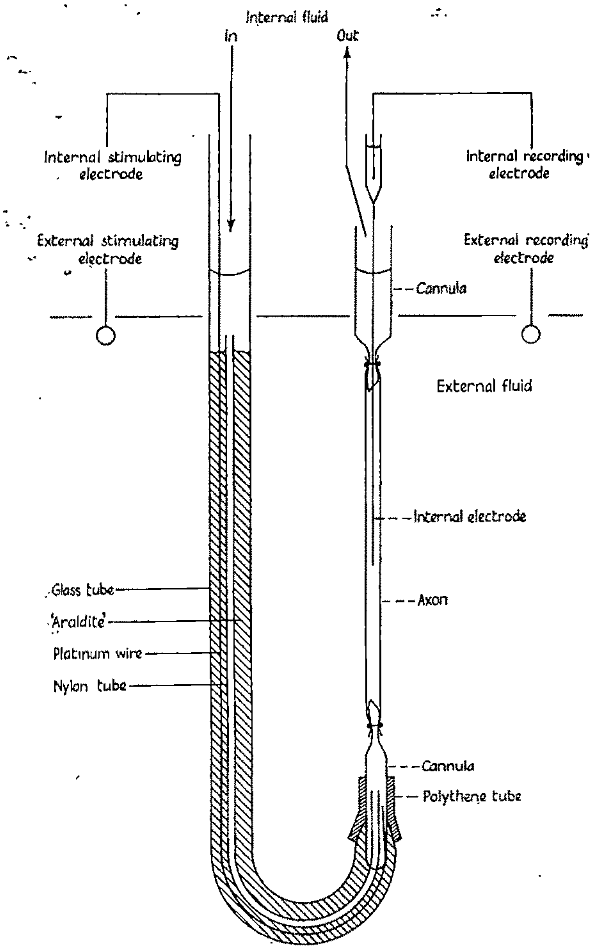

Fig. 2. Method used with internal electrodes; the drawing is not to scale

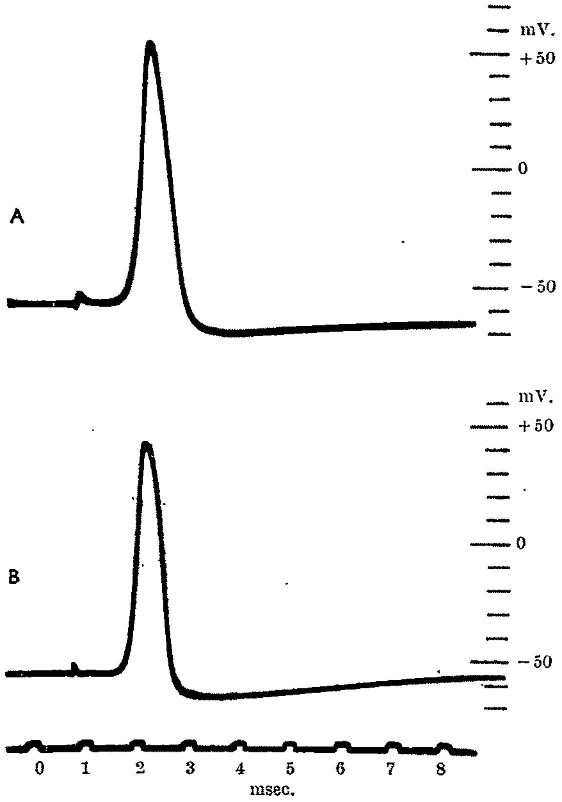

Fig. 3. *A*, action potential recorded with internal electrode from extruded axon filled with isotonic potassium sulphate (16° C.); *B*, action potential of an intact axon, with same amplification and time-scale (18° C.)
The voltage scale gives the potential of the internal electrode relative to its potential in the external solution (no correction for junction potential)

(50–70 mV.) were of the usual magnitude, and, as may be seen from Fig. 3, the time course of the action potential in a perfused axon resembled that in an intact axon. It is possible that prolonged perfusion may have some harmful effect, but a volume of 3–4 ml., which is more than one hundred times that of the fibre, could be perfused without making the fibre inexcitable. Axons filled with isotonic potassium solutions remained excitable for 3–5 hr. and were capable of carrying a large number of impulses, for example, 3×10^5 at 16° C.

Evidence that the perfusion fluid had rapid access to the surface membrane was provided by the speed with which the action potential was blocked by changing potassium for sodium ions in the perfusion fluid. With relatively short exposures, the effect of high sodium concentration was completely reversible, and both block and restoration might occur in 10–20 sec., the speed of the effect depending on the rate of flow and on the extent to which the dead space could be reduced.

Alterations in the ionic composition of the internal solution gave reversible changes in membrane potential, which were consistent with the view that the resting potential arises from the difference in concentration of potassium across a membrane which is selectively permeable to this ion[4]. Replacement of isotonic potassium sulphate with isotonic potassium chloride caused only a small decrease in resting potential, but replacement of potassium by sodium ions reduced the resting potential by about 50 mV.

Fig. 4 illustrates the effect on the resting potential of replacing potassium chloride by sodium chloride inside the axon, the external fluid being sea-water with the usual potassium concentration of 10 m*M*.

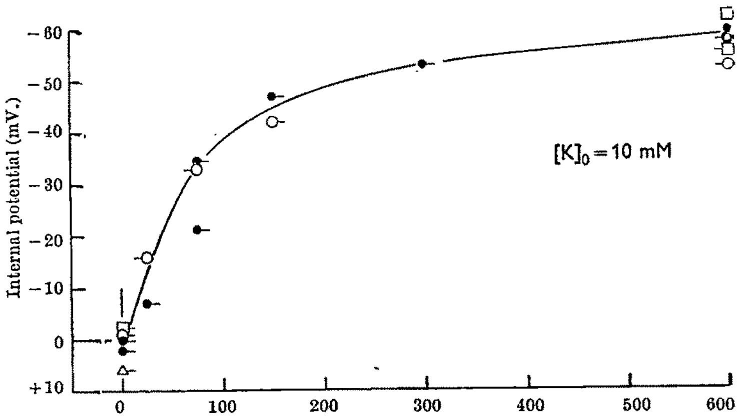

Fig. 4. Effect of internal potassium concentration on resting potential. External solution, sea-water; internal solutions, sodium chloride–potassium chloride solutions isotonic with sea-water

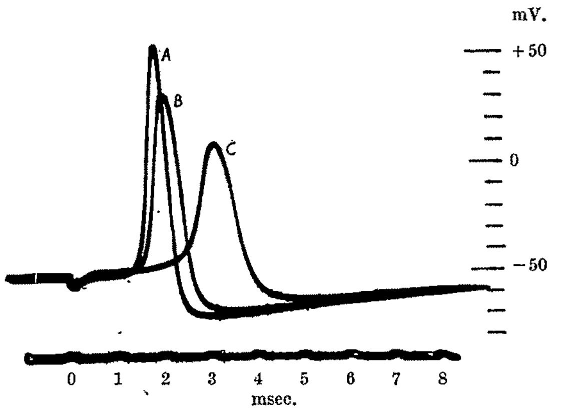

Fig. 5. Effect on action potential of replacing internal potassium with sodium ions. *A*, isotonic potassium sulphate; *B*, one quarter of the potassium ions replaced by sodium ions; *C*, half the potassium ions replaced by sodium ions. The records were taken from a long experiment and were obtained in the order *B*, *A*, *C*

With sodium chloride inside the fibre, the potential was close to zero, but it increased rapidly as potassium replaced sodium and reached a value of 40–50 mV. at an internal potassium concentration of 150 m*M*. A further rise from 150 to 600 m*M* potassium increased the resting potential by only 5–10 mV. as compared with the 35 mV. expected for a potassium electrode. This 'saturation' effect probably occurs because the permeability to potassium falls as the resting potential rises[5]. A similar hypothesis has been advanced to explain why the membrane potential ceases to behave like a potassium electrode at low external concentrations of potassium[6]. With isotonic potassium chloride outside the fibre and isotonic sodium chloride inside, the normal resting potential was reversed, the internal solution being 50–60 mV. positive to the external solution. The resting potential was close to zero if similar solutions were placed on both sides of the membrane.

The effects of varying the internal sodium concentration support the view that the action potential depends on a rise in permeability to sodium[5,7]. With sulphate as the internal anion, replacement of half the potassium by sodium caused little change in resting potential but decreased the action potential by 40–50 m*V*. Fig. 5 illustrates the effect of different concentrations of sodium ions on the action potential. From these and other records it appears that the membrane changes from a resting state, in which the permeability ratio $P_{\mathrm{Na}}/P_{\mathrm{K}}$ is about 0·1, to an active one in which $P_{\mathrm{Na}}/P_{\mathrm{K}}$ is 5–10.

The experiments described here raise the question of the extent to which the axoplasm is removed by extrusion and subsequent perfusion. Histological preparations showed that the volume of axoplasm in a perfused fibre was about 3 per cent of that in an intact fibre. A preliminary study of thin sections with the electron-microscope showed that the axon membrane and Schwann cell layer were present in perfused axons.

The general conclusion is that electrical changes in giant nerve fibres depend on differences of ionic concentration across the surface membrane and that the bulk of the axoplasm is not essential for the conduction of impulses.

We are indebted to Dr. J. S. Alexandrowicz for histological preparations, and to Dr. V. J. Howarth for help with the vapour-pressure measurements. Our thanks are also due to the Nuffield Foundation, the Medical Research Council, the Royal Society and the Wellcome Trust for financial support.

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PARAPHENYLENEDIAMINE OXIDASE ACTIVITY AND COPPER-LEVELS IN MAMMALIAN PLASMAS

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THE function and properties of the blue copper-protein cæruloplasmin have been the subject of much work since it was first isolated and purified from pig and human serum by Holmberg and Laurell[1] in 1948. Cæruloplasmin is an α_2-globulin containing 0·34 per cent of copper corresponding to 8 atoms of copper per molecule. The copper is very firmly bound to the protein, is non-dialysable and does not react with sodium diethyldithiocarbamate unless it has first been liberated by denaturation of the protein. Holmberg and Laurell[2] also showed that cæruloplasmin could oxidize *in vitro* a number of substances, including ascorbic acid, catechol, adrenaline and *p*-phenylenediamine (PPD), the latter being the best substrate. This PPD oxidase activity has been adopted as the basis of a number of methods for the estimation of cæruloplasmin in serum or plasma.

The concentration of cæruloplasmin oxidase activity in the serum has been shown to vary considerably in different clinical conditions in man; it is elevated in certain acute and chronic infections and in pregnancy, while it is usually depressed in Wilson's disease. A close correlation has been demonstrated between copper-levels in plasma and oxidase activity of the plasma of man[3] and rats[4].

Gubler and co-workers[5] have differentiated two fractions of plasma copper on the basis of their reaction with sodium diethyldithiocarbamate; these fractions were designated "direct reacting copper", in which the copper reacts directly with diethyldithiocarbamate, and "indirect reacting copper" in which the copper reacts with it only after acid deproteinization of the plasma. This indirectly reacting fraction of plasma copper, which is generally assumed to correspond to the cæruloplasmin copper, accounts for a different percentage of the total plasma copper in different species. Thus this fraction in man, rat, dog and pig represents 96, 99, 88 and 56 per cent respectively of the total plasma copper[5].

The object of the present work was to determine the copper-levels in plasma in several species of mammals including man, and to find to what extent

Table 1. Comparison of the Copper Content and *p*-Phenylenediamine Oxidase Activity of the Plasma of a Number of Mammalian Species

Species	No. of samples	Total plasma copper (μgm./ml.)	*p*-Phenylenediamine oxidase activity in 0·1 ml. plasma		
			Activity/ 0·1 ml.	Activity/ μgm. plasma total copper	Activity/ μgm. indirect-reacting copper
Man*	4	1·09	0·35	0·32	0·33
Pig†	9	2·20	0·92	0·42	0·73
Sheep†	10	1·09	0·12	0·11	0·14
Cattle†	10	0·99	0·12	0·13	0·17
Dog	1	1·12	0·15	0·14	0·16
Cat†	5	0·56	0·05	0·10	0·11

Plasma copper was estimated by the method of Gubler *et al.* (ref. 6) and oxidase activity by Ravin's method (ref. 7), in which results are expressed as E(1 cm., 530 mμ). Species in which a correlation between plasma copper and oxidase activity has been observed are marked * (ref. 3), † (McCosker, unpublished work). Oxidase activity/μgm indirect-reacting copper is calculated on the basis of the percentage of indirect-reacting copper of plasma.

the *p*-phenylenediamine oxidase activity of the plasma of different species could be correlated to the indirectly reacting fraction of the plasma copper. The results, which are summarized in Table 1, show that there are considerable species differences in the total copper-levels in plasma and in the *p*-phenylenediamine oxidase activity of the plasma when this is expressed either in terms of activity/0·1 ml. plasma (as used in Ravin's test[7]) or as activity/μgm. total plasma copper. If, however, the oxidase activity is expressed as activity/μgm. indirectly reacting copper, then we find that the species differences are even more marked and are in the order pig > man > cattle, dog, sheep and cat.

It has been assumed that the oxidase activity of the plasma of the different species of mammals (Table 1) is due to cæruloplasmin for the following reasons: (*a*) the oxidase activity observed is inhibited by sodium azide, (*b*) the oxidase activity is directly correlated with copper-levels in plasma over a wide range of copper values, and (*c*) variations in the oxidase activity and copper-levels in plasma in certain species (sheep, cattle and pig) are similar to those found in man; for example, these levels are elevated in acute infections in sheep and cattle.

To explain the marked differences obtained for the *p*-phenylenediamine oxidase activities/μgm. of indirect reacting copper of the plasmas of different mammals, which are in the order pig > man > cattle, dog, sheep and cat, we may consider three hypotheses which will then be discussed in detail: (1) the cæruloplasmin copper may correspond to the whole of the indirect reacting copper fraction, the species differences being due to enzyme activators or inhibitors characteristic to the particular plasma; (2) the cæruloplasmin may be of the same standard activity, but in different concentrations in each species; (3) the differences in activity may be a result of species differences in the copper–protein itself.

(1) The oxidase activity of purified cæruloplasmin is very sensitive to changes in *p*H, and to the concentrations of a number of different ions[8]. If the oxidase activity per molecule of purified cæruloplasmin were the same in all species, then the differences in activity encountered in the plasmas of various animals might be due to the presence of substances which either activate or inhibit the oxidase activity of the enzyme. Unfortunately, so far, only pig and human cæruloplasmin have been purified, and no comparative investigations of activity in terms of their copper contents have been carried out. Preliminary experiments in which the plasmas of sheep, pig and man were each dialysed extensively against water, failed to indicate the presence of any dialysable activator or inhibitor, while the oxidase activity of a mixture of equal proportions of plasma of two different species was that of the arithmetic mean of the activities of the two plasmas. The experiments therefore suggest that the activity of the enzyme in the plasma is not appreciably modified by a dialysable or non-dialysable substance characteristic of one plasma but not of the other.

(2) If the plasmas contain different concentrations of cæruloplasmin of uniform activity, then in the case of the pig, which has the highest oxidase activity observed so far, the cæruloplasmin might be assumed to account for the whole of the indirectly reacting copper fraction, whereas in man and other animals, which have a lower plasma oxidase activity, the cæruloplasmin copper would only account for a proportionally smaller percentage of the indirectly reacting copper. In these species one would have to postulate another copper-containing substance to account for the remainder of the indirect-reacting copper. As major changes in copper-levels in plasma under various physiological and pathological conditions are accompanied by proportional changes in *p*-phenylenediamine oxidase activity, this second copper-containing substance would have to change in concentration in the plasma at the same time and to the same extent as cæruloplasmin.

In the course of the purification of human cæruloplasmin by chromatographic methods, Curzon and Vallet[9] obtained a second protein peak with PPD oxidase activity. This fraction, which contained about 5 per cent of the eluted protein but only 2–3 per cent of the oxidase activity, was considered by them to be either "an active artefact" or a modified form of cæruloplasmin where the oxidase activity/protein ratio suggests that only half the active centres are functional. It is, however, unlikely that this small copper-containing fraction of lower activity could account for the species differences observed between the plasmas of man, pig and other animals, since in human plasma the oxidase activity/μgm. indirectly reacting copper is only about half that found in the pig, so that the second copper-containing substance postulated would have to account for about half the indirect reacting copper in man, and for an even greater proportion in other species, and yet be devoid of oxidase activity.

(3) From our present knowledge, it therefore appears that almost the whole of the indirect-reacting copper of the plasma is in the form of cæruloplasmin, and that the differences in oxidase activity observed in various animals are probably due to species differences in the copper–protein itself. That not all the 8 atoms of copper in the molecule of human cæruloplasmin have the same properties is indicated by the work of Scheinberg and Morell[10], who showed that in the presence of ascorbic acid only half the cæruloplasmin copper was exchangeable with copper-64, while Curzon[11] has shown that when human cæruloplasmin was digested with chymotrypsin, only 4 of the 8 atoms of copper became dialysable, the other 4 remaining in a core resistant to chymotrypsin.

Since, as is shown in Table 1, the PPD oxidase activity/μgm. indirect-reacting copper of the plasmas of different species which are in the order pig > man > cattle, dog, sheep and cat, bear an approximate ratio to each other of 8 : 4 : 2, and considering the evidence presented here that in human cæruloplasmin 4 of the copper atoms are more labile than the rest[10,11], it is tempting to suggest that the observed differences in *p*-phenylenediamine oxidase activity in the plasma of various mammals may be a reflexion of the number of catalytically active copper atoms in the molecule of the cæruloplasmin of each species.

I wish to thank Dr. J. Keilin for her advice and also the Animal Health Trust for a Junior Wellcome Fellowship.

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PROTECTIVE ACTIVITY OF FRACTIONS OF TUBERCLE BACILLI AGAINST ISOLOGOUS TUMOURS IN MICE

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TUBERCLE bacilli and some of their crude fractions have been reported to be capable of initiating a wide range of non-specific changes in the resistance of animals to infectious diseases[1]. Such changes are frequently found to be accompanied by a general enhancement of antibody responsiveness. They have also been shown to be associated with alterations in certain physiological processes believed to be concerned with other aspects of the clearing mechanisms by which micro-organisms as well as foreign, effete, and abnormal cells are removed from the tissues.

It was, therefore, thought of interest to determine whether the moieties of the tubercle bacillus which have proved most effective in eliciting non-specific anti-microbial immunity possess any tumour-inhibitory powers. It has already been reported that intact, living tubercle bacilli of the *BCG* strain retard the progress of several non-isologous cancers in mice[2]. In order to avoid the immunological artificialities inherent in the transplantation of homologous and heterologous tissues, or even of very old isologous ones, all the tumour transplants employed here were of fairly recent, isologous source.

The mice were of the *BALB/c Crgl* and the $C_3H/Crgl$ strains. The tumour tested in the former was a uterine sarcoma (*T*-66); those tested in the C_3H strain were two mammary carcinomas (*T*-32 and *T*-71), a hepatoma (*T*-46), and an osteogenic sarcoma (*T*-62). (The tumours are maintained in the tumour-bank of the Cancer Research Genetics Laboratory of the University of California in Berkeley; the *T* numbers refer to the tumour nomenclature used at the Laboratory.) The tubercle bacillus substances were the following: living *BCG* organisms; intact, phenol-killed and acetone-extracted *BCG*; and several methanolic extracts and their residues, obtained from the phenolized and acetone-treated bacilli. The preparation of these materials has been described elsewhere[3].

The experimental procedure was to subject groups of animals to a single intraperitoneal injection of different quantities of these substances, suspended in 0·5 c.c. saline. Control groups of saline-injected animals were included in every experiment. At different intervals after treatment, isologous tumours were transplanted subcutaneously into the animals by means of a trocar. The transplants consisted of pieces of tumour tissue approximately 0·5 mm.^{3} in size, and were placed on the right flank midway between the axilla and the last rib. Every attempt was made to ensure a maximum degree of sterility in the operation. The development of the tumours was followed by tri-weekly measurements of the two diameters evenly bisecting the palpable growths at right angles to each other.

The results obtained so far can be summarized as follows:

(1) Prophylactic effects of several types and of varying degree were exhibited by all the preparations tested, at certain dose-levels. Several of the non-living preparations were at least as effective as the living *BCG*, and in certain experimental circumstances more so.

(2) Several distinct types of protective effects were seen: an initial retardation of the onset of tumour development, followed by a normal rate of growth; a consistent reduction in the rate of growth; a total prevention of tumour development, with no traces of malignant growth found on autopsy of the animals killed several weeks or months after transplantation; a regression and disappearance of tumours after an initial period of development; an inhibition of metastatic spread; and a prolongation of life of animals which did develop massive tumours despite pre-treatment. Against some tumours only one, and against others several, of these manifestations of heightened resistance were displayed by the preparations.

(3) The dose of the materials was critical, the optimum quantities varying somewhat from one preparation to the next. On the whole, small quantities, of the order of 1·5 mgm., dry weight, or less, were most effective, thus paralleling the results obtained with these materials in protection experiments against heterologous bacterial infections. The optimum doses of the several vaccines differed somewhat *vis-à-vis* the various tumours. Excess quantities sometimes enhanced tumour development, even though the amounts were still considerably below the threshold of gross toxicity.

(4) The protection elicited by some of the preparations was as great, or greater, three months after

Table 1. Protective Effects against a Uterine Sarcoma in *BALB/c* Mice
(One-month vaccination–transplantation interval)

Vaccine	No. of mice out of 7 in which malignant isograft failed to develop*	Tumour area† average values (mm.^{2}) of tumours in those animals which permitted tumour development, at the following intervals after transplantation (days)											
		20	22	25	27	29	32	34	36	40	42	46	48‡
Saline (control)	0	28	40	67	75	88	107	105	122	192	190	198	205
Living *BCG*	2	0	0	60	60	71	68	99	110	119	120	193	233
Phenolized *BCG*, 1 mgm.	4	0	0	25	24	32	50	99	99	99	108	144	156
Methanol extract, Batch No. 1, 1·0 mgm.	0	0	0	24	64	86	115	121	117	197	210	202	252
Methanol extract, Batch No. 2, 1·0 mgm.	3	25	31	56	75	84	96	132	172	166	170	191	247
Residue, 0·5 mgm.	6	24§	36	56	63	63	88	108	120	143	121	240	256
Residue, 1·0 mgm.	0	0	28	104	166	176	216	251	253	383	440‡		

* No tumour found at autopsy 48–50 days after transplantation.
† Product of bisecting diameters.
‡ Tumours grossly necrotic, experiment terminated.
§ Tumour in only one animal of this group.

treatment as it was when only 2–4 weeks elapsed between vaccination and transplantation.

The results of four representative experiments are briefly shown here, in order to illustrate some of these findings:

Experiment 1. Groups of 7 *BALB/c* mice each were injected with one of the following substances: saline; living *BCG* grown for 7 days in liquid culture and then diluted ten-fold; 1·0 mgm. phenolized *BCG*; 1·0 mgm. of a methanolic extract obtained by extracting the organisms for 32 hr. (batch No. 1); 1·0 mgm. of an extract which went into the solvent between the 32nd and 82nd hr. of continuous extraction (batch No. 2); and 0·5 and 1·0 mgm., respectively, of a preparation of the exhaustively extracted residue. All animals received isotransplants of uterine sarcoma *T*-66 one month after vaccination. The number of animals which developed tumours, and the rate of growth of the cancers, are shown in Table 1.

Experiment 2. Groups of 10 C_3H mice each were vaccinated with the same materials described in Experiment 1. Isotransplantation with mammary carcinoma *T*-32 was performed one month later. The transplanted tumours grew in every animal, but differences were observed from group to group in the rate of growth. These are presented in Table 2.

Table 2. Protective Effects against a Mammary Carcinoma in C_3H-Mice
(One-month vaccination–transplantation interval)

Vaccine	Average tumour area* (mm.^{2}) at the following intervals after transplantation (days)						
	11	13	15	18	20	22	25†
Saline (control)	102	130	256	279	369†	—	—
Living *BCG*	32	59	75	119	120	148	207
Phenolized *BCG* 1·0 mgm.	89	122	196	251	319	332	383
Methanol extract Batch No. 1, 1·0 mgm.	32	48	73	167	156	139†	—
Methanol extract Batch No. 2, 1·0 mgm.	90	139	174	241	277	317	402
Residue, 0·5 mgm.	34	52	68	102	112	115†	—
Residue, 1·0 mgm.	62	105	168	225	232	267†	—

* Product of bisecting diameters.
† Tumours grossly necrotic, experiment terminated.

Experiment 3. Groups of 8–10 *BALB/c* mice each were injected with saline; a 1 : 50 dilution of a living, 7-day-old liquid medium culture of *BCG*; or 1·5 mgm. phenolized *BCG*. Uterine sarcoma *T*-66 was transplanted into all animals three months later. It was found that the tumours placed into some of the animals regressed spontaneously after an initial period of development, while those of others progressed unchecked. These observations are illustrated in Table 3.

Experiment 4. Groups of 10 C_3H mice each received one of the following preparations: saline; 10-day-old living *BCG*, grown in liquid medium and then diluted 1 : 10; and 0·25 and 0·5 mgm., respectively, of the residue of a methanolic extraction. Four weeks later, isotransplants of osteogenic sarcoma *T*-62 were placed into the animals. The transplants grew progressively in most of the animals, but there were considerable differences in survival-time after transplantation. These are tabulated in Table 4.

These and related findings, as well as the observation that certain fractions of the tubercle bacillus possess the ability of retarding the development of already established cancers in mice, will be reported in detail elsewhere. It might be added that the protective activities of bacillary fractions against malignant tumours may bear some relation to the occasionally reported clinical and laboratory impressions that episodes of certain infectious diseases may have a beneficial influence on already-established cancers, or may reduce the likelihood of then cancer-free individuals developing neoplastic disease after recovery from the infection.

The present findings may also have some relevance for the more fundamental problem of why spontaneously arising tumours are able to grow progressively

Table 3. Protective Effects against a Uterine Sarcoma in *BALB/c* Mice
(Three-months vaccination–transplantation interval)

Vaccine	No. of animals per group	No. of animals with palpable tumours at the following intervals after transplantation (days)										
		4	10	16	22	28	34	40	46	54	60	96
Saline	10	5	9	10	10	10	10	10	10	10	10	All have massive tumours; 5 dead
Living *BCG*	8	6	8	8	7	7	7	7	7	7	7	1 alive, tumour-free; 7 have massive tumours, of which 4 dead
Phenolized *BCG*, 1·5 mgm.	9	4	7	8	8	6	4	3	3	2	2	7 alive, tumour-free; 2 have massive tumours, dead

Table 4. PROTECTIVE EFFECTS AGAINST AN OSTEOGENIC SARCOMA IN C_3H MICE
(Four-weeks vaccination–transplantation interval)

Vaccine	Cumulative number of animals dead* at the following intervals after transplantation (days)						No. of animals free of tumours at autopsy upon termination of experiment on ninetieth day
	45	55	65	75	85	90†	
Saline	0	0	0	2	6	7	1 of 3
Living *BCG*	0	1	3	3	5	7	0 of 3
Residue, 0·25 mgm.	0	0	0	0	2	4	4 of 6
Residue, 0·5 mgm.	0	0	0	0	0	0	3 of 10

* All animals dying before termination of experiment had massive tumours.

† Experiment termination.

in some individuals but not in others. Many aspects of the behaviour of malignant tumours in man and in experimental animals are consistent with the assumption that the ability of malignant cells to grow progressively in the animal body reflects a failure of the antibody or hypersensitivity-forming mechanisms, or of the phagocytic abilities of the reticulo-endothelial system[4]. Indeed, the idea has been advanced that the function of the homograft reaction is, primarily, to prevent the colonization of neoplastic cells in the normal individual[5]. Some support for this provocative suggestion comes from the clinical observations that individuals suffering from cancerous diseases show a poor ability to respond with hypersensitivity reactions to unrelated antigenic stimuli[6].

The firm establishment of an immunological theory of progressive malignancy depends, however, on the demonstration that the changes which cells undergo in the transition from normality to malignancy are accompanied by the acquisition of one or more new antigenic determinants. Largely because of the considerable technical difficulties encountered, this has not yet been shown unequivocally. It is possible, nevertheless, to secure further support for the immunological nature of the native defences against neoplastic growth and dissemination by a number of indirect approaches. The results of the present work might be taken to provide some such circumstantial evidence: If progressive neoplastic disease is, in fact, a manifestation of immunological deficiencies, it would be expected that substances which enhance both antibody production and the efficiency of the foreign body clearing reactions will also increase resistance to the development of malignant tumours. It appears that this expectation has been realized in the tumour systems which were the subject of this investigation.

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CHOICE OF ANIMALS FOR BIO-ASSAY

In marked contrast to earlier views, it is now generally accepted that, in naturally outbreeding organisms, F_1 hybrids between two inbred strains tend to show a lower level of phenotypic variability than do the parental inbred strains. The moral for the bio-assayist in search of uniformity of response, first pointed out by Mather[1], is that F_1 hybrids are, in general, to be preferred to inbreds. We have discussed this point in the course of a review[2] of methods of controlling variation in experimental animals.

The scope of the discussion has now been enlarged by C. K. Chai[3], who rightly points out that the variance of response is often not the only biometrical attribute which is of interest when assessing the suitability of a group of animals for bio-assay. In the case of quantitative (as opposed to all-or-none, or quantal) responses, the precision of the estimates is dependent not only on the variance, s^2, of the responses at a given dose, but also on the slope, b, of the dose-response line, which measures what Chai terms the 'sensitivity' of the animals.

This point was given quantitative expression thirty years ago by the introduction of λ, defined as s/b, as a suitable criterion for the comparison of the economic worth of various quantitative assay techniques for the estimation of a given substance. Thus for this type of assay it is not the absolute values of s and b which concern us, but their relative magnitude. The smaller the value of λ the more information will a given number of animals yield.

Chai also expounds the paradoxical, and potentially confusing, point that in 'quantal', or all-or-none assay the slope is merely an inverse reflexion of the variability of response, according to the relation $s^2 = 1/b^2$. It follows that no information on 'sensitivity' can be extracted from a quantal assay, and that the precision of the estimate is inversely related to the absolute value of b^2.

It is unfortunate that in the course of his further discussion of the question, Chai intermittently loses sight of these clear and valuable distinctions. The resulting confusion is such that the reader may gain an impression that the general superiority of F_1 hybrids over inbreds in respect of s^2 is in doubt. Such an impression is not justified by examination of the evidence presented by Chai. This falls into three main categories: (1) the results of a quantitative assay of testosterone, using the weights of seminal vesicles in castrated mice as the index of response: (2) the results of quantal assay of human chorionic gonadotrophin, using the percentage of immature female mice with hæmorrhagic ovarian follicles; and (3) a tabulation of published results concerning the responses of mice to hormones.

Response to testosterone. This assay was performed on the *C57BL/6* and *C57BR/cd* inbred strains and on the F_1 hybrids between them. Only animals were used the body-weight of which at weaning was in the range 9–12 gm. Although no doubt desirable for assay purposes, such a restriction renders the data of dubious value for a comparison of variation. As we have stated previously[2]: "Since the weight of the seminal vesicle is likely to be highly correlated with body weight, this restriction may have artificially brought the inbreds and F_1 hybrids being compared within the same range of variability".

The data given in Chai's Table 1 justify his conclusion that there are no differences between the inbreds and F_1 hybrids in sensitivity, measured as the slope, b, of the dose-response line. They do not justify the conclusion that the mice are equally suitable for use in the assay. The criterion for this is, as we have already said, the ratio s/b, where s^2 is the variance of response at a given dose. The relevant comparison cannot be made accurately from Chai's Table 1, since a scale has been used which makes the variances within each group grossly dependent on the value of the mean. However, inspection of the values suggests that s^2, and therefore λ also, would prove to be less for the F_1's than for the inbreds.

The hybrid mice would, therefore, be more efficient than the inbreds for the assay of testosterone. Chai reproduces in his Fig. 2 dose-response curves for another pair of inbred strains and their F_1 hybrids from an earlier publication on the same assay. Unfortunately, even from his published numerical data it is impossible to estimate λ; and the statistical manipulation of the data by which he obtained Fig. 2 is totally misleading, since λ must be computed in the scale in which the validity requirements are satisfied.

Response to chorionic gonadotrophins. In assessing the results of this experiment, we shall follow Chai's textual statement that the dependent variate was the percentage of animals with hæmorrhagic ovarian follicles, and not, as stated in the legend to Table 2, the percentage of follicles. The assay is quantal, and consequently irrelevant to the context in which Chai places it, namely, differences in sensitivity. As will be recalled, in contrast to the case of quantitative assay, the slope of the dose-response line in a quantal assay yields no information on sensitivity, but is an inverse measure of variability. Interpreting in this sense Chai's values for b, we find a four-fold difference in variance between the two parental inbred strains, with the F_1 closely resembling the less variable of the two. For all five groups of mice used, the values of the slopes are very low (0·62–1·27), and although the crucial information on their standard errors is not given, it seems likely that the five estimates of slope are not, in fact, significantly different.

Tabulation of published assay results. Chai's compilation is stated to refer to "sensitivity or magnitude of response", the latter being a measure of sensitivity on the assumption that the dose-response line passes through the origin. Several quantal assays appear in his Table 2, although, as has been stated, such assays yield no information on sensitivity. (The results of Claringbold and Biggers shown in Chai's Table 2 are only partially quoted, some in error. They compared both œstrone and œstradiol-3 : 17β (not œstrone-3 : 17β, an impossible compound) by both intravaginal and subcutaneous routes of administration. Only half these results are quoted—œstrone (intravaginal) and œstradiol-3 : 17β (subcutaneous)—and in the line incorrectly labelled œstrone-3 : 17β one inbred strain (*C57*) is omitted.) If we omit the quantal assays, and further confine our attention to relevant comparisons (that is, between F_1's and their parental strains), we are left with thirteen cases. In seven the hybrids are intermediate in "sensitivity or magnitude of response", in two they resemble the superior of the two parental strains and in four they are superior to both parental strains. Surprisingly, Chai bases on this result the generalization that "F_1 hybrids are not, in general, preferable to inbred mice in hormone assay in so far as the b value is concerned". A more illuminating formulation might be that, since they are apparently not inferior in this respect (and may even prove, on average, to be superior), their known tendency towards lower variability justifies the conclusion of earlier writers that F_1's are, as a rule, preferable to inbreds for bio-assay.

It is likewise indisputable that, "For the assay of a given substance, the choice of assay animal—either an inbred or an F_1 hybrid—cannot be made in advance; it has to be based either on existing data or determined experimentally". But our contention has been, and is now, that existing data sufficiently establish a generalization which can serve as a useful, although by no means an absolute, guide to the choice of experimental material. Chai's emphasis on the empirical approach is very welcome. It does not, however, gain in effectiveness if the known facts in this field are made to appear more arbitrary and unaccountable than they really are.

A word remains to be said about the position of random-bred animals. In 1939, Emmens showed that a random-bred colony of mice was less variable in response to œstrogen than an inbred strain (Strong's *CBA*). (He did not show, as Chai reports, "that some F_1 hybrid mice were less variable in response to œstrogen than their inbred parental strains".) Other relevant tests on random-bred animals are listed in our review. Chai misquotes us as concluding that random-bred strains are more uniform than inbreds. Our actual statement[2] was ". . . unlike the comparison with F_1 hybrids, no general law can be asserted on the comparison of inbred strains with random-bred stocks". This still holds to-day. Moreover, even in animal colonies primarily concerned with bio-assay, uniformity and sensitivity are not the only criteria that matter. If a 'mouse efficiency unit' were defined so as to include the economics of fecundity, viability and growth, there are many bio-assays for which a healthy and prolific random-bred strain might provide the most economical answer.

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Drs. J. D. Biggers, A. McLaren and D. Michie have commented on my article, "Response of Inbred and F_1 Hybrid Mice to Hormones", published in *Nature* of February 20, p. 514. The first of their comments supports my view that, in evaluating information of an animal in quantitative bio-assay, the slope of the dose-response curve needs to be considered. Some of their other comments, however, seem to be not much concerned with a choice between inbred and F_1 hybrid animals in bio-assay. Although I do not believe that clarification of the points which they have criticized will contribute much to the real issue at hand, I wish to make a brief defence of my position on these points. Following this I will discuss the unjustification of their conclusions.

Sensitivity. I have applied the basic principles determining the best measure of response in bio-assay as they were outlined by Gaddum and fully illustrated later by other biometricians in evaluating the bio-assay information obtainable in an animal experiment. It is necessary to draw a clear distinction between quantal and quantitative assays in terms of the importance of b. The papers published by Drs. Biggers, McLaren and Michie concerning information of an assay animal have been merely concerned with variation, and I do not think there is any need for them to restate the history of the statistics concerned. The qualitative experimental assays given in my article apply to the variance based on the relationship of $b^2 = 1/s^2$. I purposely avoided a discussion of variation in such assays and considered mainly the sensitivity measured by the slope of the dose-response curve. I do not believe that my presentation in this manner has caused confusion to readers. It may appear to be confusing to Biggers *et al.* because they have the impression that inbreds are more variable than F_1 hybrids in biological responses.

Androgen experiments. My presentation of the testosterone experiments was mainly to illustrate the point that the F_1 hybrids are not 'necessarily' more sensitive than inbreds as measured by the slope of the response curve. It is likely, when the sensitivity values of the parents differ widely, that the value of the F_1 hybrid will fall between them because of additive effects of polygenes. When the values of the parental strains are similar in sensitivity to a given stimulus, the values of the F_1 hybrids may be either greater, smaller or similar to the parental strains, depending on the degree of dominance, genic and gene-environment interactions. I used the *BALB/c*, *A/Jax* experimental results to illustrate the former case and the experiment with *C57* strains to illustrate the latter. I had no intention of concluding that inbreds or F_1 hybrids were more sensitive one than the other, but rather wished to give a possible interpretation of the results based on the polygenic theory of inheritance.

I did not transform the response to render the variance independent of the means as I was not concerned with variation, but simply gave the actual means and variances for the sole purpose of plotting the means. Crude as my method may have been, I think I illustrated my point by comparing the slopes. In the experiment using *BALB/c* and *A/Jax* mice, I tried many different transformations of the responses; the logarithmic transformation gave the best results although it was not fully satisfactory. Finney, in his *Statistical Method in Biological Assay*, p. 76, has pointed out that "In practice, slight heterogeneity of variance will not affect the statistical analysis very much, and a transformation that is approximately correct will be adequate . . .". In concordance with this statement, I used only the means in the comparison of bio-assay information between the inbred and hybrid mice. Also, there were no indications, from glancing at the variances, that the F_1 hybrids were less variable than the inbreds.

I judge from their criticism of my use of mice within a limited range of body-weights that Drs. Biggers, McLaren and Michie have confused 'normal variation' with 'variation in response to stimulus'. I used mice within a given range of body-weight as an aid in adjusting dosage of hormone, and to eliminate the need for correcting for differences of body-weight in the analysis of the data. Should the smaller variation in response of the F_1 hybrids be due merely to their being more uniform in body-weight, the increased variance in response could be corrected statistically. It is more likely that the differences in variation, if any, are a reflexion of physiological regulating abilities, which are, in turn, a reflexion of genetic-environment interaction.

Gonadotropin experiment and tabulations of published results. The comments concerning my experiment on response to gonadotropin and my tabulation of published data do not contradict my interpretation and conclusion that F_1 hybrids are not 'necessarily' more sensitive than inbreds. Furthermore, there seems to be no theoretical basis for them to be so.

It is mentioned that I quoted only half the results of Claringbold and Biggers. I did this because, in both their methods of administration of œstrogen, the relative order of response of inbred and F_1 hybrids was the same. I did mis-state Emmens's conclusions, and appreciate Drs. Biggers, McLaren and Michie's correction. However, I neither misquote nor mis-state the conclusion of Drs. McLaren and Michie, who have stated: " . . . On the contrary, the published facts suggest that inbred strains are usually more variable than inter-strain hybrids, with random-bred colonies occupying an intermediate position" (*J. Genetics*, 54, No. 3, p. 454). This obviously means that random-bred animals are less variable than inbreds. Dr. Biggers *et al.* pointed out that the name of the œstrogen was incorrectly cited from their paper and the title of Table 2 of my communication published in *Nature* was not clear. I sincerely regret this carelessness.

Unjustification. Drs. Biggers, McLaren and Michie draw their conclusions without considering the importance of the 'slope' of the dose-response curve in quantitative assay; they relied on 'variation' alone. They cited thirteen papers (*Nature*, 182, 77; 1958), including experiments using such animals as mouse, fowl and *Drosophila* for making comparisons between F_1 hybrids and inbreds. Among these thirteen papers only three dealt with biological responses: one, my paper on response to androgen, one by McLaren and Michie on the response to 'Nembutal', and the other by Claringbold and Biggers on the response to œstrogen. I have discussed my paper above and omit further discussion of it here. In the 'Nembutal' experiments (McLaren, A., and Michie, D., *J. Genetics*, 54, 440; 1956) the authors tested inbreds, F_1 hybrids and 'Mousery' mice (random-bred) by giving the drug to the same mice on days 1, 2, 3, 8, 9 and 10, and to 'fresh mice' on day 10. They observed the response each day, but only day 1 and day 10 'fresh mice' data were used for analysis.

The remaining five days records were omitted from analysis. I calculated the variances using the data which they excluded and found the combined variances (with the degrees of freedom in parentheses) to be: 0·0346 (45) for *C57BL*; 0·0849 (43) for *C3H*; 0·0396 (44) for the F_1 hybrid; and 0·0213 (44) for 'Mousery' animals. The variance for the F_1 hybrid is smaller than that for the *C3H* strain, but greater than that for *C57BL*, although not statistically significant. The variance for the 'Mousery' mice appears to be the smallest among them. The relative order is obviously different from that which they obtained (estimate of variance: 0·0552 for *C57BL*; 0·0778 for *C3H*; 0·0165 for F_1 hybrids; and 0·0176 for 'Mousery') based on two-days records. However, perhaps their selection of data was due to their lack of understanding of the biological nature of the experiment.

The other ten papers cited by Biggers, McLaren and Michie dealt with normal variation and not with variation of biological responses. Such data might be a basis for speculation, but could not be 'definitive' evidence for conclusions about biological responses. There seems to be a lack of distinction between normal variation and biological response.

It is clear that at the present time no one has sufficient amounts of critical evidence for the generalization which these investigators have made. It is true that Mather suggested the possibility of using F_1 hybrids in bio-assay, and Lerner pointed out that heterozygotes have better self-regulating ability than homozygotes. Moreover, I believe that under ordinary environmental conditions and without treatment, the F_1 hybrids may be more homogeneous in morphological traits than inbreds, but to equate this morphological homogeneity with response to chemical or biological stimuli requires further experimental evidence.

It is well established that there are species differences in sensitivity and that, in bio-assay, preference is given to certain species for certain assays; for example, metamorphosis in the tadpole for assay of thyroid hormone; comb-growth of chicken for androgen; crop-gland secretion of pigeon for lactogenic hormone; and uterus growth in mice for œstrogen. I believe that there are differences also in sensitivity between races, breeds and strains within species as well. Indeed, some differences have been shown to exist between mouse strains, as already illustrated in my article in *Nature*, 185, 514; 1960. Inbred strains can be very useful for assays of certain substances. This has been well illustrated and discussed by Becker and Berg (*Poultry Sci.*, 38, 362) in a paper in which they compared inbred and hybrid chickens in high and low planes of nutrition.

Finally, I reiterate that I do not underestimate the potentiality of F_1 hybrids for use in bio-assays. It has been claimed that the heterozygotes are better 'buffered' than the homozygotes, but differences between them in the mechanisms of reactivity are poorly understood. Our understanding of genetic pathways in the polygenic systems are practically nil, and reliable data from comparisons in bio-assay values between inbreds and F_1 hybrids are limited. Assay substances from synthetic and biological sources are numerous, and their end-points in assay vary widely from physiological and morphological measurements to rates of survival. In these circumstances, it seems wise to use an empirical approach, as I have, rather than to draw conclusions which might mislead the experimenter into believing that there is more evidence available for his making a choice of animal material than actually exists.

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Dr. Chai has now raised a criticism of an earlier paper of ours (*J. Genet.*, 54, 440; 1956). As explained in the paper in question, estimates of variance of response derived from animals which have acquired a degree of resistance through previous exposure to the drug have no simple interpretation. Our "selection of data" consisted of using only estimates of variance derived from mice not previously exposed to the drug. Furthermore, it is not statistically legitimate to pool variance estimates, as Chai has done, when the estimates are derived from the same mice on different occasions and when the responses on different occasions are correlated. As for Chai's distinction between "variation of biological responses" and "normal variation", in so far as these two categories can usefully be distinguished, it is not easy to see why they should not be subject to the same underlying mechanisms of genetic and developmental homœostasis. We have discussed these matters more fully elsewhere (*New Biol.*, 19, 48; 1955).

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Development of resistance to drugs, particularly those affecting the central nervous system, is well known. The analysis which Drs. McLaren and Michie made, testing the significance of this, was not convincing, for their interpretation (that response to 'Nembutal' is affected by previous dose) was based on only part of their results. The primary concern in pooling or comparing variances of different groups should be that the variance is independent of the mean, not that there was, or was not, previous exposure to the drug. McLaren and Michie used the logarithmic scale for correcting the dependence of the variance on the mean. This transformation was not adequate, as they themselves stated, ". . . this applies too strong a correction and results in a negative correlation between variance and mean". When I analysed their results, I noticed that they pooled the variance-estimate in the two-day tests and compared the variances without considering that the means were different and the transformation not adequate. Transformation of data for biological responses is often a troublesome matter and it is difficult to obtain an adequate scale. I reiterate that although "variation of biological response" and "normal variation" may be subject to the same underlying genetic and developmental mechanisms, further experimental evidence is necessary before this can be accepted as fact. Furthermore, there is little information available regarding differences in variability between inbred and F_1 hybrids in characters which have different evolutional significance. Speculation with regard to homœostasis, a concept which is theoretical and controversial, should not be substituted for proof.

C. K. CHAI

LETTERS TO THE EDITORS

ASTRONOMY

Origin of Jupiter's Great Red Spot

THE Great Red Spot in the southern hemisphere of Jupiter's otherwise ever-changing surface of dense cloud has baffled astronomers for many years[1,2]. The purpose of this communication is to outline the results of elementary dynamical and hydrodynamical considerations which suggest a straightforward explanation of the phenomenon. Contrary to generally accepted views[3], the non-uniform rotation of the Great Red Spot does *not* rule out the possibility that its origin is in the 'solid planet'; I believe that quite a shallow 'topographical feature' of the surface of the 'solid planet' could account for the observations. The theory should be amenable to further observational tests, and an experiment is now in hand to check the chief theoretical argument on which it is based.

The mean radius of Jupiter is about 70,000 km. The Great Red Spot, which is elliptical in shape, having its long axis along zenocentric latitude 22° S., occupies 30° of longitude and 10° of latitude. According to observations of the motion of markings on Jupiter's visible surface[2], which go back more than a century, the rotation of this surface about the axis of the planet is non-uniform, being a complicated function of latitude which is unsymmetrical about the equator. The period of rotation of the equatorial zone is 5 min. less than that of other regions, and astronomers find it convenient to refer to the more rapidly rotating part of the disk as System I and the other parts as System II. Their average periods with respect to position and time are, respectively, 9h. 50m. 30s. and 9h. 55m. 40s. In System II, spatial variations of the mean period with respect to time range from 9h. 55m. 5s. to 9h. 55m. 54s. As regards variations in time of the rotation periods in System II, these are erratic and have a range from ±5 sec. to ±10 sec. depending on position on the disk. Within System I, the equatorial zone, the rotation is most rapid roughly on the equator itself. Temporal fluctuations in period over a range ±15 sec. are indicated by the observations.

During the past hundred years or so, the period of the Great Red Spot has varied between 9h. 55m. 31s. and 9h. 55m. 44s., the average value being 9h. 55m. 38s.; the corresponding amplitude of this drift in longitude with respect to the mean motion is 1,080°, and on the present hypothesis the motion of the Spot is also that of the 'solid planet'. The average (with respect to position) period of the South Tropical Zone, in which the Spot is embedded, has varied between 9h. 55m. 27s. and 9h. 55m. 36s. The corresponding average linear velocity of the South Tropical Zone with respect to the Spot is about + 2 m./sec., the positive sign indicating that this motion is in an eastward (westerly) direction. Points on the equator rotate about the axis of the planet at 12 km./sec.

The mean density of Jupiter is 1·34 gm./cm.^{3}. According to Wildt's theoretical model[4,8], the atmosphere has a depth d (say) of 12,000 km. and a mean density of 0·35 gm./cm.^{3}. The corresponding moment of inertia of the atmosphere about the rotation axis exceeds 0·2 of that of the whole planet. Assuming that the motions of the visible surface are representative of the flow at lower levels (and this assumption has some theoretical backing) it is possible to estimate the order of magnitude of temporal fluctuations in the angular momentum of the atmosphere. This turns out to be comparable with the fluctuations of the angular momentum of the 'solid planet' as evinced, on my hypothesis, by the motion in longitude of the Great Red Spot, and it follows therefore that transfer of angular momentum between atmosphere and 'solid planet' might account for this motion. Evidently, Jupiter's 'day' is subject to quite large variations, probably due to meteorological causes.

The variation of latitude of the Great Red Spot expected on the present model is utterly negligible, and this accords well with observation.

Hydrodynamical theory suggests that Jupiter rotates so rapidly that the effect on the general circulation of the atmosphere of quite a shallow 'topographical feature' of the 'solid planet' will be attenuated very slowly with height, and thus the 'feature' will make its presence manifest at the level of heavy cloud at the top of the atmosphere. Specifically, the atmospheric flow cannot surmount the 'feature' if h, the height of the 'feature' above (or depth below) the general level, exceeds:

$$h_c \equiv \alpha d(U/L\,\Omega) \qquad (1)$$

where α is a number of order unity, U is a characteristic wind speed in the vicinity of the 'feature', L is a typical horizontal dimension of the 'feature' and Ω is the angular speed of rotation of the solid planet. When this criterion is satisfied, there will be a stagnant column of fluid stretching, not necessarily in a vertical direction, from the 'topographical feature' at the bottom of the atmosphere all the way to the top of the atmosphere. This column of fluid will partake of the motion of the 'topographical feature' and virtually no mixing will occur between the fluid within the column and that without. Although effects of this kind were predicted and demonstrated long ago by Sir Geoffrey Taylor[5], the foregoing criterion for 'Taylor columns' to occur is only based on crude theoretical arguments of my own. The criterion is now being tested in my laboratory.

If L = 25,000 km., d = 12,000 km., U = 2 m./sec. and $\Omega = 1{\cdot}5 \times 10^{-4}$ rad./sec., according to equation (1), h_c = 6 km. Although geometrically shallow, stresses in the 'solid planet' due to the presence of a 'topographical feature' having this height would exceed 10^8 dynes/cm.^{2}. If the 'feature' cannot be supported elastically, it may be necessary to invoke slow convection in the 'solid planet' to account for it, assuming that to forces of slow period the 'solid' is really a very viscous liquid. The coefficient of viscosity of the 'solid' would then have to exceed 10^{19} gm. cm.$^{-1}$ sec.$^{-1}$.

Equation (1), which shows h_c is *inversely* proportional to L, suggests a possible explanation of the uniqueness of the Great Red Spot, for if there were no ‘topographical feature’ having L in excess of 40,000 km. and h nowhere substantially exceeding the critical value for the Spot, other ‘topographical features’ would only produce spots on those occasions when U has a sufficiently low value. This appears to be borne out by the occasional appearance of short-lived spots, slightly smaller than the more permanent Great Red Spot, and it will be of importance to scrutinize the observations more closely in this connexion. It is not permissible to apply equation (1) to very shallow ‘topographical features’, because then friction has to be taken into account.

Considerations of the atmospheric flow around the hypothetical column of fluid underlying the Great Red Spot account for the presence of the Red Spot Hollow[2] on the equator-ward side of the Spot and for the bizarre nature of the interaction of the Spot with the South Tropical Disturbance[2]. Atmospheric turbulence resulting from the presence of this column will be most intense on the poleward side of the column, and this may bear on the interpretation of radio astronomical observations of Jupiter[6,7]. Recently, bursts of radio noise have been reported, and the electromagnetic energy in a typical burst estimated at about 10^{18} ergs. As the kinetic energy of a typical turbulent eddy in Jupiter's atmosphere (about 10^{24} ergs) is several orders of magnitude bigger than this, one wonders whether radio bursts are due to lightning flashes associated with thunderstorm activity, or to strong interaction between this turbulence and Jupiter's ionosphere.

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ASTROPHYSICS

Anomalies in the Light Curves of Meteors resulting from Fragmentation

IN one of a series of papers on the physical theory of meteors, Jacchia[1] has pointed out that while the observed decelerations and light curves of bright meteors are in good agreement with the simple ballistic theory, the fainter meteors photographed with the Baker Super Schmidt cameras show startling anomalies. He has adduced evidence to show that the theory of progressive fragmentation of the meteoroid offers a simple explanation of the observed anomalies.

In a recent communication[2], I have shown that the observed light curves of a random sample of 360 meteors studied by Hawkins and Southworth[3] suggest that the luminous efficiency factor τ in the luminosity equation for meteors has to be regarded as a variable along the trail of an individual meteor and proportional to the atmospheric density ρ at every point of the trail. It was shown that the normalized light curve, when the theory is thus modified, fits better with observational data than the corresponding curve of the conventional theory. For a single-body meteoroid, if we denote by α and β the height-intervals from the level maximum luminosity (corresponding to stellar magnitude $M_{\max.}$) to levels of equal luminosity (corresponding to stellar magnitude M) on the ascending and descending branches of the light curve, then the ratio $\alpha/(\alpha + \beta)$ is related to $M - M_{\max.} = \Delta M$. For a given value of ΔM, the values of this ratio as given by the conventional and modified theories are nearly the same as can be seen from Table 1.

Table 1

ΔM	$\alpha/(\alpha+\beta)$		ΔM	$\alpha/(\alpha+\beta)$	
	Conventional theory	Modified theory		Conventional theory	Modified theory
0·5	0·631	0·613	4·0	0·814	0·798
1·0	0·680	0·663	4·5	0·827	0·813
1·5	0·713	0·691	5·0	0·839	0·827
2·0	0·737	0·720	6·0	0·858	0·846
2·5	0·761	0·747	7·0	0·871	0·861
3·0	0·782	0·769	8·0	0·883	0·873
3·5	0·799	0·788			

I directed attention to the fact that, according to the single-body theory, the height-interval from the level of maximum luminosity to the level of complete ablation is independent of the mass of the meteoroid and is uniquely related to the scale-height a of the atmosphere, being equal to $1 \cdot 1a$ according to the conventional theory and $0 \cdot 7a$ according to the modified theory. The effect of fragmentation on the light curve is, in general, to shorten the ascending part of the curve (α), and, in a good many cases, to lengthen the descending part of the curve (β). Without going into details, it may be mentioned that the ratio $\alpha/(\alpha + \beta)$ will decrease from the theoretical value as a result of fragmentation. I have therefore made use of this parameter as a rough index to study the behaviour of the 360 meteors listed by Hawkins and Southworth. The value of the ratio is found to be less than 0·6 for nearly two-thirds of the meteors. Rejecting these, the values of α and β for the remaining meteors are plotted in Fig. 1 against the appropriate values of ΔM. By this process of selection, I have eliminated the large majority of the fragmented meteors, so that the behaviour of the light curves of those remaining may be expected to show a close

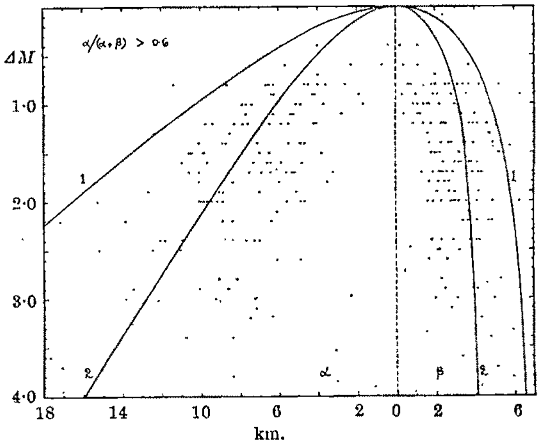

Fig. 1

degree of approximation to the theoretical light curve. Curves 1 and 2 in Fig. 1 are the normalized light curves according to the conventional and modified theories for a scale-height value of $a = 6\cdot38$ km. It is clearly seen that the observational data are in accord with the light curve 2 of the modified theory.

To study the behaviour of meteors brighter than those in the random sample of Hawkins and Southworth, I have examined the data of 144 meteors listed by Whipple[4]. Whereas less than 3 per cent of the meteors in the random sample had the end-points of their trails below 75 km., nearly 50 per cent of Whipple's meteors had their trails ending between 75 and 40 km. For about a third of these meteors the ratio $\alpha/(\alpha + \beta)$ was found to be less than 0·6. Rejecting these, and plotting the data of the remaining meteors in a manner similar to that of Fig. 1, it is found that the light curves of the brighter meteors too are in better conformity with the normalized light curve of the modified theory than with that of the conventional theory.

The Draconid shower meteors of October 1946 gave, for the first time, the clearest indication of the need to revise the concept of the single-body meteoroid, and to replace it with the concept of the meteoroid being 'a cluster of breaking fragments' (refs. 1 and 5). The data relating to these meteors taken from the paper of Jacchia, Kopal and Millman[6] have been plotted in Fig. 2. Comparison of Figs. 1 and 2 brings out strikingly the effect of fragmentation on the light curves of meteors.

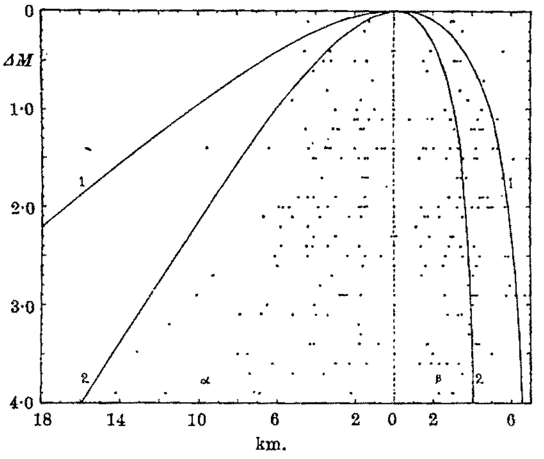

Fig. 2. Draconid shower meteors (Jacchia, Kopal and Millman)

For the luminous efficiency factor τ in the luminosity equation for meteors, Whipple derived the relation:

$$\log \tau = \log \tau_0 + \log v = -9\cdot07 + \log v$$

where v is velocity of the meteor in cm./sec. It is significant to note that the value of $\log \rho$ at 100 km. for the Rocket Panel Atmosphere (1952) is $-9\cdot065$, which is practically equal to $\log \tau_0$ in Whipple's equation. It is also of interest to note that the mean value of $\log \rho$ between 120 km. and 80 km. for the Rocket Panel Atmosphere is $-9\cdot02$. Whipple's equation is, therefore, very nearly equivalent to:

$$\tau = \tau_0 v \sim \bar{\rho} v$$

where $\bar{\rho}$ is the atmospheric density at 100 km. or the mean atmospheric density between 120 and 80 km. The observational data relating to light curves of meteors are in better accord with the relation:

$$\tau \propto \rho v$$

where ρ is a variable along the meteor trail.

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Nanometeorites

THIS communication is a preliminary report of our work on a high-altitude airborne particle collection obtained in the Arctic on November 15, 1960. The collector was exposed from a *U*-2 aircraft at an altitude of 60,000 ft. at longitude 144° W. and at latitudes ranging from 71° to 73° N. for a duration of 366 min.

The particle collector was of an impact type which could simultaneously expose four collection surfaces each with a different effective collection area and consequently different particle-size cut-offs. The smallest particles which could be individually collected were approximately 0·2μ in diameter. The particles were collected directly on high-purity 'Formvar' films approximately 70 Å. thick supported by 200 mesh/in. copper screening. The exposed screens were examined by electron microscopy without any chemical treatment after exposure. The moving and exposed parts of the collector were either coated with 'Teflon' or constructed from 'Teflon' to eliminate the need for lubrication for de-icing.

Examination of the exposed samples revealed enormous numbers of ultra-small, high-density particles of the order of 75 Å. in size in addition to the solid and 'fluffy' particles larger than 0·2μ normally seen in previous high-altitude collections at lower latitudes.

Fig. 1 shows an electron micrograph of a portion of a cluster of these ultra-small particles, a 0·1μ scale and unresolved background material with some holes from which very small spherical particles appear to have evaporated. Table 1 shows approximate particle-size distributions for these high-density spherical particles and for some ultra-small irregular particles which were also found.

These extremely small particles and the background material have also been studied by selected-area electron diffraction. The high-density spherical particles have given diffraction patterns indicating

Table 1

Diameter size-range (Å.)	No. of small spherical particles per size-range	No. of irregular solid particles per size-range
0–50	1.092	107
50–100	735	137
100–150	256	145
150–200	72	127
200–250	16	57
250–300	2	10
300–350	—	3
350–400	—	1

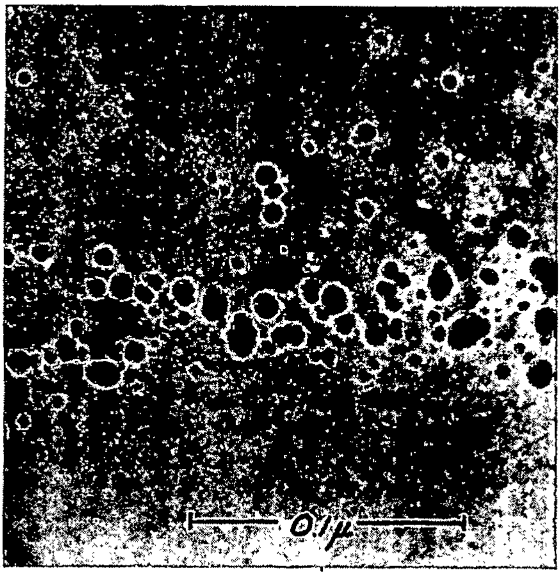

Fig. 1

compositions of nickel monoxide and also of taenite. The background material and the irregular particles appear to be partly nickel monoxide and ferric oxide.

The following additional comments concerning these ultra-small particles are appropriate:

(1) They are always found clustered, suggesting that at the altitude of collection they were within a larger particle which has afterwards evaporated, probably under electron-beam bombardment in the electron microscope.

(2) The small spherical type and the background material have been occasionally detected by us in other high-altitude aircraft collections and in balloon collections during the past four years.

(3) This collection was made during a period of unusual solar activity in the auroral zone. Major solar flares were observed on November 12 and November 15, 1960.

(4) The ultra-small particles appear to be about 100,000 times more numerous than particles 0·2μ in size or larger.

(5) The four simultaneously exposed collection areas showed an approximately correct decrease in particle numbers per unit area with an increase in collection aperture and particle-size cut-off, indicating freedom from contamination of samples.

(6) Previous high-altitude collections at lower latitudes have indicated that, at altitudes greater than 50,000 ft., one is substantially free of contamination by terrestrial particles and that numbers of particles collected are roughly proportional to duration of exposure.

We feel that the evidence is strong for the existence of a previously unsuspected class of meteoric bodies entering the Earth's atmosphere. Since these ultra-small bodies are approximately a thousand times smaller in diameter than the expected sizes of micrometeorites, and since the forces acting on them are likely to be critically dependent on their electrical charge and velocity in the Earth's ionosphere and magnetic field, we propose, for convenience, to label these ultra-small particles nanometeorites.

Nanometeorites may in part result from meteorite ablation, or meteor fragmentation. We are inclined, at present, to favour a lunar dust origin for these particles. A full report on nanometeorites will be published at a later time.

We wish to thank Mr. Herbert Cohen and Mr. Joseph Frissora, of the Space Instrumentation Division of the Technology Instruments Corporation of Acton, Mass., for the design and construction of this particle collector, and Dr. James Friend, of Isotopes, Inc., at Westwood, New Jersey, for arranging to have this collector flown for us by the Armed Forces Special Weapons Agency.

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GEOPHYSICS

Possible Identification of Atmospheric Waves associated with Ionospheric Storms

It has been noticed that ionospheric storm effects are not simultaneous at polar and more temperate regions. The purpose of this communication is to present the results of an inquiry into the nature of travel of these storms.

Mason[1] has described the general properties of night-time storms at Christchurch, and has noticed that one class of storm has relatively simple characteristics. These were storms with magnetic sudden commencement followed by easily identifiable storm features in the ionosphere. The latter were characterized by rise in virtual height of *F* layer and drop in critical frequency often accompanied by oblique echoes and enhanced *E* layer ionization. Such storms were chosen for study as they gave the least ambiguous effects.

Night-time ionograms were studied at Christchurch (43° 34′ S., 172° 48′ E.) and at Campbell Island (52° 33′ S., 169° 09′ E.) in order to measure the northward component of any travelling phenomena. Periods immediately preceding and following a local night-time magnetic sudden commencement with *K*-index disturbance of 5 or more, during July 1957–February 1961, were used. These times included the eighteen months of the International Geophysical Year, during which the standard of recording was particularly suited to the project. The criteria of an ionospheric storm were a sudden drop in the critical frequencies and a rise in virtual height of the *F* layer, and other features—oblique echoes, retardation of extraordinary ray, and night *E*—noted. The first rise in virtual height of the *F* layer was taken as the onset of the storm, though the changes in height were usually more extreme at Campbell Island than at Godley Head.

32 storms in all were studied; but for the following reasons 24 were discarded: (*a*) 7 were discarded as their onset-time was obscured by continuing effects of earlier storms; (*b*) 8 were discarded because sunrise occurred before storm onset; (*c*) 4 were discarded as onset at Campbell Island was obscured by polar cap absorption; (*d*) 5 were discarded as no storm developed at Christchurch.

The remaining 8 storms were considered sufficiently reliable to measure the apparent velocity between

Table 1. THE APPARENT VELOCITY OF STORM INDICATIONS DEDUCED FROM THE IONOGRAMS

Time of storm (G.M.T.)	Maximum K-index of disturbance	K-index over considered period	Time of onset at Campbell Island (G.M.T.)		Time of onset at Christchurch (G.M.T.)		Apparent velocity (m./sec.) from Campbell Is. to Ch'ch.	
			Lower limit	Upper limit	Lower limit	Upper limit	Lower limit	Upper limit
Dec. 19, 1957, 09·37	5	4	10·50	11·00	11·31	11·83	428	592
July 8, 1958, 07·50	7	6	07·58	08·00	08·38	08·40	438	485
Sept. 3, 1958, 08·42	7	4–5	08·50	09·00	09·20	09·30	460	921
Feb. 11, 1959, 07·55	6	4–6	08·40	08·50	09·20	09·30	368	613
Mar. 26, 1959, 08·42	7	4–5	09·00	09·10	09·34	09·47	392	767
Aug. 16, 1960, 14·09	6	4–5	14·42	14·55	15·17	15·30	384	837
Oct. 24, 1960, 14·54	6	5	15·17	15·30	16·02	16·15	318	575
Nov. 12, 1960, 13·50	9	5	16·05	16·15	16·40	16·45	460	737
Mean:							406	691

these two stations. With ionograms taken only at certain fixed times, the velocity may only be stated between limits. Table 1 gives the list of storms with sudden commencement times, followed by onset times at Campbell Island and Christchurch. The spherical distance between these two stations was 1,105 km. at a height of 150 km., and using these data the velocity limits were calculated.

The K-index first used was that describing the maximum disturbance period in the course of the magnetic storm, but for this work, the K-index for the actual period involved from sudden commencement until storm onset was more appropriate. These figures were more consistent with a correlation between delay time and magnetic activity, the event on July 8, 1958, having greatest activity and least delay until storm onset. As the K-index figures were only available for successive 3-hr. periods daily they are still not a sufficiently accurate measure for detailed work of this nature.

In several cases the readings from ionograms were taken from 15-min. records, giving correspondingly wider limits to the observed velocity.

It is obvious from Table 1 that the northward component of apparent velocity is well defined and, as this is also the most probable direction for any storm effects originating in the polar or auroral regions, this will be taken as the apparent velocity.

From the results it is suggested that this velocity is that of a neutral gaseous wave, being of the same order as that found for the limiting velocity of the disturbance from the Johnston Island nuclear explosion, that is, 470 m./sec. at 150 km. (ref. 2). It is further suggested that the apparent vertical movement of the ions is caused by the interaction of the ionized component of the disturbed atmosphere with the magnetic field. The dissipation of this wave would also explain the more pronounced effects at Campbell Island than at Christchurch, and also the five cases where no storm developed at Christchurch.

This work is part of the research programme of the Christchurch Geophysical Observatory, Department of Scientific and Industrial Research, New Zealand, under the superintendent, Mr. J. W. Beagley. I wish to thank Mr. C. H. Cummack for his guidance and also Mr. G. A. M. King for helpful discussions.

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GEOLOGY

Granite beneath the Northern Pennines

A FULLY cored borehole sited at Rookhope (*NY*35/937,428), West Durham, has proved the existence of granite at 1,281 ft. depth directly beneath Lower Carboniferous (Viséan) strata. The drilling, which is still in progress, is financed by the Department of Scientific and Industrial Research, and forms part of a programme of geological and geophysical investigation by the Durham Colleges Geology Department.

The background of the operation is as follows. The lead–zinc–fluorine–barium deposits, which occur as veins and replacements in the Carboniferous rocks of the northern Pennines, were shown by K. C. Dunham[1,2] to exhibit a concentric pattern of regional mineral zones, suggesting that mineralization was due to heated solutions, rising at a limited number of centres, and thereafter distributed laterally along channels conditioned by hard beds in the Yoredale sequence. Acid magmatic activity at depth was considered to be a possible source of the solutions. Later on, M. H. P. Bott and D. Masson-Smith[3], following up a strong negative Bouguer anomaly, first noticed during a traverse by J. Hospers and P. L. Willmore[4], made a detailed gravimeter survey of the region. This revealed a negative gravity anomaly of 35 milligals amplitude closely similar in character to the gravity field over exposed granitic batholiths with roof cupolas, such as the Cornubian batholith. Furthermore, certain features of the anomaly were considered to be inconsistent with interpretation as a sedimentary basin. Under the Alston Block, the cupolas indicated by the gravity survey correspond exactly with the postulated emanative centres of the mineralization. When the gravity survey was published, it was suggested that the most probable explanation was that a granite of Permo-Carboniferous age underlay the area, and that it not only supplied the mineralization, but also contributed to the devolatilization of the coal seams in the area. Using a method of calculation devised in collaboration with R. A. Smith, Bott[5] showed from the magnitude of the second derivative of the anomaly that the top surface could not be deeper than 3,500 ft. below Weardale. No upper limit to the depth can be placed from the gravity data, but having regard to the fact that no contact metamorphism is seen in the exposed Carboniferous rocks except near the Whin Sill, he concluded that the probable depth was between 2,000 and 3,000 ft. The postulated granite has become known as the 'Weardale granite'.

The borehole commenced near the top of the Great Limestone, 350 ft. from the surface position of the hanging wall of the Red Vein, one of the most powerful W.N.W. channels of mineralization in the district, with a yield so far of 460,000 tons of fluorspar and 48,000 tons of galena. The collar of the boring is also 500 ft. from the hanging wall of the E.N.E. Boltsburn Vein, a weak but very persistent fracture, which has yielded 143,000 tons of galena, mainly from associated replacements. Both veins dip towards the boring, which was sited so as to pass through them at depth. Below the Great Limestone, the base of which is taken as the Viséan–Namurian boundary in northern England, a normal Middle Limestone Group succession was encountered. Two quartz-dolerite sills were proved in these beds; the Little Whin Sill, 6 ft. thick, is intruded into the Three Yard Limestone, and the Great Whin Sill, 200 ft. thick, lies beneath the Jew Limestone. The base of the Middle Limestone Group remains to be defined exactly when the faunal study of the cores has been carried out, but the expected change from dark blue-grey limestone to pale grey limestone shows the approximate position of this boundary. The Lower Limestone Group is represented by an alternation of light grey limestones, shales and sandstones; no massive Melmerby Scar Limestone such as is present below Cross Fell is developed; but the typical 'pseudo-breccia' lithology characteristic of this limestone is present in some of the bands. Beneath these light-coloured limestones, black shale with very thin quartz- and feldspar-bearing conglomerate beds rest directly on weathered granite which in a few feet gives place to fresh, well-foliated microcline-quartz-muscovite-oligoclase rock. The foliation is nearly horizontal, and aplite and pegmatite bands occur at various angles.

So far, ten intersections of metalliferous mineralization have been made. Of eight in the Carboniferous, three are horizontal replacements in limestone and one of these is 16 ft. thick, rich in green fluorite and sphalerite. Two typical Pennine Zone I veins, carrying pyrrhotite, fluorite, quartz, have been intersected within the granite at 1,349–1,363 ft. and at 1,585–1,656 ft., the latter being accompanied by extreme alteration of the granite.

Two definite conclusions may be drawn at this stage: (1) the granite so far proved is of pre-Carboniferous age; (2) the mineral veins were fed from some source beneath that part of the granite so far drilled. To these may be added the suggestion that the buried pre-Carboniferous cupolas served to localize the upward flow of mineralizing fluids into the Carboniferous rocks.

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Isotopic Ages of the Weardale Granite

AGE determinations have been carried out on muscovite from the Weardale Granite[1] by both the rubidium–strontium and potassium–argon methods. One specimen from a depth of 1,396 ft. in the borehole was measured by both methods, while another from 1,314 ft. was measured by the potassium–argon method only.

Isotopic dilution techniques were employed for the determination of rubidium, strontium and argon. A flame photometric method was employed to determine potassium. The results for the rubidium–strontium method are given in Table 1, and those for the potassium–argon method in Table 2.

Table 1. RUBIDIUM-STRONTIUM DETERMINATIONS ON MUSCOVITE

Depth of sample (ft.)	Rubidium (p.p.m.)	Normal strontium (p.p.m.)	Radiogenic strontium (p.p.m.)	Age (m.yr.)
1,396	763	19·4	1·15	360 ± 12
1,396	757	19·6	1·15	365 ± 12

Decay constant of rubidium-87 = $0{\cdot}1475 \times 10^{-10}$ yr.$^{-1}$

Table 2. POTASSIUM–ARGON DETERMINATIONS ON MUSCOVITE

Depth of sample (ft.)	K_2O (per cent)*	Weight fused (gm.)	Radiogenic argon (p.p.m.)	Atmospheric argon (per cent)	Age (m.yr.)
1,396	10·01	2·034	0·240	17·4	370 ± 10
1,314	10·26	3·291	0·239	24	356 ± 12

Decay constants of potassium-40: $\lambda_K = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$
$\lambda\beta = 4{\cdot}72 \times 10^{-10}$ yr.$^{-1}$
Isotopic abundance of potassium-40 = 0·0119 atom per cent
* Potassium analyses by Dr. N. J. Snelling.

All the results agree within the limits of error. Agreement between the two methods makes it highly probable that the weighted mean of the four figures is close to the true age of emplacement of the granite.

The following points may be noted: (i) The weighted mean of 362 ± 6 million years corresponds to a Middle or Late Devonian age of emplacement, according to the latest time-scales[2,3]. The isotopic results therefore agree with the borehole evidence for the pre-Carboniferous age of the granite. (ii) The age of the Northern Pennine mineralization from the field evidence[4] is undoubtedly post-Carboniferous and probably pre-Tertiary. From lead isotope measurements by Moorbath[5] the mean model age of Northern Pennine galenas is 280 ± 30 million years, indicating that the mineralization is in fact of Hercynian age. Thus, from the lead isotope data and the absolute ages, as well as from the observations reported in the preceding communication, the northern Pennine mineralization is not connected with the emplacement of the Weardale granite. (iii) The weighted mean of 362 ± 6 million years for the Weardale granite is significantly younger than the dates for the Shap granite given by Kulp *et al.*[6]. These authors obtained 391 ± 7 million years by the potassium–argon method and 381 ± 7 million years by the rubidium–strontium method. Unpublished rubidium–strontium results obtained at Oxford on the Skiddaw granite suggest that the latter may be contemporaneous with the Weardale granite.

We are indebted to Prof. Dunham for supplying the material for this investigation.

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CRYSTALLOGRAPHY

Highly Oriented Boron Nitride

Pyrolytic graphite deposited on a hot target[1] has been found to have a structure with highly oriented crystallites[2]. We have now prepared, similarly, by vapour phase pyrolysis and deposition on a hot target, a boron nitride the structure of which also has a high degree of orientation of its crystallites. Utilizing boron-bearing gases and regulating the parameters of the treatment, we have been able to control the deposit of the pyrolytic boron nitride.

The X-ray diffraction pattern of pyrolytic boron nitride, Fig. 1, shows the structure to be composed of crystallites which are highly oriented with respect to each other and essentially parallel to the deposition surface. The stacking of the basal planes within the crystallites is considered to be turbostratic as indicated by the expanded unit cell height. On the other hand, the diffraction pattern of normal boron nitride reflects the random orientation of the crystallites, but within these the basal planes are well ordered. The diffraction patterns are compared in Fig. 1 with the powdered sample scan intensity having twice the scale factor of the pyrolytic. The trace of the pyrolytic material has been amplified to enhance the low-intensity peaks.

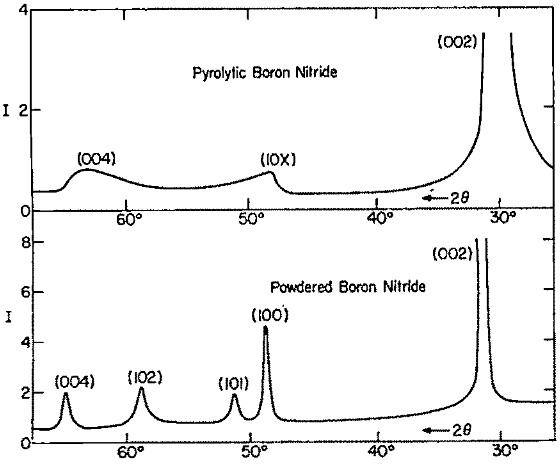

Fig. 1

Boron nitride has a simple hexagonal lattice with unit cell dimensions of $a = 2{\cdot}50399 \pm 0{\cdot}00005$ Å. and $c = 6{\cdot}6612 \pm 0{\cdot}0005$ Å. (ref. 3). The height of the unit cell of the pyrolytic material has been measured as 6·72 Å. using iron-filtered cobalt $K\alpha$ radiation on a General Electric *XRD*-5 diffractometer. Our experience indicates that the a-dimension of the unit cell, the chord of the hexagonal net in the basal plane, is not significantly different in pyrolytic graphite from natural graphite. Therefore, we do not expect any significant change in the edge-length of the pyrolytic boron nitride compared with the published data; this value has been assumed for calculation. The measurement of the edge-length of the pyrolytic unit cell is planned. Crystallite size has been determined to be approximately 140 Å. The theoretical density of normal boron nitride is reported[3] as 2·27 gm./c.c., compared with 2·26 gm./c.c. for pyrolytic boron nitride calculated with the above data.

Spectrochemical analysis of the pyrolytic material shows a relatively pure material with only trace concentrations of impurities. Observed impurities are magnesium, molybdenum and silicon at levels of about 10 parts per million or less.

The material is deposited on the hot target as a dense, fine-grained piece. Thin pieces are transparent and fairly thick (1/2 mm.) pieces are translucent. Samples have been obtained with either white or yellow colouring. Specimens of the white and yellow material have been examined by X-ray and chemical analysis and by neither method can we discriminate between the colours. Microstructure of the material shows the columnar cones characteristic of those found in the vapour deposition of the pyrolytic graphite. Fig. 2 is a photomicrograph of a representative section, the surface of the initial deposit being at the apex of the growth cones. Examination of the optical characteristics has been initiated, but results are not available for this preliminary report.

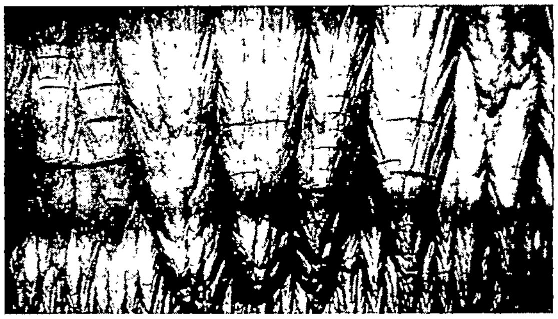

Fig. 2

These are the initial results from the investigation of pyrolytic boron nitride. The work reported is one phase of a broader programme to gain better understanding of the single crystal properties of selected refractory materials such as graphite, graphitic alloys, carbides, nitrides and related compounds for which adequate single crystals are not available. The technique of pyrolytic preparation produces samples with highly oriented structures that can be used in place of single crystals for investigation of some properties of the materials. Thus, the preparation of the highly oriented boron nitride is an approach to, and will permit the examination of, single-crystal characteristics of boron nitride so that the effect of its lattice anisotropy can now be investigated. It is expected that the wide interlayer spacing compared with the interatomic distance within the plane will generate significant anisotropy of the fundamental physical properties of boron nitride.

We thank R. Mozzi for his assistance and co-operation in obtaining diffraction data.

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PHYSICS

Rates of Recombination in Hydrogenic Plasmas

In common with other experimental determinations of rates of recombination of ions with electrons[1], recent spectroscopic observations of the afterglow of the *Zeta* discharge have indicated that these rates are much greater than simple theory predicts. When comparison has been made with the theory of radiative recombination[2-4], including recombination into all the excited levels of the atom, the theoretical value falls short of the experimental. Another primary process is three-body recombination. This is the inverse of ionization by electron collision to which it is related by the principle of detailed balance in thermodynamic equilibrium. The theory of collisional ionization was developed classically by Thomson[5], and if three-body recombination-rates into the ground-state are computed from this theory they also are found to be quite inadequate to explain the experimental observations.

In this communication there is proposed a more complex recombination mechanism which is based on well-known atomic collision processes, and which may help to explain the observed values. The three-body recombination-rate (derived from the classical ionization-rate) is proportional to the fourth power of the principal quantum number of the state into which the electron falls. Thus there is the possibility of three-body recombination into an excited state followed by spontaneous radiative decay being an effective recombination mechanism. This possibility is the kernel of the physical problem investigated here.

Specifically computations have been made of the rate at which the ground-states of hydrogen atoms and of hydrogen-like boron ions (B V, $Z = 5$) are populated as a result of the following processes among the first 20 quantum states of the atom (or B V), the continuum of free electrons and the corresponding bare nuclei: (1) collisional ionization; (2) three-body recombination; (3) collisional transitions between bound states; (4) radiative recombination; (5) spontaneous radiative transitions. The plasma was assumed to be optically thin.

The total rate at which the ground-state is populated is the sum of: (1) direct three-body recombination; (2) direct radiative recombination; (3) collisional transitions from all the excited states; (4) spontaneous decay from all the excited states.

This rate therefore depends on the instantaneous populations of all the excited states. Thus the solution was required of the following set of equations:

$$\frac{\mathrm{d}n_{0,i}}{\mathrm{d}t} = (\text{rate of populating state } i) - (\text{rate of depopulating state } i)$$

where $n_{0,i}$ is the population of the state having main quantum number i. Low-energy collisions are assumed to be sufficiently frequent to ensure that the substates are populated relatively according to their statistical weights. These equations were solved using the steady-state approximation $\left(\frac{\mathrm{d}n_{0,i}}{\mathrm{d}t} = 0, i \neq 1\right)$.

This is legitimate for hydrogen atoms and hydrogen-like ions because transitions among the excited states and the continuum are much more probable than transitions to and from the ground-state. Excluded from the computed rate of populating the ground-state are processes where the ground-state electron is raised to an excited state by collision and then returns to the ground-state by either a radiative or collisional transition.

In deciding the uppermost quantum state to be taken into account I noted the equivalence of situations (*a*) where states have a Saha distribution of populations and (*b*) where there is an imaginary continuum of electrons having a Maxwellian distribution of velocities, extending over the same energy region, and being suitably quantized. Thus, in making the discrete states merge into the imaginary continuum at the twentieth state, it was necessary to check in each case considered that at least the states immediately below twenty were Saha-populated. This was shown to be so for the range of conditions considered.

Cross-sections, due to M. J. Seaton (private communication), and *f*-values calculated by Green *et al.*[6], were used. Seaton derived his expression specifically for ions, but its similarity to the classical Thomson expression justifies its use for atoms. The calculations were done on a *Mercury* (Ferranti) electronic computer.

The results are presented in Fig. 1 in terms of a correction factor which is the amount by which the ground-state radiative recombination-rate has to be multiplied to give my computed-rate of populating the ground-state.

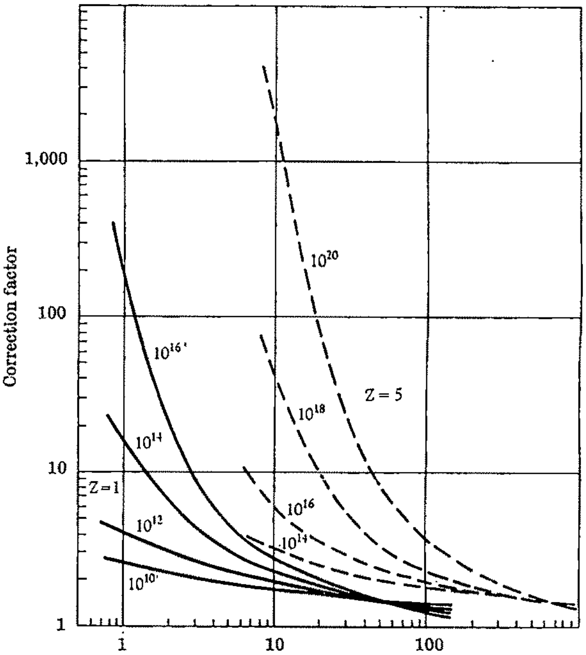

Fig. 1. Computed rates of populating the ground-states. The curves are for ions having nuclear charges 1 and 5 and for various electron densities (cm.$^{-3}$)

$$\text{Correction factor} = \frac{\text{Computed rate of populating the ground-state}}{\text{Radiative recombination-rate into the ground-state}}$$

The departures of the populations of the states from their Saha values is illustrated in Fig. 2 ($kT_e = 1$ eV.). In this connexion it is useful to designate a particular state which approximately marks the lower limit of the Saha region of the term scheme as the 'collision limit'. I define it as the lowest state for which the bound electron has a greater probability of making a transition to an upper state than to a lower state. The collision limits are marked on Fig. 2. The broken curve of this diagram depicts

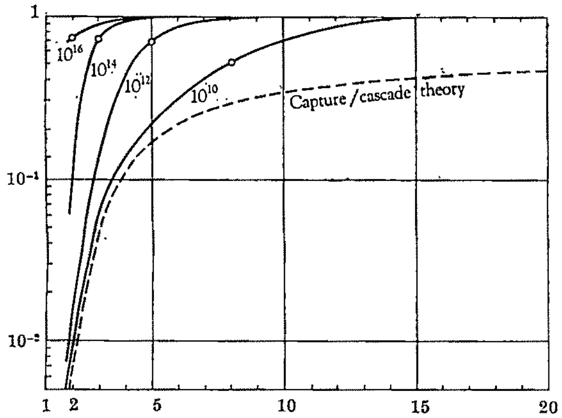

Fig. 2. Populations of the excited states of hydrogen in a recombining plasma at an electron temperature $kT_e = 1$ eV. The curves are for various electron densities (cm.$^{-3}$)

$$b_i = \frac{\text{Calculated population of state } i}{\text{Saha population of state } i}$$

O, Collision limits.

the case where radiative processes only are taken into account. This case was worked out by Seaton[7].

The set of curves of Fig. 2 illustrates clearly the effect of increasing density on the mechanism by which electrons leave the continuum and become bound. In the limit of very low density, radiative recombination is the only mechanism, and the states are populated according to the capture-cascade theory. However, the curves of Fig. 1 do not tend to unity because radiative recombination into upper states followed by spontaneous decay make a contribution. Such processes are more important at low density and this explains the cross-over of the curves of Fig. 1. As the density increases, the upper states progressively become populated more closely according to the Saha distribution. This is due to the increasing influence of collision processes (including three-body recombination).

The computed values for the rate of populating the ground-state may be compared with the experimentally observed ion decay-rates for the hydrogen plasma of *Zeta*[8]. For the conditions of the measurement the plasma parameters were (to a factor 2) $n_e = 10^{14}$ cm.$^{-3}$, $kT_e = 1$ eV. The rate at which the ion population decayed was about ten times greater than the radiative recombination-rate, in agreement with my computed value.

Since the work reported here was done, there have appeared two reports on similar calculations[9,10]. The results of Bates and Kingston are in good agreement (±30 per cent) with mine. Besides providing useful confirmation of this other work, I have investigated the effect of ionic charge, have extended the calculations to higher-electron temperature, and have presented the computed populations of the excited states.

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Doubling of the Proton Magnetic Resonances of Certain Organophosphorus Compounds

We have found that some of the proton magnetic resonances of the alkyl groups of several types of organophosphorus compounds are doubled. This doubling is in addition to the resonance multiplication that would be predicted from coupling with other magnetic nuclei in these compounds. So far we have observed resonance doubling for three types of structures. Type (I) is exemplified by 2-propyl phenylphosphinyl chloride. In compounds of this type, the alkyl group is directly linked to the phosphorus atom. Type (II) is exemplified by di-2-butyl phenylphosphate or di-2-butyl phenylphosphonate. In such compounds the alkyl group is linked through an oxygen atom to the phosphorus atom. Type (III) is exemplified by di-2-propyl phenylphosphonite, in which the phosphorus is trivalent.

It seems probable that the resonance doubling with type (I) compounds is due to the same sort of hindered rotation that produces resonance multiplication in substituted ethanes[1,2]. However, in the case of type (I) compounds the axis of rotation is the bond between the α carbon atom and the phosphorus atom rather than between two carbon atoms. A Fisher–Hirschfelder–Taylor model of the 2-propyl phenylphosphinyl chloride shows that if one of the rotational isomers is energetically preferred over the other two, then, on the average, one of the methyl groups is closer to the benzene ring than is the other. Since the benzene ring possesses a relatively strong induced magnetic field, the methyl groups would, on the average, be in different magnetic environments, and two sets of resonance signals would result.

We have, at present, only an incomplete concept of the structural features that cause resonance doubling in type (II) compounds. It seems likely that the same structural effects that cause doubling in type (I) also cause the resonance doubling for type (II). However, the situation is complicated by the intervention of an oxygen atom between the phosphorus atom and the α carbon atom of the alkyl group. The resonance doubling in type (II) compounds was observed only when the molecule contained one aromatic substituent and when the alkyl group was linked through an internal carbon atom. Di-1-alkyl phenylphosphates and di-1-alkyl phenylphosphonates did not exhibit the doubling. Qualitative proton

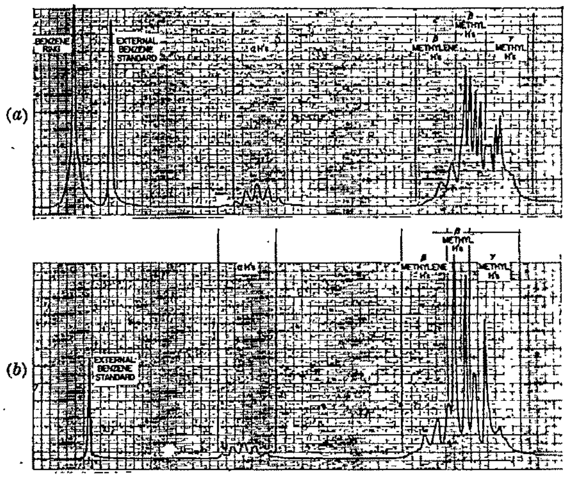

Fig. 1. (*a*) Di-2-butyl phenylphosphate. (*b*) Tri-2-butyl phosphate

resonance spectra of (*a*) di-2-butyl phenylphosphate and of (*b*) tri-2-butyl phosphate are given in Fig. 1. These spectra were run at 40 Mc./s. on a Varian nuclear magnetic resonance spectrometer, model *V*-4300*B*.

There is no background of experience with this type of resonance doubling in structures similar to that of type (III). However, it is perfectly evident that there must be conformations of type (III) that represent lower potential energies than other conformations. If in these conformations the two methyl groups in a 2-propyl radical are not interchanged with respect to their proximity to the benzene ring, then resonance doubling can occur. This is true since the average magnetic environment of the protons of the two methyl groups would be different.

We are continuing our investigations of the proton magnetic resonances of these three types and related compounds.

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RADIATION CHEMISTRY

Photoemission into Liquid *n*-Hexane

RECENT measurements of conduction[1] and of ion transit time[2,3] in liquid *n*-hexane have made use of the fact that electrons can be released into the liquid by the incidence of ultra-violet illuminations on the cathode surface. These measurements have been interpreted with the assumption that the ultra-violet light can be passed through the liquid to the cathode surface without producing ions in the liquid or otherwise disturbing it. Even in the most highly purified *n*-hexane it is not certain that this is true, particularly when subject to electric stress. The method described here attempts to overcome this difficulty.

Ultra-violet light is directed through a quartz window, attached to the liquid cell, on the inside of which is deposited a thin metal film cathode. Electrons are ejected from the opposite surface of the film into the liquid. By depositing a film of suitable thickness it is possible to produce a reasonable emission current without appreciably irradiating the liquid. Other advantages are that the area of the cathode illuminated can be controlled accurately and localized within the uniform field region, an important factor if current saturation is to be examined; and the spacing between electrodes in the cell can be made very small if desired, since the need for a large spacing for oblique illumination is removed. This will allow much higher fields to be attained with reasonable voltages.

The test cell was constructed from a short length of 2-in. diameter glass tubing, with a ground-glass flange at one end to hold the anode assembly. The opposite end was carefully ground to ensure that the electrodes were parallel. Both electrodes were sealed to the cell with cold-setting 'Araldite' since it was found that neoprene 'O' rings contaminated the liquid. The anode was a stainless steel disk, 1 in. in diameter; and in the present experiments the cathode film was aluminium with a transmission for visible light of about 50 per cent. The spacing between the electrodes was $3{\cdot}74 \times 10^{-2}$ cm., and they were parallel to within ± 2 per cent over the uniform field region. Finally, the cell was incorporated into a simple distillation system which allowed the liquid to be degassed at a pressure of about 10^{-3} mm. mercury.

After 24 hr. at a stress of 10 kV./cm., the residual conductivity of the liquid was less than 10^{-18} mho./cm., and on irradiating the outside of the cathode with ultra-violet light the current increased initially by a factor of 10^3. However, this did not remain constant but decreased slowly over a period of 20 min. by a factor of about 3. On switching off the ultra-violet illumination, slow-time effects were also observed in the decrease of conductivity. Morant[1] found similar slow variations for the case of direct illumination of the cathode through the liquid.

By reversing the polarity it was found that the current injected into the liquid with the metal film as cathode was about 100 times that injected with the film as anode. The fact that there was a current when the film was positive indicated that some radiation was passing through the film and either releasing electrons from the stainless steel electrode or photoionizing the liquid. The ratio of the two currents remained the same when the cell was emptied and evacuated, and this suggests that the currents originated at the electrodes and not in the bulk of the liquid. More careful control of the film thickness should allow the amount of radiation passing into the liquid to be reduced.

With the cell evacuated the current from the film became saturated with increasing voltage, and was about 10^{-8} amp. In the liquid for the same field and illumination intensity the current was approximately 10^{-11} amp. The variation of photocurrent with increasing field up to 700 V./cm. in *n*-hexane has been reported by Morant[1], and with the present experimental arrangement a similar variation was observed over the same field-range (curve *a*, Fig. 1). At higher fields,

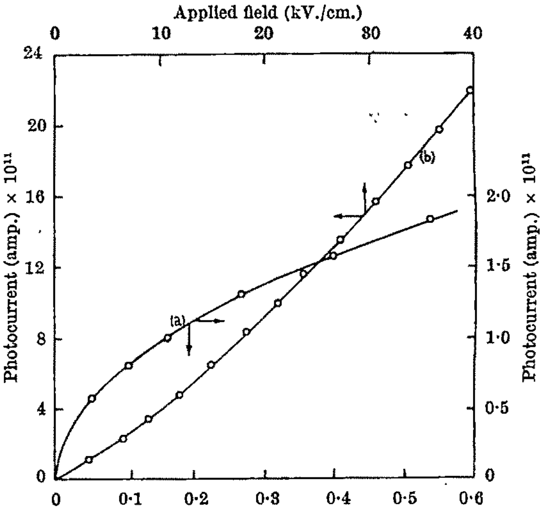

Fig. 1. Photocurrent injected into *n*-hexane for various field strengths with constant ultra-violet illumination

however, there is a marked change, and the current increases with increasing field (curve *b*, Fig. 1). Above 10 kV./cm. the current varies with field as $E^{1 \cdot 21}$.

Although the equipment used was not designed for mobility measurements, an estimate of the mobility of the negative ion in *n*-hexane can be made from the transient growth of current immediately after switching on the ultra-violet light, and the value so obtained (about 8×10^{-4} cm.²/V. sec.) is in rough agreement with other published data[2,3].

The experiments described here show that it is possible to inject an appreciable current into a dielectric liquid without having to subject the liquid to intense radiation. The fact that the current can be confined to the centre of the uniform field region is of great importance in the study of transient phenomena in which electrode edge effects can give rise to appreciable error. Also, the long time decay of current which is observed after the ultra-violet light is switched on would not be important if pulsed illumination were used, and the amount of charge injected into the liquid during the pulse would be considerably greater than that calculated from the magnitude of the steady current.

This work is part of a programme which is receiving financial support from the Department of Scientific and Industrial Research. I wish to thank Prof. M. W. Humphrey Davies for facilities provided, and Dr. T. J. Lewis for his encouragement and helpful discussions.

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The Importance of Hydroxyl Radicals as Intermediates in the Cross-linking of High Polymers by γ-Irradiation

We have irradiated thin gelatin films (thickness 0·5–1·0 mm.) with cobalt-60 γ-rays at a dose-rate of $4 \cdot 2 \times 10^4$ r. hr.$^{-1}$ at 10° C. The film was either suspended in a stoppered bottle containing oxygen or nitrogen or immersed in a liquid which filled the bottle. After irradiation of the film, attempts were made to dissolve it in 19 times its own weight of water at 40° C., and the viscosity of any resultant solution was measured. No increase in relative viscosity was observed when the irradiation was carried out in an atmosphere of oxygen or nitrogen, or when the film was suspended in liquid paraffin, benzene, ether, *cyclo*hexanone, pyridine, acetone or an alcohol, even when the dose exceeded 1 mega-roentgen. In the presence of water, however, the gelatin became cross-linked, as was shown by the fact that it was merely swollen by treatment with boiling water.

In a second series of experiments, gels prepared by dissolving 50 gm. of gelatin in 1 litre of water at 40° C. followed by 1 hr. in an ice-bath were irradiated at 10° C. immediately after covering with a layer of an organic liquid. Liquids such as methanol, glycerol and ether markedly reduced the cross-linking in that part of the gel which they had penetrated. These experiments indicate that cross-linking of gelatin induced by radiation is dependent on generation of free radicals by the action of the γ-rays on water, and that these radicals attack gelatine in reactions which probably involve abstraction of hydrogen atoms, thereby forming free radicals on the gelatin chains which become the cross-linking sites. Any organic liquid present diminishes the cross-linking either by competing with the gelatin for radicals from the water or by interfering with the cross-linking process.

If, as our results suggest, radicals play an important part in radiation-induced cross-linking of polymers, the otherwise anomalous results of Okada, Ito and Amemiya[1] may readily be explained. These authors observed that an atmosphere of nitrous oxide promotes the cross-linking of polyethylene induced by γ-irradiation, whereas an atmosphere of oxygen retards it. Dainton *et al.*[2] have shown that nitrous oxide reacts rapidly with hydrogen atoms (reaction 1) with formation of hydroxyl radicals and nitrogen,

$$H\cdot + N_2O \rightarrow HO\cdot + N_2 \qquad (1)$$

and it is known that oxygen 'scavenges' hydrogen atoms to form $HO_2\cdot$ radicals which are likely to be less effective than $HO\cdot$ radicals in causing cross-linking.

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CHEMISTRY

Prediction of Retention Data in Gas–Liquid Chromatography from Molecular Formulæ

As a result of the introduction of the R_{x9} method[1-3] of presenting gas–liquid chromatographic retention ratios, it has been possible to derive a simple method of predicting retention data from a knowledge of molecular weight and structure. Preliminary investigations have indicated its value in the structural analysis of organic compounds.

The retention index (I) of Kovats[4] is closely related to R_{x9} by the following expression (ref. 2):

$$I' = \frac{\log R_{x9}}{b} + 9$$

where I' is $I/100$ and b the slope of the log plot for *n*-paraffins. I' can be interpreted as the carbon number of a hypothetical *n*-paraffin having the same retention as the solute to which I' refers. The formula of the hypothetical *n*-paraffin equivalent to the solute is $C_{I'} \cdot H_{2I'+2}$ and will have a molecular weight given by $M_e = 14 \cdot 026\, I' + 2 \cdot 016 = 14 \cdot 026 \dfrac{\log R_{x9}}{b} + 128 \cdot 25$. This can be regarded as the 'effective' molecular weight of the solute. We have found the difference between the effective and true molecular weights of the solute (ΔM_e) to be a useful parameter for correlating retention data with chemical structure for the following reasons:

(1) ΔM_e is a conceptually simple parameter having the familiar dimensions of molecular weight.

(2) It is virtually constant throughout a homologous series (Table 1).

(3) It has no, or only a slight linear, variation with temperature (Table 2).

(4) ΔM_e is an additive function of the structural features of a molecule (Table 3) (except when specific group–group interactions occur).

Table 1. ΔM_e VALUES OF HOMOLOGOUS SERIES (65° C.)

Compound or class	Stationary phase	R_{x_9}	b	ΔM_e
Acetone ,	DNP	0·069	0·377	+27·0
Methyl ethyl ketone	DNP	0·163	0·377	+26·7
Methyl *n*-propyl ketone	DNP	0·345	0·377	+24·9
Methyl *n*-butyl ketone	DNP	0·841	0·377	+25·3
Butan-1-thiol	Squalane	0·152	0·391	+8·7
Pentan-1-thiol	Squalane	0·381	0·391	+8·7
Hexan-1-thiol	Squalane	0·939	0·391	+9·0
Heptan-1-thiol	Squalane	2·323	0·391	+9·1
Oct-1-ene	PEG 400	0·740	0·301	+9·1
Dec-1-ene	PEG 400	2·725	0·301	+9·3
Dodec-1-ene	PEG 400	10·02	0·301	+8·9
n-Alkane thiols	DNP	—	0·377	+20·0*
	Squalane	—	0·391	+8·9*
	PEG	—	0·301	+49·4*
n-Alcohols	DNP	—	0·377	+40·0*
	PEG	—	0·301	+106·7*
Alk-1-enes	DNP	—	0·377	+1·6*
n-Alkane bromides	DNP	—	0·377	−24·2*
n-Alkane cyanides	DNP	—	0·377	+45·8*
n-Alkane acetates	DNP	—	0·377	+8·3*

*Mean value for at least 4 homologues.

Table 2. VARIATION OF ΔM_e WITH TEMPERATURE

Compound	On 20 per cent di-nonyl phthalate/ 'Celite'		
	20° C.	65° C.	100° C.
t-Butanol	+21·7	+18·5	+16·0
Di-*t*-Butyl peroxide	−39·7	−40·6	−41·5
Butan-1-thiol	+19·2	+19·7	+20·1
Oct-1-ene	+1·9	+2·3	+2·1

The column b factors were 20°, 0·477; 65°, 0·377; 100°, 0·311.

Table 3. EFFECTIVE MOLECULAR WEIGHTS OF $\alpha : \omega$ DITHIOLS ON SQUALANE AT 65° C.

Compound	Calculated*		Found	
	M_e	R_{x_9}	R_{x_9}	M_e
Ethane-1 : 2-dithiol	111·9	0·350	0·320	110·5
Propan-1 : 3-dithiol	126·0	0·866	0·838	125·5
Butan-1 : 4-dithiol	140·0	2·268	2·268	140·0

* Using ΔM_e for SH group of 8·88.

(5) For a proposed structure, M can be computed and ΔM_e for the various structural features obtained from correlation tables. Thus M_e and hence R_{x_9} can be predicted. This has proved invaluable in identifying the products of a reaction where only a few alternatives are possible. Thus, once ΔM_e functional group correlation charts are available, retention times can be predicted without any prior chromatography. This is not practicable with the methods at present available.

(6) ΔM_e values of selected functional groups may be used to characterize chromatographic columns, particularly when the retention data are to be published. Thus, it should be possible to use the gas-liquid chromatography data confidently in the literature for the solution of everyday analytical problems.

(7) In general, ΔM_e values need to be expressed to fewer significant figures than I' contributions.

Work on the compilation of ΔM_e values and the application to column characterization, structural analysis and intramolecular interactions is proceeding in these laboratories and will be published in due course.

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Trimerization of Nickel-(II) Acetylacetonate in Solution

FOLLOWING Bullen, Mason and Pauling's confirmation of the trimeric structure of anhydrous nickel-(II) acetylacetonate[1], we report evidence for the persistence of the trimeric structure in solution.

We have prepared the anhydrous compound from the dihydrate both by vacuum sublimation and by azeotropic drying of toluene solutions, the latter method providing a particularly convenient means of obtaining water-free solutions. Cryoscopic molecular weight determinations on solutions of the anhydrous compound in benzene (dried over anhydrous magnesium perchlorate) gave the following results:

Weight of benzene (gm.)	23·12	23·12	23·12
Weight of acetylacetonate (gm.)	0·0985	0·2050	0·3185
Depression of freezing point (° C.)	0·027	0·059	0·093
Molecular weight	819	770	759

the mean molecular weight of 779 comparing well with a theoretical value for the trimer of 771.

Addition of water to solutions of the anhydrous compound in toluene precipitated the dihydrate; addition of ethanol precipitated the corresponding di-ethanolate and of pyridine the di-pyridine adduct, the solubility of which was high enough to permit spectrophotometric examination of the equilibrium:

$$\text{anhydrous compound} + \text{pyridine} \rightleftharpoons \text{pyridine adducts.}$$

Absorption curves for various ratios of the reacting species passed through one of two distinct isosbestic points, indicating a two-stage addition, but with considerable overlap of the two stages. However, by taking advantage of the variable reactivity of different heterocyclic bases we have been able to examine the two stages independently; thus, 4-methyl-pyridine gave virtually complete conversion to the 1 : 1-adduct and incomplete conversion to the 1 : 2-adduct, whereas 2 : 6-dimethyl-pyridine gave incomplete conversion to the 1 : 1-adduct without detectable formation of the 1 : 2-adduct. The form of the equilibrium constants for the two stages:

$$k_1 = \frac{[\mathrm{Ni(acac)_2.base}]^3}{[\{\mathrm{Ni(acac)_2}\}_3] \times [\mathrm{base}]^3};$$

$$k_2 = \frac{[\mathrm{Ni(acac)_2.base_2}]}{[\mathrm{Ni(acac)_2.base}] \times [\mathrm{base}]}$$

correspond to the reactions:

$$\{\mathrm{Ni(acac)_2}\}_3 + 3\,\mathrm{base} \rightleftharpoons 3\,\mathrm{Ni(acac)_2.base}$$

$$\mathrm{Ni(acac)_2.base} + \mathrm{base} \rightleftharpoons \mathrm{Ni(acac)_2.base_2}.$$

The constancy of the equilibrium constants obtained over a wide range of completion of each reaction is shown in Tables 1 and 2.

Bullen, Mason and Pauling's crystal structure determination shows that each nickel atom in the anhydrous trimer is octahedrally co-ordinated by six oxygen atoms, trimerization presumably occurring so as to maintain the co-ordination number of the nickel atoms. It is rather surprising therefore that the intermediate 1 : 1-adducts appear to be monomeric where we might expect dimerization to occur. Recent X-ray analyses of zinc acetylacetonate monohydrate[2] and a triarsine nickel bromide complex[3], however, suggest that five co-ordination may be more common than is often supposed.

Our observation that the trimeric structure, and hence the octahedral co-ordination of the nickel

Table 1. First-stage Equilibrium Constant. Total [Ni] = 25 mgm.-at./l.

[Base](mmole/l.)	0·0	3·0	6·1	9·3	12·6	16·0	19·5	23·1	26·8	30·5	34·5	∞
Optical density (675 mμ)	0·770	0·750	0·730	0·710	0·685	0·666	0·645	0·635	0·610	0·595	0·585	0·550
Per cent Ni as adduct	0·0	9·1	18·2	27·2	38·6	47·3	57·0	61·5	73·0	80·0	84·0	100
k_1 (mole^{-1})	—	4,800	3,300	3,300	7,200	5,100	5,700	2,550	4,500	3,900	2,700	—

Average k_1 = 4,200 moles^{-1}. Infinity value calculated.

Table 2. Second-stage Equilibrium Constant. Total [Ni] = 25 mgm.-at./l.

[Base](mmole/l.)	25·0	30·0	40·0	50·0	65·0	75·0	85·0	95·0	105·0	120·0	∞
Optical density (575 mμ)	0·320	0·360	0·472	0·520	0·670	0·710	0·755	0·770	0·790	0·830	0·950
Per cent Ni as adduct	0·0	6·3	24	32	56	62	69	71	75	81	100
k_2 (mole^{-1})	—	20	35	28	49	47	52	46	49	56	—

Average k_2 = 42 moles^{-1}. Infinity value calculated.

atoms, is preserved in solutions of anhydrous nickel acetylacetonate clarifies the difficulties which have been met in attempts to interpret the absorption spectra of these solutions. Both Jørgensen[4] and Maki[5] have commented on the similarity between the absorption spectra of the anhydrous and hydrated compounds, interpreting this in terms of ligand-field effects due to axial perturbations by the solvent in solutions of the anhydrous compound or to the smallness of the axial perturbations in the dihydrate. It is now apparent that both substances are examples of nickel-(II) cations in an octahedral environment of six oxygen atoms and the similarity of spectra is to be expected.

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Mullite Formation in the Aluminium Fluoride–Silica System (AlF_3–SiO_2)

The role of mullite, the conditions and kinetics of its formation are among the main problems of ceramic theory. The equilibrium conditions of the alumina–silica system have been established[1-3]. Grofcsik and Vago[4,5] have given an answer to the problems of kinetics on the basis of results obtained from their investigations carried out on silica gel and alumina. Several authors have dealt with the mineralization of mullite formation too[6-8]. The examination of the AlF_3–SiO_2 system suggests a way which may clear up the problem of possible mullite-formation mechanisms in ceramic products.

The following materials have been used: reagent grade aluminium fluoride with or without structural water, silica gel produced by the hydrolysis of ethyl silane, cristobalite obtained by 1,300° C. heat treatment of the latter, rock crystal and silica glass. All materials were finer than 60μ with similar particle-size distributions. Mixing was done mechanically in an agate mill, the heat treatment in an oxidizing atmosphere.

The gross process may be described by the following equation:

$$12\ AlF_3 + 13\ SiO_2 = 2(3Al_2O_3.2SiO_2) + 9\ SiF_4$$

using materials with structural water[9] by the reaction:

$$AlF_3 + SiO_2.\,xH_2O \rightarrow 3Al_2O_3.2SiO_2 + HF$$

is mentioned, too.

Examinations, however, have been carried out with a slight excess of silica. The samples were measured separately and dried at 105° C.

The reaction kinetics, as a function of heating time and temperature, have been determined by differential thermal analysis and thermogravimetric analysis. The formation of mullite has been detected microscopically and by X-ray diffraction patterns and determined quantitatively by a Muller diffractometer.

The following statements are made:

(1) The formation of silicon fluoride in the AlF_3–SiO_2 system begins at 600 ± 15° C. and is intense at 850–1,000° C.

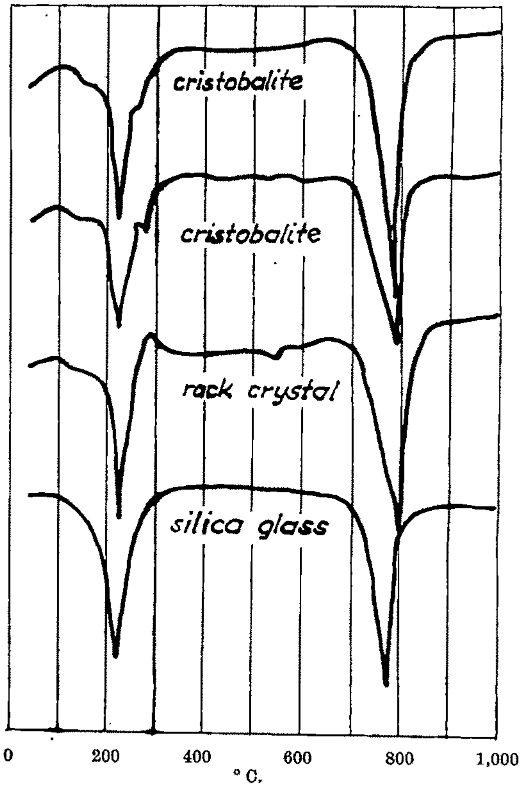

Fig. 1. Differential thermal analysis of various compositions

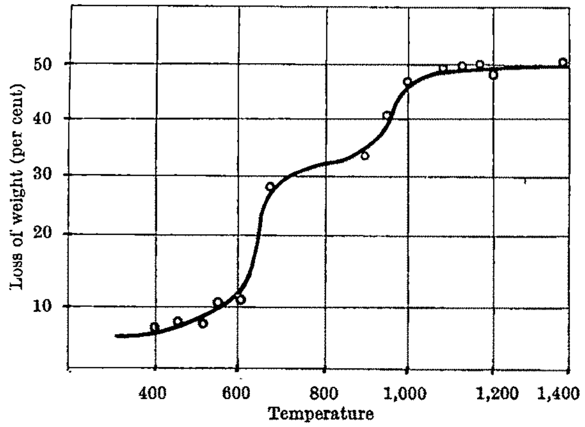

Fig. 2. Aluminium fluoride–cristobalite; loss of weight as a function of temperature

(2) The beginning and development of the reaction is a function of the particle size and the space between particles of the mix.

(3) The beginning of mullite formation does not coincide with the silicon fluoride formation, having a temperature higher by 300–400° C.

(4) The activation energy of mullite formation is lowest in the case of silica glass and higher with all other silica modifications.

(5) The initial temperature of mullite formation is 900 ± 15° C., using a mix of silica glass and aluminium fluoride (particle size less than 60μ) and agglomerated by a pressure of 50 kgm./cm.².

(6) The quantity of developed silicon fluoride increases at the start exponentially with temperature and is followed by a temporary quasi-steady state. The complete development of silicon fluoride and the simultaneous mullite formation needs further activation energy.

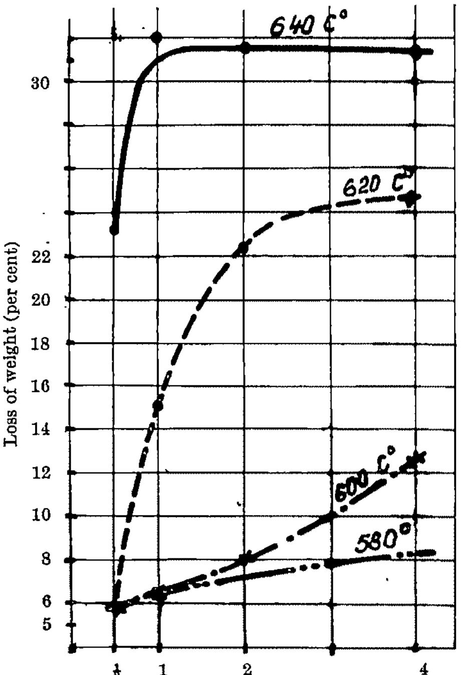

Fig. 3. Aluminium fluoride–cristobalite; loss of weight as a function of temperature and time of thermal treatment

Table 1. X-Ray Diffraction Pattern of the Silica Gel AlF_3 Mixture, Heat treated 2 hr.

d (kX.)	Intensity	Material	d (kX.)	Intensity	Material
3,718	3	$2Al_2O_3.2AlF_3.3SiO_2$	1,872	3	$2Al_2O_3.2AlF_3.3SiO_2$
3,216	3	,,	1,684	3	,,
3,050	1	,,	1,626	2	,,
2,951	3	,,	1,536	3	,,
2,502	1	,,	1,473	1	,,
2,377	3	,,	1,453	1	,,
2,191	1	,,	1,423	2	,,
2,127	3	,,	1,406	2	,,
2,062	2	,,	1,369	3	,,
1,993	1	,,	1,333	1	,,

Loss of weight against temperature plots, being similar in the case of all silica modifications, are shown in Figs. 2 and 3.

(7) Two endothermal effects are shown by differential thermal analysis in the case of a raw mix made of water containing aluminium fluoride and different silica modifications; the first from 105° to 330° C. and the second from 680° to 850° C. (Fig. 1).

(8) In the case of the aluminium fluoride–silica system the formation of silicon tetrafluoride ensures conditions in which mullite develops more rapidly than in the alumina–silica system. The velocity of mullite formation shows a rapid increase with temperature.

A crystalline and an amorphous fluorine-bearing body is formed at 600 ± 15° C. as proved by X-ray diffraction patterns of materials heat-treated in the interval 600–900° C. (Table 1).

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BIOCHEMISTRY

Reactions of Cytochrome-c with Methyl Linoleate Hydroperoxide

It is now well known that the oxidation of unsaturated fatty acids and esters is catalysed by a variety of hæmatin protein compounds[1,2]. The effect of cytochrome *c* has been of particular interest to us since we have evidence which conflicts with existing theories regarding the effect of cytochrome *c* on olefinic oxidation.

In our attempts to elucidate the apparent catalytic effect of cytochrome *c*, it was discovered that under certain conditions it could act as an antioxidant, particularly at *p*H's greater than 6·0. With freshly made emulsions of linoleic acid[3] prepared by adding phosphoric acid to a previously heated and then cooled solution of potassium linoleate in a slight excess of alkali, increasing amounts of cytochrome *c* produced longer and longer induction periods, and provided that sufficient cytochrome *c* was added, oxidation could be delayed for hours. Table 1 summarizes the kind of result that has been obtained by measuring the oxygen uptake of 3·3 ml. of an *M*/20 suspension of linoleic acid in *M*/20 phosphate buffer with gelatine as a stabilizer in Warburg manometers at 30°.

Table 1

Concentration of cytochrome *c*	Time (in min.) required for linoleic acid suspension to absorb :						
	0–10 c. mm.	10–50 c. mm.	50–100 c. mm.	100–200 c. mm.	200–300 c. mm.	300–400 c. mm.	400–500 c. mm.
$3\cdot50 \times 10^{-5}$ *M*	12	60	7	4½	4	5	5
$3\cdot15 \times 10^{-5}$ *M*	9	40	3	4	4½	5	5
$2\cdot80 \times 10^{-5}$ *M*	9	33½	5	3½	4½	5½	6½
$2\cdot45 \times 10^{-5}$ *M*	3	25	2½	4	5½	6½	9
$2\cdot05 \times 10^{-5}$ *M*	2	4	2½	5	6½	8	10½
$1\cdot75 \times 10^{-5}$ *M*	2	3	3	6	7½	10	12½

Oxygen uptake in the absence of catalyst is very slow, hours being required for the absorption of the first 10 c. mm. or so. The above results, however, show that cytochrome *c* at high concentrations has an antioxygenic effect in comparison with its behaviour at low concentrations. At $3\cdot5 \times 10^{-5}$ *M*, 72 min. are required for the absorption of the first 50 c. mm. and the maximum rate of absorption is reached after about 83 min. At half this concentration of cytochrome, the first 50 c. mm. of oxygen were absorbed in 5 min. and the rate of absorption was at its maximum at this time.

Changes in cytochrome *c* during oxidation experiments have been followed spectroscopically and have shown rapid falls in concentration coinciding with termination of the induction periods.

It seems to us that these phenomena can only be explained by assuming that cytochrome *c* catalyses the breakdown of unsaturated fatty acid peroxides into inactive products, but that during this catalytic process, the cytochrome becomes 'denatured', which is not unusual in enzymatic reactions of this kind, and that the 'denatured' cytochrome has the typical property of catalysing olefinic oxidation. The results that we have obtained depend on the suspensions used being free from traces of peroxides. It has been found, for example, that suspensions prepared by shaking linoleic acid with gelatin solution in an inert atmosphere do not exhibit the above phenomena with these concentrations of cytochrome *c*, and that the amount of heating the potassium soap is subjected to during the preparation of the precipitated suspension has an influence on the length of the induction period produced in the presence of cytochrome. It is thought that trace amounts of peroxide are removed from the linoleic acid in the present instance when the potassium soap is heated in order to bring it into solution.

The effect of cytochrome *c* on pure *trans-trans* methyl linoleate prepared as previously described[4] has now been studied. Working with a 5×10^{-3} *M* suspension of the pure hydroperoxide in a very weak solution of sodium dodecyl sulphate, it has been observed that cytochrome *c* at a concentration of $1\cdot6 \times 10^{-4}$ *M* will cause the almost instantaneous destruction of 80–90 per cent of the peroxide, and that about a third of the cytochrome is 'denatured' at the same time. Oxygen gas is not liberated during the reaction but there is a loss of conjugated di-ene unsaturation, just slightly less than the loss of peroxide. The reaction is not affected in any way by diluting the peroxide with an equal quantity of pure methyl linoleate, or by a change in *p*H over the range 5·8–8·0. Hæmatin and porphyrin *c* are both inactive; the former is slowly destroyed by the peroxide.

Work is continuing with the object of identifying the products of the reaction; in particular, it is hoped to identify the reduced hydroperoxide and to obtain further knowledge of the 'denatured' cytochrome *c*.

This work was carried out as part of the programme of the Department of Scientific and Industrial Research.

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Isolation of Peptides containing Hydroxyproline from Animal Tissues

The idea that fibrogenic cells secrete peptide which, in extracellular spaces, is built into collagen fibre has been postulated repeatedly in the course of the past thirty years[1,2]. There is no direct evidence, so far, of the existence of a peptide which, as a precursor of collagen, should contain the typical collagen imino-acid hydroxyproline. Peptides containing hydroxyproline isolated from urine[3,4] can be considered rather as products of collagen catabolism than as steps in the course of biosynthesis of collagen.

In 1959 we found peptide-bound hydroxyproline in ultrafiltrates of extracts from different animal tissues; for elucidation of its biological significance we have presented some work[5–7] which favours the hypothesis that peptides represent non-protein precursors of collagen protein.

In this communication we give the results of experiments dealing with the isolation and identification of peptides containing hydroxyproline.

We have experimented mostly with the skin of 2–4-months-old pig embryos. Apart from that we have investigated the granulation tissue after the application of carrageenin under the skin of guinea pigs, and we have examined growth-zones of bones in bovine embryos. Results presented in this communication involve peptides from pig embryo skin.

Fresh tissue was homogenized, immediately after freezing with liquid air, by pulverization in a mortar and shaken in a mixture of ethanol and 0·5 per cent of acetic acid (3 : 1 v/v). The suspension warmed to boiling point was kept overnight at 0° [8]. After centrifugation, the ethanol in the supernatant was removed by low-pressure distillation at a temperature not exceeding 20° and condensed by freeze-drying. For deproteination we used a metal ultrafilter with membrane 'Nojax' cellulose casings, Visking Corp., Chicago, at a pressure of 7 atm. approximately and a temperature of 1°. The ultrafiltrate was independent of the total ultrafilterable hydroxyproline condensed again by freeze-drying. Fractionation of ultrafiltrate was carried out first by column chromatography (100 cm. × 1 cm.), using 'Dowex 50 × 4', mesh 200 in ammonia cycle. The column was developed by passing the system through a solution of 0·2 *M* ammonium formate (*p*H 3·3) and 0·2 *M* ammonium acetate (*p*H 5·3) into the mixing chamber used for gradient elution at 20–30 ml./hr. and effluent was collected in 5-ml. fractions. Fractionation was followed by determining the content of free and bound hydroxyproline in different fractions. Bound hydroxyproline was ascertained from the difference in hydroxyproline

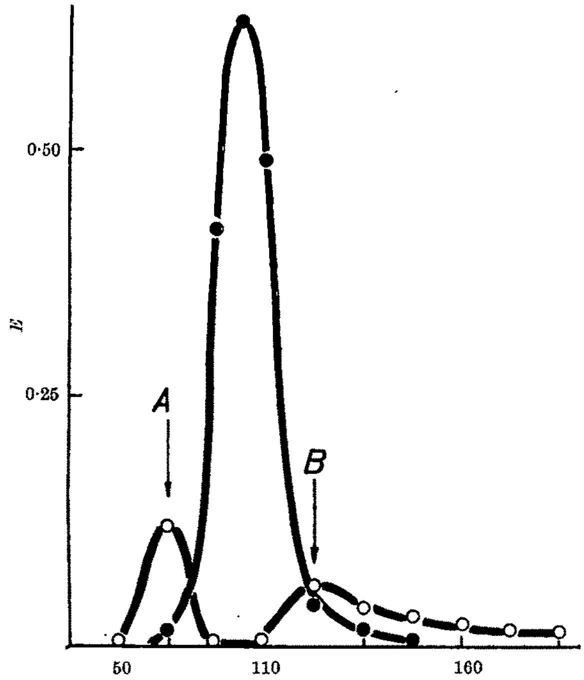

Fig. 1. Free hydroxyproline and peptide in ultrafiltrate of extract from pig embryo skin fractionated on 'Dowex 50 × 4'. ○, Peptide-bound hydroxyproline; ●, free hydroxyproline; the amount of hydroxyproline is given by extinction of sample.

content before and after acid hydrolysis of the sample (3 hr., 140°, 6 *N* hydrochloric acid). Fractions containing bound hydroxyproline were re-chromatographed on Whatman 4 (descending) in butanol/acetic acid/water (4 : 1 : 1). Peptides and amino-acids were detected by 0·1 per cent ninhydrin solution in acetone, by a specific method for detecting hydroxyproline[9] and after chloration of peptide bonds by iodostarch reagents. Strips of chromatograms containing peptides were eluted by water and re-chromatographed in a similar way. Hydroxyproline[10] and proline[11] were determined colorimetrically in aliquots of extract. The amino-acid composition of individual peptides was ascertained after acid hydrolysis, using paper chromatography as mentioned.

As shown in Fig. 1 with column chromatography, we found two fractions, *A* and *B*, with peptide hydroxyproline. By preparative paper chromatography we identified in fraction *A* at least four other peptides in which the content of proline and hydroxyproline was determined. It was found that molar relation of proline to hydroxyproline in individual peptides deviates from 5 : 1 to 1 : 1. Owing to the fact that the ratio of both imino-acids mentioned is 1 : 1 in mature mammal collagens, this finding is in good agreement with the generally accepted opinion, that hydroxylation of proline proceeds after its incorporation into peptide. Homogeneous spots of peptides A_1–A_4 obtained by re-chromatography were hydrolysed and the amount of amino-acid determined. The list of amino-acids is given in Table 1. This examination proved that peptides with greater mobility on paper chromatography contain a lower number of different amino-acids. But glycine, glutamic acid, proline and hydroxyproline were always found. Some spots have not been identified so far.

Table 1. AMINO-ACID COMPOSITION OF INDIVIDUAL PEPTIDES OF FRACTION *A*

A_1	A_2	A_3	A_4
Lys	Lys	Gly	Gly
Arg	Gly	Glu	Glu
Gly	Glu	Hypro	Hypro
Glu	Hypro	Ala	Pro
Hypro	Ala	Pro	
Ala	Pro		
Pro	Leu		
Val			
Leu			

Peptide hydroxyproline formed 20–30 per cent of total ultrafiltrable hydroxyproline.

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Demethylation in the Metabolism of (−)-Nicotine *in vivo*

THE physiological disposition of nicotine randomly labelled with carbon-14 has been subjected to investigation by many authors[1–3]. After intravenous administration to mice[1] and to dogs[2] analyses of the expired air showed no radioactive carbon dioxide. In an extension of this work, in which degradation of nicotine was investigated, the methylamine fraction of dog urine was examined for radioactivity and found[3] to contain none.

If the foregoing results are accepted as being completely definitive for the metabolism of nicotine, the inevitable conclusion is that nicotine undergoes an unusual metabolism with no degradation to single-carbon metabolites. This conclusion would tend to contradict our work[4] in which demethylcotinine, 5-(3′-pyridyl)-pyrrolidone-2, has been identified in urine after administration of nicotine. The need for a direct experimental resolution of this apparent contradiction has led to our employment of labelled nicotine containing a high level of activity in the methyl group.

Nicotine-methyl-^{14}C was conveniently synthesized in high yield by methylating (−)-nornicotine with formaldehyde-^{14}C in the presence of formic acid. This methylation reaction has been previously employed as a quantitative analytical procedure[5].

Male albino rats, weighing approximately 250 gm. and housed in glass metabolism cages, received the labelled nicotine intraperitoneally (0·2 mgm./100 gm.). The approximate radioactivity of the dose was 24·8 × 10^3 c.p.m. Expired carbon dioxide was collected in sodium hydroxide traps and then precipitated as barium carbonate for counting at infinite thickness. Radioactivity was readily demonstrated in the expired air. During the first 6-hr. period after administration of the nicotine, the respiratory carbon dioxide from three animals contained an average of 5·7 per cent of the administered radioactivity with individual values: 7·5, 4·0, and 5·6 per cent. Further elimination as radioactive carbon dioxide was observed in a subsequent 18-hr. period to give cumulative values of 12·8, 6·2 and 8·4 per cent of the administered dose. In addition, a small but significant amount of carbon-14 activity was present as urea (as determined from carbon dioxide evolved by urease) in the individual 24-hr. urines (0·26, 0·17, and 0·24 per cent).

The fact that previous workers did not obtain any indication that nicotine is degraded to carbon dioxide may be attributed to a lower level of methyl-^{14}C activity of the nicotine used in their experiments. Although our work with nicotine-methyl-^{14}C has not included any experiments on the dog, and does not indicate the precise point at which demethylation occurs, the fact that demethylcotinine arises from both (−)-nicotine and (−)-cotinine[4] in the dog points to the importance of single-carbon compounds in the metabolism of nicotine in this species.

Since the radioactivity of randomly labelled nicotine-^{14}C is excreted[2] to the extent of 94 per cent in the urine of rats, the addition of carbon-14 determined as carbon dioxide in our investigations gives a total elimination which approaches 100 per cent. This provides convincing evidence that, after single doses, storage of nicotine or its metabolites occurs to only an extremely limited extent.

We wish to thank the American Tobacco Co. and the Tobacco Industry Research Committee for generous support.

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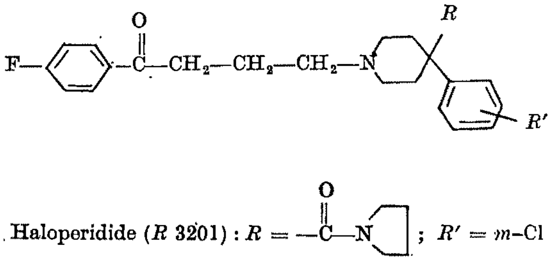
Haloperidide (*R* 3201), a Highly Potent and Selective Anti-emetic Agent in Dogs

HALOPERIDIDE, the proposed generic name for 4′-fluoro-(4-(4′-pyrrolidin-amido-4′-*m*-chlorophenyl)-piperidino)-butyrophenone (serial number *R* 3201), was synthesized in the Organic Chemistry Department of this Laboratory. It is a new member of the butyrophenone series of neuroleptic compounds (Fig. 1). The prototype of this series, haloperidol (*R* 1625), has been described in a number of pharmacological and clinical publications. It has been shown that haloperidol is pharmacologically related to the potent neuroleptics of the phenothiazine-series[1–6].

Both haloperidol and perphenazine are potent inhibitors of the emetic effect of apomorphine in dogs[2,3,5]. After subcutaneous injection, haloperidol has a quicker onset and a considerably longer duration of action than perphenazine, the estimated potency ratios varying considerably as a function of the time-interval between the injection of the drug and of the apomorphine challenging dose (Table 1). Haloperidide is considerably more active than either haloperidol or perphenazine in this test. It has a somewhat longer duration of anti-emetic action than perphenazine, but the onset of its effect is much shorter (Table 1).



Haloperidide (*R* 3201): *R* = —CO—N ; *R′* = *m*-Cl

Haloperidol (*R* 1625): *R* = OH; *R′* = *p*-Cl

Table 1. POTENCY RATIOS IN DOGS

Hr. after subcutaneous injection	Drug	*A* anti-emetic potency	*B* 'jumping box'	Ratio *A*/*B*
1–5	perphenazine	1	1	1
	haloperidol	1	4	1/4
	haloperidide	10	4	5/2
25–30	perphenazine	1	1	1
	haloperidol	4	6	2/3
	haloperidide	20	4	5

In an effort to evaluate the selectivity of these anti-emetic effects, we investigated the influence of the three drugs on avoidance and escape behaviour of trained dogs in a 'jumping box' situation. The details of the procedure, similar to the well-known Warner technique for rats, are described in a previous communication[6].

Briefly, the 'jumping box' consists of two compartments, separated by a partition. The floor of each compartment is a grill which can be charged electrically. The animal can escape shock or avoid it by jumping into the other compartment when presented with a warning signal (buzzer; maximum duration of 15 sec.). After adequate training, the dogs are treated at random at weekly intervals with selected doses of each compound, the effects on reaction-time, avoidance and escape being investigated 1, 5, 25 and 30 hr. after dosage.

The potency ratios in Table 1 are derived from two separate Latin square experiments. In one experiment four doses of haloperidol and four doses of haloperidide were investigated in eight dogs (8 × 8 design). In the other experiment six dogs were treated with three doses of haloperidol and three doses of perphenazine (6 × 6 design). The results obtained with all three substances are qualitatively similar. At low dose-levels, reaction times are prolonged. With increasing dosage, this effect leads to gradual and transient loss of learned avoidance and of escape behaviour.

It is concluded that haloperidide is a more potent, more selective, faster and longer acting antagonist of the emetic effects of apomorphine than perphenazine.

It is also more potent and more selective than haloperidol, but shorter acting.

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Effect of Erythropoietine on Incorporation of Formate labelled with Carbon-14 into the Nucleic Acids of Normal Rabbit Tissues *in vitro*

It has previously been shown that the blood and urine of rabbits suffering from hæmolytic anæmia contain a mucoprotein humoral factor, hæmopoietine or erythropoietine, which on injection into normal animals stimulates erythropoiesis[1]. The present experiments were undertaken to find out what effect this factor might have on nucleic acid biosynthesis in normal rabbit tissues *in vitro*. A crude preparation of erythropoietine was obtained from the urine of animals made anæmic with phenylhydrazine. The urine was brought to *p*H 4·5 with hydrochloric acid, and 4 volumes of ethanol were added. After standing overnight at 4° C. the precipitate was centrifuged down, washed with ethanol and ether, and dried. The dry powder was extracted with a small volume of Krebs–Ringer bicarbonate buffer. The protein concentration of this 'anæmic urine extract' was determined by the biuret method. A 'normal urine extract' was similarly prepared from the urine of normal rabbits. The effects of both extracts on the incorporation of formate labelled with carbon-14 into the nucleic acids of bone marrow, spleen and liver *in vitro* were determined by the methods of Smellie *et al.*[2].

Table 1. Incorporation of ^{14}C-formate* into Nucleic Acid Bases of Normal Rabbit tissues *in vitro*

Additions	Product analysed	Specific activity (counts/min./μmole)					
		Bone marrow		Spleen		Liver	
		RNA	DNA	RNA	DNA	RNA	DNA
Nil	Adenine	2,495	1,183	426	188	128	406
	Guanine	539	631	167	113	100	102
	Thymine		24,500		317		1,026
'Normal urine extract'†	Adenine	980	536	489	159	240	486
	Guanine	335	395	202	108	167	—
	Thymine		16,200		438		675
'Anæmic urine extract'†	Adenine	3,080	963	3,750	662	3,055	734
	Guanine	—	374	1,205	372	1,220	425
	Thymine		28,400		730		671

Incubation time, 4 hr. * Isotope concentration, 5 μc./ml. † Concentration of extracts, 2·3 mgm. protein/ml. RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

The results obtained are shown in Table 1. In agreement with results previously reported[3], the pattern of incorporation in normal bone marrow is characterized by the very high activity of the deoxyribonucleic acid thymine relative to the purines of both ribonucleic acid and deoxyribonucleic acid. In contrast, in spleen and liver deoxyribonucleic acid the activity of the thymine is generally only slightly greater than that of the purines. In previous papers[2,3] it has been argued that these observations may indicate that bone marrow, unlike the other tissues, may be incapable of synthesizing purine nucleotides fast enough to meet its own requirements. Bone marrow also differs from spleen and liver in its response to the urine extracts. The 'anæmic extract' produces virtually no effect on it, while the 'normal extract' tends to depress the activity of the nucleic acid purines and to a lesser extent, of the deoxyribonucleic acid thymine. In liver and spleen, on the other hand, the 'normal extract' has little or no effect, while the 'anæmic extract' causes a dramatic increase in the activity of the purines in both nucleic acids. In spleen, though not in liver, the anæmic extract also increases the activity of the deoxyribonucleic acid thymine.

These observations suggest that *in vivo* erythropoietine may stimulate *de novo* nucleic acid synthesis in liver and spleen, but not in bone marrow. However, Smellie *et al.*[4] have found that, in the rabbit, hæmolytic anæmia results in increased incorporation of $^{32}PO_4$ into the nucleic acids, not only of spleen, but also of bone marrow. An explanation of this latter observation may perhaps be found in the relationship between liver and bone marrow. Lajtha and Vane[5] have shown that *in vivo* hepatectomy greatly diminishes incorporation of ^{14}C-formate into the nucleic acid purines of rabbit bone marrow. Clearly, therefore, the liver must play some part in the synthesis of the purines which the bone marrow requires (for example, for nucleic acid synthesis), but which it cannot synthesize for itself. It is therefore quite possible that erythropoietine might stimulate erythropoiesis in the bone marrow indirectly by acting on the liver. The increased incorporation of ^{14}C-formate into liver nucleic acid purines produced by erythropoietine in the present experiments might be a reflexion of such action.

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Adrenocorticotrophin and the Sugar-level in Plasma in Young Fowls

THIS communication is prompted by the finding of Urist and Deutsch[1] that large doses of freeze-dried adrenocorticotrophin of mammalian origin were without effect on the blood sugar of adult cocks (Black Minorcas) and hens (White Leghorns). Some years ago, work in this laboratory required the intramuscular injection of Brown Leghorn pullets and cockerels (5 weeks of age, body-weight 500 gm.) with large doses of long-acting adrenocorticotrophin ('Acthar', Armour). It was found that the hormone could produce a marked rise in the level of plasma sugar in these young birds, which were kept in brooders with free access to food and water throughout the experimental period.

Trichloroacetic acid filtrates of the plasma were analysed by my adaptation[2] of Jones and Pridham's method[3] (1954). Injections (0·5 ml.; 20 I.U.) of 'Acthar' were made into the pectoral muscle and the birds returned to the brooder. These injections were repeated on the two following days. The birds at no time showed signs of distress or lack of appetite. On the fourth day, blood was again taken for analysis; in some instances further samples were taken on the 5th, 8th and 18th days after the start of the experiment. Control birds were not injected.

The results are summarized in Table 1. While injection of adrenocorticotrophin was followed on the 4th day by a marked rise in the plasma sugar in seven out of eight experiments, by the 5th day the sugar had fallen to, if not below, the normal level. The responses of the two sexes were indistinguishable. It will be noted that the effect of the long-acting 'Achthar' was not observed 48 hr. after the last injection.

The present findings, along with those of others, on the effects of adrenocorticotrophin, raise the question whether the stage of development of the fowl is a factor in relation to the effects of the hormone. Urist and Deutsch[1] found no effect on the sugar-level of whole blood from adult birds when this hormone was administered, in contradistinction to mammalian species. Perek and Eckstein[4], and Perek and Eilat[5] showed that the adrenals of the young immature bird failed to respond to adrenocorticotrophin with respect to ascorbic acid depletion, again in contradistinction to the mammal, due to the activity of a lymphoepithelioid organ of as yet incompletely defined function, the bursa of Fabricius. This, situated at the dorsal aspect of the proctodæum, involutes during the approach to maturity. The ascorbic acid of the adrenal of the bursectomized chick is responsive to adrenocorticotrophin. (*Note added in proof.* H. S. Siegel and W. L. Beane (*Poultry Sci.*, 40, 216; 1961) have also observed hyperglycæmia subsequent to intramuscular adrenocorticotrophin-gel in 6–7-week-old fowls.)

Table 1. EFFECTS ON REDUCING SUGAR OF PLASMA OF INTRAMUSCULAR 'ACTHAR' GEL ON FIVE-WEEK-OLD PULLETS AND COCKERELS
Injections were given on days 1, 2 and 3

Exp. No.	Injection Days	Plasma reducing sugar (mgm./100 ml.) *D*1	*D*4	Change *D*1 to *D*4	*D*5	*D*8	*D*18
1	'Acthar'	205	240	+ 35	—	—	—
2		310	950	+640	—	—	—
3		270	480	+410	—	—	—
4		240	450	+210	—	—	—
5		295	670	+375	230	210	230
6		250	530	+280	210	225	220
7		275	410	+135	205	220	230
8		290	710	+420	200	200	220
—	Nil (controls)	220	250	+ 30	—	—	—
—		225	255	+ 30	—	—	—
—		240	235	− 5	—	—	—
—		250	240	− 10	—	—	—
—		230	230	0	—	—	—
—		230	235	+ 5	—	—	—
—		200	190	− 10	—	—	—
—		190	205	+ 15	—	—	—
—		190	200	+ 10	—	—	—

Drs. J. Beattie and J. G. Campbell and I find that bursectomy does not alter the positive response of the 5-week-old bird to long-acting adrenocorticotrophin. Adults, 24 hr. after 40 I.U., show 500–600 mgm. of reducing sugar per 100 ml. of plasma along with diuresis and glycosuria. On the other hand, I have so far failed to find any hyperglycæmic effect, on young intact and bursectomized birds, after freeze-dried adrenocorticotrophin.

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Presence of Sialic Acid in a Granuloma Pouch

THE presence of sialic acid in connective tissues has already been described[1]. It is well known that by means of a granuloma pouch newly formed connective tissue can be induced; this is very important for the evolution of inflammation. The presence and the concentration of sialic acid in the granuloma pouch have now been investigated.

Granulomas were induced in 150-gm. male rats by the use of 10 ml. of air and 0·4 ml. of croton oil solution (4 per cent in olive oil). The animals were killed 1, 3, 5 and 8 days after treatment. The dissected granulomas were homogenized in acetone and stirred for 48 hr.; after centrifugation, the sediment suspended in ethyl ether was dried in vacuum. The powder so obtained was employed to determine nitrogen and sialic acid content by Svennerholm's method[2].

For the last determination the powder was hydrolysed with 0·1 *N* sulphuric acid at 80° C. for 1 hr.; the hydrolysate was filtered on a sintered glass filter and chromatographed on a 'Dowex 2 × 8' column; after elution with 1 *M* acetate buffer *p*H 4·6, the eluate was tested by the resorcinol reaction and sialic acid was identified by the absorption spectrum of the reaction.

A control determination was carried out using an untreated region of the abdomen (non-inflamed tissue).

Table 1. PRESENCE OF SIALIC ACID IN A GRANULOMA POUCH INDUCED IN RAT

Days after treatment	Values referred to 100 mgm. of acetonic powder: Granuloma pouch	Non-inflamed tissue
1	480 μgm.	96 μgm.
		91 ,,
3	426 ,,	78 ,,
	473 ,,	
5	319 ,,	
	386 ,,	
8	313 ,,	47 ,,
	343 ,,	

N-acetyl neuraminic acid, kindly supplied by Prof. G. Blix, was used as a standard to determine the sialic acid quantitatively. The results are presented in Table 1.

The results indicate that the content of sialic acid in a granuloma pouch is much greater than in a corresponding abdominal region not involved in the pathological process.

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Content of Sialic Acid in Serous Glands of Equines

In histochemistry it is well known that an objection to the specificity of the periodic acid–Schiff reaction for mucopolysaccharides is due to the possible interference of some amino-acids. This objection is particularly important when the periodic acid–Schiff reaction is applied to serous glands, in which the secretion is essentially proteins. However, Junqueira *et al.*[1] found material giving a positive reaction in the parotids of many mammals, and concluded that substances other than proteins were present in the secretion of parotid gland. This observation opened up an interesting problem of cellular biology, regarding the possibility of a multiple secretion from serous glands.

In order to confirm this observation, a comparative histochemical investigation of material positive to the periodic acid–Schiff reagent and the ribonucleic acid content of some serous glands was carried out by Bignardi *et al.*[2]. This investigation, using pancreas, parotid and orbital glands of equines, demonstrated that the intensity of periodic acid–Schiff reaction varied inversely with the ribonucleic acid content. On the basis of general views on the function of ribonucleic acid in protein biosynthesis, this result was interpreted as indirect evidence that positive reaction to the periodic acid–Schiff reagent was really due to carbohydrate material.

Since more direct evidence for this view could be obtained by chemical analysis of some constituent of mucopolysaccharides, we have investigated the content of sialic acid in serous glands. The emphasis on sialic acid was due to its presence in submaxillary mucin[3] and to its strong reactivity for periodic acid. (In a very recent paper of Quintarelli *et al.*[4], a relation between sialic acid and positive periodic acid–Schiff reaction has been reported.)

Sialic acid has been determined in orbital glands (lacrimal gland and gland of the third eyelid), parotid and pancreas of ass and horse.

The glands were removed and stored under acetone. About 50 mgm. (dry tissue) of each gland was used for sialic acid determination, which was carried out by Svennerholm's method[5]. The tissue was homogenized with 5 ml. of 0·1 *N* sulphuric acid by Potter–Elvehjem homogenizer and refluxed at 80° C. for 1 hr. The hydrolysate was centrifuged and the supernatant chromatographed on 'Dowex 2 × 8'; the sialic acid was eluted by acetate buffer 0·1 *M*, *p*H 4·6, and sialic acid determined by the resorcinol reaction.

Table 1. Content of Sialic Acid in Serous Glands of Equines
(Values are referred to 100 mgm. of dry tissue)

Animals	Lacrimal gland	Gland of the third eyelid	Parotid	Pancreas
Ass 1	500 μgm.	410 μgm.	195 μgm.	118 μgm.
2	420 ,,	362 ,,	167 ,,	127 ,,
3	430 ,,		181 ,,	
Horse*				
1	621 μgm.	408 μgm.	157 μgm.	134 μgm.
2	778 ,,	427 ,,	138 ,,	168 ,,

* The presence of sialic acid in parotid gland of the horse has been reported by Svennerholm (ref. 5).

The standard curve was obtained with N-acetylneuraminic acid isolated from sheep submaxillary gland and kindly supplied to us by Prof. G. Blix. The results are reported in Table 1.

The results show that the highest content of sialic acid is in the orbital glands, a low content in parotid and the lowest in pancreas. Since positive periodic acid–Schiff reaction is also highest in glands of the orbit, is less in the parotid and is weakly positive in the pancreas, there seems to be a relation between the periodic acid–Schiff reaction and sialic acid content.

The presence of sialic acid and, probably, of a sialopolysaccharide, in serous glands shows that these glands elaborate not only protein but also carbohydrate material.

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In vivo Metabolism of N-Acetylhexosamine ^{35}S-Sulphate Esters

In a previous report[1] evidence was presented indicating that the monosulphate ester obtained as the principal product of the direct sulphation of N-acetyl-D-glucosamine with chlorosulphonic acid was identical with N-acetyl-D-glucosamine 6-O-sulphate prepared by definitive synthesis. In addition, following preliminary observations on the structure of the monosulphate ester prepared by the direct sulphation of N-acetyl-D-galactosamine[1], independent results from work on tritylation (triphenylmethylation)[2], and infrared spectroscopy[3,4], indicate that this material is also a 6-O-sulphate ester. Since available information suggests that N-acetyl-D-glucosamine 6-O-sulphate and N-acetyl-D-galactosamine 6-O-sulphate exist *in vivo* as part of the polymer chains of keratosulphate and chondroitin sulphate-C respectively[5,6], it was of interest to examine the *in vivo* metabolism of these N-acetylhexosamine sulphate esters.

The direct action of chloro-^{35}S-sulphonic acid on N-acetyl-D-glucosamine and N-acetyl-D-galactosamine yielded in each case a mixture of the mono-^{35}S-sulphate derivative together with the di-^{35}S-sulphate and unchanged parent compounds[1]. These preparations were fractionated by zone electrophoresis on

Table 1. DISTRIBUTION OF RADIOACTIVITY IN RAT URINE, FÆCES AND CARCASE AFTER THE INJECTION OF N-ACETYL-D-GLUCOSAMINE 6-O-^{35}S-SULPHATE

Results (expressed as percentage of the injected dose) are average values for 3 animals in each instance. The radioactivity of the doses corresponded to $1{\cdot}3 \times 10^5$ counts/min. (10 mgm.) and $2{\cdot}6 \times 10^5$ counts/min. (20 mgm.) measured after hydrolysis as an 'infinitely thick' plate of $Ba^{35}SO_4$

Sex	Dose (mgm.)	Distribution in urine								Distribution in fæces				Residual ^{35}S in carcase after 96 hr.
		Inorganic sulphate fraction				Combined inorganic and ester sulphate fraction								
		24 hr.	48 hr.	72 hr.	96 hr.	24 hr.	48 hr.	72 hr.	96 hr.	24 hr.	48 hr.	72 hr.	96 hr.	
Male	10	7·2	2·1	1·3	0·6	89·5	2·7	1·9	0·9	0·3	0·1	0·07	0·02	3·1
Male	20	5·3	3·2	1·9	1·1	90·8	4·4	2·8	1·6	0·2	0·1	0·09	0·04	1·7
Female	20	4·9	2·4	1·0	0·8	91·2	3·2	1·4	1·2	0·2	0·14	0·06	0·04	2·5

Table 2. DISTRIBUTION OF RADIOACTIVITY IN RAT URINE, FÆCES AND CARCASE AFTER THE INJECTION OF N-ACETYL-D-GALACTOSAMINE 6-O-(^{35}S) SULPHATE

Results (expressed as percentage of the injected dose) are average values for 3 animals. The radioactivity of the doses corresponded to $1{\cdot}8 \times 10^5$ counts/min. (10 mgm.) and $3{\cdot}6 \times 10^5$ counts/min. (20 mgm.) measured after hydrolysis as an 'infinitely thick' plate of $Ba^{35}SO_4$

Sex	Dose (mgm.)	Distribution in urine								Distribution in fæces				Residual ^{35}S in carcase after 96 hr.
		Inorganic sulphate fraction				Combined inorganic and ester sulphate fraction								
		24 hr.	48 hr.	72 hr.	96 hr.	24 hr.	48 hr.	72 hr.	96 hr.	24 hr.	48 hr.	72 hr.	96 hr.	
Male	10	5·6	2·7	1·1	0·3	87·3	3·1	1·5	0·7	0·41	0·15	0·08	0·03	4·2
Male	20	4·4	2·6	1·5	0·9	93·8	2·9	2·1	1·3	0·27	0·07	0·02	—	2·8
Female	20	5·2	1·9	1·7	0·5	92·9	2·3	2·5	0·8	0·38	0·11	0·04	0·01	2·6

cellulose columns[2] and the 6-O-^{35}S-sulphate esters finally isolated as the potassium salts. Chromatography of the products on Whatman No. 1 filter paper using downward irrigation with butan-1-ol/acetic acid/water, 50 : 12 : 25 (by vol.) or *iso*butyric acid/0·5 *N* ammonium hydroxide, 5 : 3 (by vol.) showed that each contained only a single component. The N-acetyl-D-glucosamine ester labelled with sulphur-35 was identical in chromatographic mobility with authentic N-acetyl-D-glucosamine 6-O-sulphate in both the solvents named above.

Male and female M.R.C. hooded rats (aged 2–3 months and weighing 130–150 gm.) were injected intraperitoneally with aqueous solutions of the labelled esters. The animals were housed separately in the funnel-type of metabolism cage and the urine and fæces collected at 24-hr. intervals up to 96 hr. Samples of the urine and fæces were taken for the determination of radioactivity according to Dodgson and Tudball[7]. At the end of the experimental period the animals were killed by a blow on the back of the head and the carcase used for the determination of retained radioactivity. The results of these experiments are recorded in Tables 1 and 2.

The striking feature of the metabolism of both N-acetyl-D-glucosamine 6-O-^{35}S-sulphate and N-acetyl-D-galactosamine 6-O-^{35}S-sulphate is the rapid elimination of radioactivity, 87–94 per cent of the injected doses appearing in the combined inorganic and ester sulphate fraction of urine in the 24-hr. period immediately following administration. The appearance of 9·0–11·5 per cent of the dose as inorganic ^{35}S-sulphate in the urine during the 96-hr. period after the injection of both esters is also of interest since it supports previous observations of the release of inorganic ^{35}S-sulphate in the urine of rats following the administration of labelled polysaccharides containing N-acetylhexosamine ^{35}S-sulphate residues[8,9]. It was found that radioactivity was retained in the carcase after 96 hr. in all instances. However, in view of the appearance of inorganic ^{35}S-sulphate in the urine, it is not possible to differentiate between the specific uptake of the intact labelled esters into the keratosulphate and chondroitin sulphate-C fractions of rat and a general incorporation of inorganic ^{35}S-sulphate into all the ester sulphates of rat tissues. A detailed account of these and other observations will be given elsewhere.

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Identification of Sugars in the Manna of Northern Iraq

MANNA is found in the Middle East in the Sinai peninsula and in Northern Iraq. Bodenheimer and Theodor[1] claimed that the manna they found on the leaves of *Tamarix* in Sinai is the excretion of the scale insects *Trabutina mannipara* and *Najacoccus serpentinus*. However, it is generally believed in northern Iraq (Allos, H. F., private communication) that manna is an exudate of *Fraxinus ornus* L. (West, W. A., private communication), probably from insect-produced wounds. The exudate usually accumulates on the leaves until they fall to the ground. The sugars are extracted from the raw material with boiling water and mixed with eggs to make a popular dessert. While sucrose and invert sugar have been identified in manna collected from Sinai[2], the Iraqi manna was shown to contain sucrose and melezitose[3] as well as trehalose[4].

Table 1. IDENTIFICATION OF SUGARS IN MANNA

Fraction		R_g values*		Response to reagents†						Hydrolysis products‡	Identity of Unknown
				I	II	III	IV	V	VI		
Monosaccharides	1	Unknown	112	*bn*	+	+	+	–	–		Fructose
		St'd fructose	111								
	2	Unknown	98	*b*	+	–	–	–	+		Glucose
		St'd glucose	100								
Disaccharides	3	Unknown	88	*bn*	–	+	+	–	–	Glucose + fructose	Sucrose
		St'd sucrose	88								
	4	Unknown	80	*b*	–	–	–	–	–	Glucose	Trehalose
		St'd trehalose	78								
Trisaccharides	5	Unknown	50	*g–bn*	–	+	+	–	–	Glucose + galactose + fructose	Raffinose
		St'd raffinose	53								
	6	Unknown	36	*g–bn*	–	–	+	–	–	Glucose + fructose	Melezitose
		St'd melezitose	36								
Polysaccharides	7	Unknown	—	–	–	–	–	–	–	Glucose	—

* Chromatograms were developed in butanol/pyridine/water (2 : 2 : 1); other developers gave comparable results.

† I, Aniline-diphenylamine (*b*, blue; *bn*, brown; *g*, grey; II, silver nitrate; III, 2 : 4-dinitrophenylhydrazine; IV, phloroglucinol; V, vanillin; VI, ammonium molybdate.

‡ These were identified by their R_g values and response to above-mentioned reagents.

Samples of manna collected in the Suleimaneyeh District in northern Iraq were kindly supplied to us by Dr. H. F. Allos. The sticky mass was dark grey in colour and contained some soil particles and leaf residues. Approximate analysis showed it to contain 5·9 per cent moisture, 0·2 per cent fat, 2·8 per cent protein, 5·2 per cent ash, 6·7 per cent fibre and 79·2 per cent carbohydrates (by difference).

The sugars were extracted from the manna with hot 80 per cent ethanol and the extract was desalted on 'Amberlite' resins '*IR*–120' and '*IRA*–410', then concentrated under vacuum to a thick syrup. The extract was chromatographed on a charcoal column, according to Whistler and Durso[5] and mono-, di- and tri-saccharides were eluted with water, 5 and 15 per cent ethanol, respectively. The adsorbent was then treated with 30 per cent ethanol to elute polysaccharides. Each of the four fractions was concentrated under vacuum and chromatographed on Whatman No. 4 paper strips (4 cm. × 60 cm.). The di-, tri- and poly-saccharide fractions were hydrolysed by refluxing with 50 volumes of *N* sulphuric acid for 6 hr., neutralizing with solid barium carbonate to congo red paper, centrifuging and chromatographing the supernatant. Paper chromatography was also carried out on the sugar extract from the manna.

The sugar constituents of the manna were identified according to:

(1) Their R_g values as compared with those of standard sugars and sugar derivatives developed simultaneously. The following developers were used and gave comparable results: 2-propanol/water (4 : 1), 2-propanol/pyridine/acetic acid/water (8 : 8 : 1 : 4), propanol/ethyl acetate/water (7 : 1 : 2), butanol/pyridine/water (2 : 2 : 1), phenol/water (4 : 1) and ethylacetate/pyridine/water (2 : 1 : 2).

(2) Specific reactions of sugars and sugar derivatives on the chromatogram:

(I) Aldoses and ketoses by aniline–diphenylamine spray, according to Buchan and Savage[6].

(II) Reducing sugars by the silver nitrate reagent described by Trevelyan and his associates[7].

(II) Fructose, sucrose and raffinose by spraying with saturated solution of 2,4-dinitrophenylhydrazine[8].

(IV) Ketoses by phloroglucinol–trichloroacetic acid mixture according to Horrocks and Manning[9].

(V) Sugar alcohols by vanillin–perchloric acid reagent[10].

(VI) Phosphate esters by ammonium molybdate spray[11].

The results indicate the presence of glucose, fructose, sucrose, trehalose, raffinose and melezitose, as shown in Table 1. While the polysaccharide fraction hydrolysed into glucose only, it gave no colour with iodine and showed no reducing power.

It is of interest to note that with the exception of glucose and fructose, which together constitute about 10 per cent of the carbohydrates in manna, all the other sugars are non-reducing.

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Molecular Mechanisms for Hydrolytic Enzyme Action

FROM kinetic and other studies of hydrolyses catalysed by enzymes several important conclusions have emerged. One of these, coming particularly from *p*H studies, is that both an acid (–*A*–*H*) and a basic (–*B*) group are in some way involved in the reaction. A second conclusion is that there are two reaction intermediates, an addition (Michaelis) complex (ES) and an acylated enzyme (ES'), so that the process may be represented as :

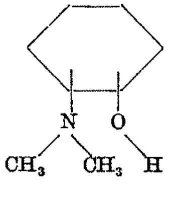
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} \underset{+P_1}{ES'} \xrightarrow{k_3} E + P_2$$

The site that becomes acylated has been a matter of some controversy, both imidazole and serine being possibilities.

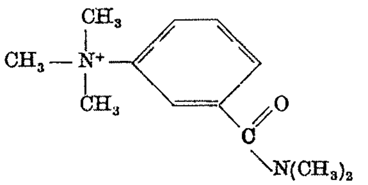
We have recently carried out a series of investigations on the enzyme acetylcholinesterase, and have studied the effects of *p*H changes and of various inhibitors. On the basis of a kinetic analysis, the details of which will be published elsewhere, we have arrived at certain conclusions which seem to be consistent with the results for other enzyme systems. These conclusions relate to the detailed configuration of the active centre of the enzyme and to the way in which the substrate and inhibitors interact with the various components of the active centre. Acetylcholinesterase has an important advantage from the point of view of elucidating these problems owing to its possession of an anionic site which serves as an 'origin' from which distances to other parts of the active centre can be deduced.

By working with two substrates, for one of which $k_2 > k_3$ and for the other $k_3 > k_2$, it has proved possible to separate effects on ES from those on ES'. Proceeding in this way, so far as *p*H changes are concerned, we find that the basic group on the free enzyme has a *pK* of 6·5 and the acid group one of 9·3. In the Michaelis complex ES the basic group is no longer able to add on a proton at *p*H 6·5, but in the acyl enzyme ES' the proton can be added on at about this *p*H. The group of *pK* 6·5 is believed to be the imidazole group, and our conclusion is that this group interacts with the carbonyl group of the substrate in the Michaelis complex, but does not do so in the acyl enzyme. There must therefore be a transfer of the carbonyl group to another group, presumably serine, during the process $ES \rightarrow ES'$.

There are two types of inhibitor for this enzyme ; those of the first class, which is exemplified by *cis*-2-dimethylaminocyclohexanol (I), give inhibition of the mixed competitive and non-competitive types, whereas those of the second, of which prostigmine (II) is an example, show pure competitive inhibition.



(I)



(II)

In (I) the distance between the nitrogen atom and the OH group is about 2·5 Å., whereas in (II) the distance between the nitrogen atom and the carbonyl carbon atom is about 5 Å. The difference between competitive and non-competitive inhibition is conventionally ascribed to differences in binding of inhibitor to the enzyme-substrate complex ; but this interpretation is excluded by our kinetic results for this system. We find that inhibitors of both classes can become attached to E and to ES' but not to ES. The difference between the types is that inhibitors of type I block the deacetylation reaction, whereas those of type II do not do so.

Support for this conclusion is provided by work we have done on the effects of the various inhibitors on the inhibition by substrate that is found with this enzyme. The results show that substrate inhibition is due not to the formation of a complex in which two substrate molecules are attached to the enzyme, but to the attachment of a substrate molecule to the acetyl enzyme with blocking of the deacetylation process. It can be shown from the steady-state equations that if an inhibitor blocks deacetylation the square of the concentration of substrate, $[S]_{\text{opt.}}$, corresponding to the maximum velocity, should vary linearly with [I] ; if it does not, $[S]_{\text{opt.}}$ itself should vary linearly with [I]. These predictions have been confirmed for inhibitors of each type, studied by Augustinsson[2], Augustinsson and Nachmansohn[3], and ourselves.

These facts lead to a clear-cut picture of the active centre of the enzyme and of the course of reaction. We conclude that when type I inhibitors become attached to the free enzyme they do so at the anionic site and at the acid (*A–H*) site, a hydrogen bond being formed between the hydrogen of the acid site and the oxygen atom on the inhibitor. The presence of the substrate prevents a similar attachment in ES, but since in ES' the acyl group of the substrate has become transferred to serine a two-point attachment of the inhibitor is now possible. Since deacetylation is now blocked, the hydrogen atom of the acid site must be required for this process.

In type II inhibitors the attachment to the free enzyme is at the anionic site and at the imidazole nitrogen, which forms an electrostatic bond with the C=O dipole of the inhibitor. Again, no attachment to ES is possible. For ES' we conclude that attachment is only at the anionic site ; the acyl group on the serine prevents attachment at the imidazole nitrogen. Since the inhibitor does not interact with the hydrogen atom of the acid site there is no inhibition of deacetylation.

These facts, and the deductions from them, lead to the picture of the active centre shown in Fig. 1. The acid and basic sites are situated 2·5 Å. and 5·0 Å. respectively from the anionic site, and the serine OH group is somewhat farther away. The figure also shows how, in our view, the substrate becomes attached to the enzyme. A more detailed

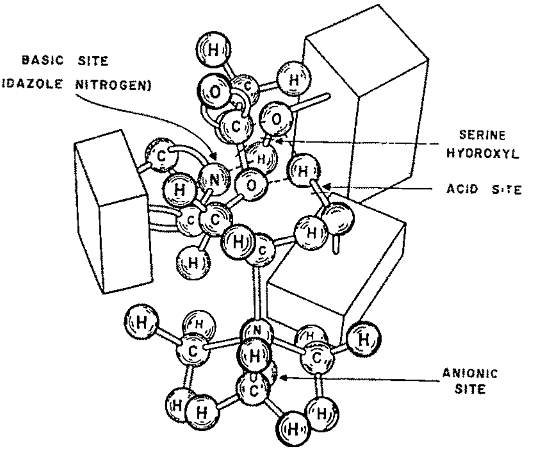


Fig. 1. Proposed arrangement of functional groups at the active centre of acetylcholinesterase. The substrate is shown in position, and electrostatic attractions occur at the dotted lines and also between the imidazole nitrogen atom and the carbonyl carbon atom. The dotted lines also indicate bonds formed during the acetylation process; the three bonds broken are marked on the diagram

discussion of the mechanism of the acetylation and deacetylation processes has been given elsewhere[4].

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PHYSIOLOGY

Spontaneous Potentials from Explants of Human Adult Cerebellum in Culture

Using techniques and supernatant similar to those used to demonstrate spontaneous potentials from explants of chick embryo cerebellum in tissue culture in Kahn tubes[1], spontaneous potentials have been demonstrated in explants from the surface of the upper postero-lateral aspects of human adult cerebellum. The explants were thin slices of the full thickness of cerebellar grey matter together with some of the related white matter. The control used was dead chick cerebellar tissue, side by side with the living culture material in the same incubator, with identical amplification and recording apparatus. The living brain tissue gave the same form and magnitude of potentials on any recording channel which was tried. The form of the potentials is shown in Figs. 1 and 2. Fig. 1 shows the typical activity after 12 hr. in culture. This consists of pairs of potentials having a complex wave-form. The first of the potentials in each pair has a magnitude of 75 μV. and the other, which follows after an interval of 12–15 sec., has about one-third of this potential. The interval between the pairs of spikes is about 45 sec. Fig. 2 shows the typical activity after 24 hr. in culture. The pairing of the spikes is much less common, and when it does occur the interval between the two members of the pair is very irregular. The interval between the potentials is more irregular and varies about 20 sec. The potentials at this time have a magnitude of about 20 μV. The culture continued to have spontaneous electrical activity until more than 72 hr. after explantation. No record of spontaneous potentials from adult brain in culture have been found in the literature. Further investigations of these potentials are in progress.

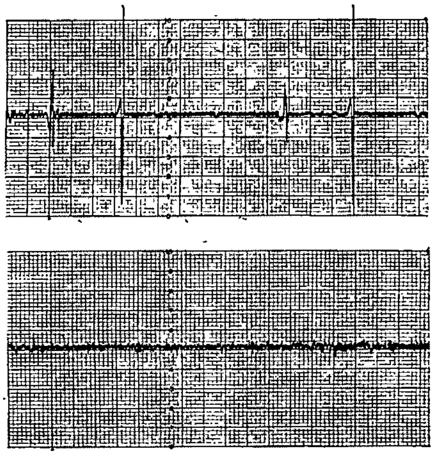

Fig. 1. Upper trace, spontaneous potentials from an explant of adult human cerebellum 12 hr. after explantation. Vertical lines 1 sec. apart; amplitude scale—distance between two horizontal lines equals $1\frac{1}{2}$ μV. Lower trace, control of dead cerebellar tissue under identical conditions and with identical time and amplitude scales

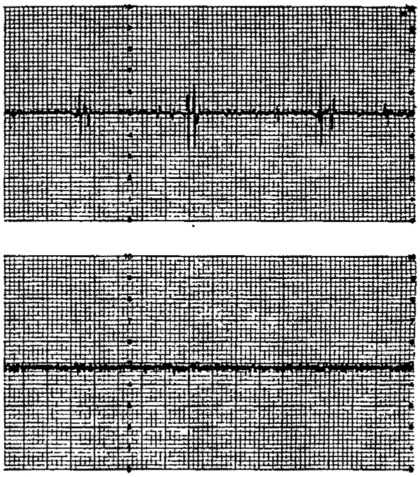

Fig. 2. Upper trace, spontaneous potentials from an explant of adult human cerebellum 24 hr. after explantation. Vertical lines 1 sec. apart; amplitude scale—distance between two horizontal lines equals $1\frac{1}{2}$ μV. Lower trace, control of dead cerebellar tissue under identical conditions and with identical time and amplitude scales

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Assay of 5-Hydroxytryptophan Decarboxylation in Intact Brain

Intraperitoneal injection of 5-hydroxytryptophan (5HTP) shortly before death causes a considerable post-mortem rise of 5-hydroxytryptamine (5HT) in the brain of rats. This may be explained by the fact that 5HTP decarboxylation continues during the first few hours post-mortem whereas monoamine degradation by monoamine oxidase is probably considerably reduced[1]. In the work reported here the question was investigated whether this 5HTP induced post-mortem increase of brain 5HT might be used as a test for decarboxylase inhibitions. D,L-α-Methyl dihydroxyphenylalanine (α-methyl DOPA)[2–6] served as a model inhibitor.

Male Wistar rats weighing 60–80 gm. and fasted for 16 hr. were given 75 mgm. D,L-5HTP/kgm. by subcutaneous injection $\frac{1}{2}$ hr. before decapitation. The 5HT content was determined by a spectrophotofluorometric method[7]: (*a*) in brain homogenized in 0·1 *N* hydrochloric acid immediately after death; (*b*) 2 hr. after incubation of the severed heads at 37° C. in small closed wet chambers. The 5HT difference between group (*a*) and (*b*) served as a measure for decarboxylase activity. α-methyl-DOPA was injected intraperitoneally at various intervals before decapitation and the post-mortem rise of 5HT compared with that of controls.

In 5HTP pretreated rats the level of brain 5HT measured immediately after decapitation was 1·00 ±

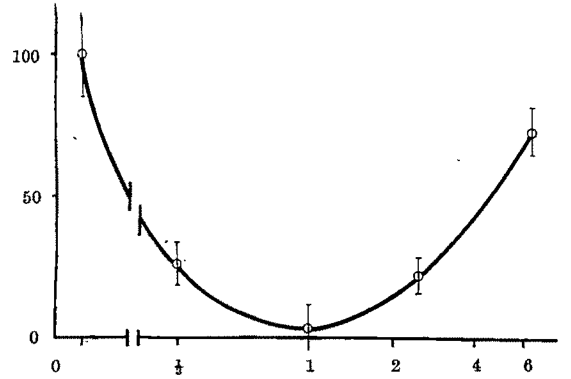

Fig. 1. Inhibition of post-mortem decarboxylation of 5-hydroxytryptophan (5HTP) by D,L-α-methyl dihydroxyphenylalanine (α-methyl DOPA) in intact rat brain in relation to time. Abscissa, hours after intraperitoneal injection of 105 mgm./kgm. α-methyl DOPA (logarithmic scale); ordinate, percentage 5-hydroxytryptamine (5HT) increase during the first 2 hr. after decapitation as compared with animals not receiving α-methyl DOPA. Subcutaneous injection of 75 mgm./kgm. D,L-5HTP ½ hr. prior to decapitation. Each point represents average and standard error of nine 5HT determinations

0·06 γ/gm. (controls 0·63 ± 0·02 γ/gm. 5HT). Incubation of the severed heads at 37° C. caused a further increase of 5HT of 0·86 ± 0·10 γ/gm. (considered as 100 per cent). α-methyl DOPA inhibited the post-mortem increase of 5HT considerably. The dose-dependent effect was maximal after 1 hr. and almost completely abolished after 6 hr.; 50 per cent inhibition was obtained by about 25 mgm./kgm. within 1 hr. (Figs. 1 and 2).

These findings show that the 5HTP induced post-mortem rise of 5HT is suitable for the investigation of decarboxylase inhibitors in brain. The results are in agreement with those obtained by measurement of decarboxylase activity in brain homogenates after injection of α-methyl DOPA into mice[6]. This method has the following advantages:

(1) Decarboxylase exerts its action in intact brain and not in disintegrated and diluted tissue (homogenates, supernatant, etc.).

(2) Monoamine oxidase activity does not interfere to any great extent, since under the almost anaerobic conditions post mortem it is nearly completely abolished[1]. Pharmacological inhibition of monoamine oxidase (for example, with iproniazid) is not necessary for the measurement of decarboxylation.

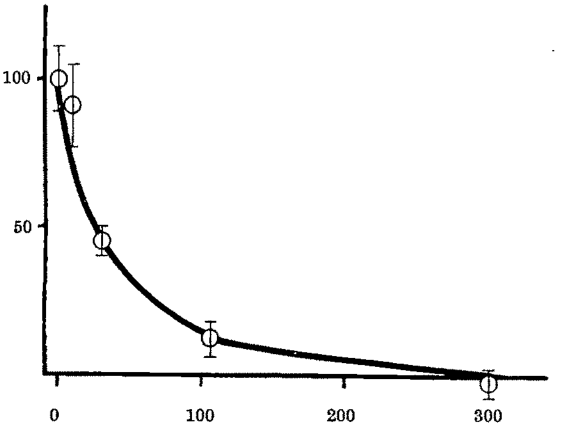

Fig. 2. Inhibition of post-mortem 5-hydroxytryptophan (5HTP) decarboxylation in intact rat brain, by D,L-α-methyl dihydroxyphenylalanine (α-methyl DOPA) in relation to dose. Abscissa, dose of α-methyl DOPA in mgm./kgm.; ordinate, percentage 5-hydroxytryptamine (5HT) increase within 2 hr. after decapitation as compared with animals not receiving α-methyl DOPA. Intraperitoneal injection of α-methyl DOPA 1 hr., subcutaneous injection of 75 mgm./kgm. D,L-5HTP ½ hr. before decapitation. Each point represents average and standard error of 5–11 5HT determinations

(3) The sensitivity is probably superior to that obtained by measurement of endogenous 5HT in brain. In the post-mortem method with rat brain 50 per cent decarboxylase inhibition can be obtained by about 25 mgm. α-methyl DOPA. A slight but significant decrease of endogenous 5HT in mouse brain, however, becomes evident only after 100 mgm./kgm. of the inhibitor[4].

Investigations on the decarboxylation of dihydroxyphenylalanine using the post-mortem method described are under way.

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Sodium Transport and Oxygen Consumption in the Mammalian Kidney

IN the isolated frog skin, it was found by Zerahn[1] and by Leaf and Renshaw[2] that the amount of sodium actively transported had a fixed relationship to the net oxygen consumption (sodium/net oxygen ratio), as about 18 sodium ions were transported per molecule of oxygen consumed under a variety of experimental conditions. Net oxygen consumption was defined as the difference between the total oxygen uptake and the basal uptake, that is, the oxygen uptake when no sodium transport took place. In the mammalian kidney, active re-absorption of sodium (anions and water following passively) represents on a molar basis the overwhelming part of all tubular transport processes. On the assumption that sodium re-absorption does therefore require the bulk of the oxidative energy supplied, we have determined the sodium/net oxygen ratio in the intact kidney of the dog.

The basal oxygen consumption of the dog kidney, here defined as the oxygen consumption when no filtration and hence no re-absorption take place, was determined in four anæsthetized dogs during hypotension induced by bleeding. The absence of filtration was evident from an inulin extraction close to zero. During twelve observation periods, in which the mean arterial blood pressure varied from 30 to 67 mm. mercury, renal blood-flow (in ml./gm./min.) was measured by the free outflow method, and the arteriovenous oxygen difference (in μmol./ml.) was determined manometrically[3]. It can be seen from Fig. 1 that the arteriovenous difference for oxygen rose sharply as blood-flow fell to less than 1 ml./gm./min., causing relatively constant consumption of oxygen over a wide range of variation in the rate of perfusion. This value was 0·215 ml. oxygen/gm. kidney/min. or 1·0 μmol./gm./min. (*S.E.* of mean, 0·04), and was considered to represent the basal oxygen uptake of the kidney. A basal oxygen consumption of a similar

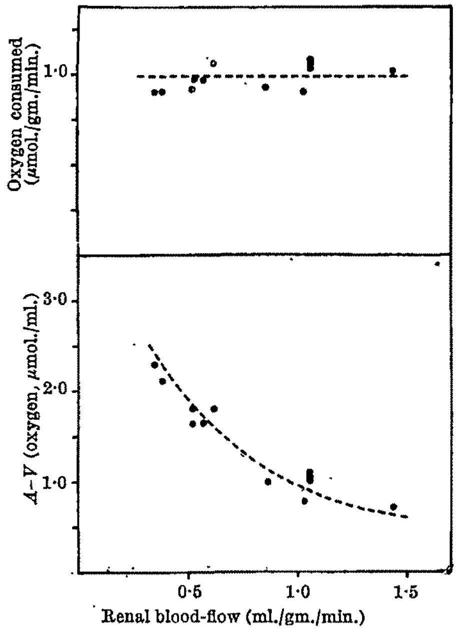

Fig. 1. Relationship between renal blood flow (in ml./gm./min.) and the arteriovenous oxygen difference (in μmol./ml.) of the non-filtering kidney during hæmorrhagic hypotension. The calculated oxygen consumption has a mean value of 1 μmol./gm. kidney/min. and is seen to remain relatively independent of wide variations in the rate of blood perfusion

magnitude has been found in various exocrine glands of the dog: submaxillary[4] 1·4 and 1·2, parotid[5] 1·4, and pancreas[6] 1·3, μmol./gm./min.

The relationship between sodium re-absorption and suprabasal (net) oxygen consumption of the normally filtering kidney was determined on eight anæsthetized dogs. The femoral artery, the left renal vein and both ureters were cannulated, and standard clearance techniques were used involving inulin and sodium *p*-aminohippurate. Sodium re-absorption (in μequiv./gm. kidney/min.) was calculated from the amount filtered minus the amount excreted in the urine. The net consumption of renal oxygen (in μmol./gm. kidney/min.) was calculated as the product of the arteriovenous difference for oxygen and renal blood-flow, subtracting the basal oxygen consumption of 1·0 μmol./gm./min. The results are shown in Table 1. Despite large variations in the rate of sodium re-absorption, the sodium/net oxygen ratios remained fairly constant in the eight dogs. The mean ratio was 28·5 (*S.D.* 2·9) and is in agreement with the value of about 30 which can be estimated from data in the literature on normal man.

Table 1. RENAL OXYGEN CONSUMPTION AND SODIUM RE-ABSORPTION OF THE DOG

Dog No.	No. of observations	Sodium re-absorption (μequiv./gm./min.)	Total oxygen consumption (μmol./gm./min.)	Net oxygen consumption (μmol./gm./min.)	Sodium/net oxygen ratio
8	4	91·3	4·0	3·0	30
12	7	172·5	6·9	5·9	29
13	3	192·1	8·6	7·6	25
15	6	113·5	5·2	4·2	27
16	6	126·0	5·9	4·9	26
17	5	122·4	5·5	4·5	27
V	3	100·6	3·9	2·9	35
VII	3	103·1	4·6	3·6	29
Average		127·7	5·6	4·6	28·5 (*S.D.* 2·9)

The present results thus suggest that the intact mammalian kidney, like the isolated frog skin, has a fixed relation between sodium transport and net oxygen consumption. The sodium/net oxygen ratio has a somewhat larger value in the kidney (about 29) than in frog skin (about 18), but it is of interest that the consumption of one equivalent of oxygen (1/4 mole of oxygen) generates sufficient energy for the transport of several equivalents of sodium in both systems.

The concept that the renal oxygen uptake can be divided into a relatively small but constant basal uptake and a variable functional uptake, which is dominated by, and bears a constant relationship to, tubular sodium re-absorption, may explain some classical observations in renal physiology.

A large arteriovenous oxygen difference has been found in uncompensated heart disease, where the filtration fraction is large and the renal blood-flow reduced[7]. Conversely, a small arteriovenous oxygen difference results from infusion of albumin, which causes a reduction of the filtration fraction and an increase in blood-flow[8]. It is evident from the present hypothesis that variations in the filtration fraction may influence the arteriovenous oxygen difference; but it is important to consider also variations in renal blood-flow, since the basal oxygen consumption of the kidney must influence the arteriovenous oxygen difference the more the lower the rate of perfusion. Theoretically, variations in the arteriovenous oxygen difference $(A-V)O_2$, can be predicted if the following variables are determined: renal blood-flow (RBF), hæmatocrit (H), filtration fraction (FF), plasma sodium (P_{Na}), excretion fraction of sodium (E_{Na}), and renal weight (W):

$$(A-V)O_2 \text{ in ml./100 ml.} =$$

$$2{\cdot}24\left[W/RBF + \frac{1}{29}(1-H)(1-E_{Na})P_{Na}FF\right]$$

In contrast with other organs, a moderate reduction of renal blood-flow results in a decrease in renal uptake of oxygen. Since the filtration and hence re-absorption of sodium is also reduced in this situation, the observed correlation is readily explained. Only when blood pressure is reduced below the limit where filtration ceases, the kidney—like any other organ—extracts more oxygen per millilitre of blood in response to a reduction of the rate of perfusion, maintaining constant consumption of oxygen.

Thus, in both situations the kidney conforms to the general physiological principle first formulated by Barcroft[4], that oxygen consumption is directly related to function. Hence the seemingly puzzling difference between the oxidative metabolism of the kidney and other organs becomes merely a consequence of the unique fact that renal work is determined by, and roughly correlated to, renal blood-flow.

Renal oxygen consumption is not correlated to tubular *para*-aminohippurate transport or to urinary urea excretion[9]. These observations fit well into the theory of the dominant role of sodium transport, since no gross changes in the re-absorption of sodium occurred during the experiments, in which the effect of variations in renal handling of these two substances were studied.

The efficiency of the kidney is approximately 1 per cent when thermodynamic considerations are applied to the composition of the urine relative to plasma[10].

Such calculations disregard the oxygen cost of isotonic re-absorption of sodium. If renal oxygen uptake is stoichiometrically related to the quantity of sodium re-absorbed, then the oxygen uptake cannot be expected to bear a fixed relation to the thermodynamically calculated (minimal) work involved in producing the final urine. Hence the concept of 'renal efficiency' may be considered physiologically meaningless.

This discussion has directed attention to some of the implications of applying the concept of a fixed sodium/net oxygen ratio to the mammalian kidney. More experiments are clearly needed in order further to investigate the validity and possible limitations of this concept.

This work was supported by grants from the Danish State Research Foundation and from King Christian Xth Foundation.

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Demonstration of Nucleases on Mammalian Skin Surface and in Saline Extracts of Hair

In the course of experiments on the effects of β-irradiation on the nucleases of guinea pig epidermis we washed thin strips of peeled tissue (25–60μ thick) before homogenization, in an attempt to demonstrate release of enzyme[1]. Approximately 50 per cent of total deoxyribonuclease (DNase) and 75 per cent of ribonuclease (RNase) activity were thus removed from normal or irradiated epidermis, indicating that considerable amounts of these enzymes may be in the stratum corneum or in the interstitial fluid. This led us to test for the presence of enzymes on the closely clipped skin surface of living animals. For this purpose we adapted the plastic cup technique used by Peiss *et al.*[2] and Szakall[3] for the study of water and ion exchange between a chemically defined medium and the skin. Albino guinea pigs of either sex (500–900 gm.) were fed a vitamin C-enriched diet, anæsthetized with veterinary 'Nembutal' and clipped with an electric shaver. Plastic cups were secured to appropriate areas of the animals' flanks by means of ½ in. 'Trimtex' rayon elastic, and small alligator clamps or paper clips were used to fasten the elastic (No. *S*-2 plastic stoppers were supplied by Pioneer Plastics, Box 8066, Arlington Branch, Jacksonville 11, Fla.). Two small needle holes were punched 180° apart and about 6 mm. from the closed end of each cup; 1·5 ml. of 0·9 per cent saline was injected with a No. 22 needle and syringe, and samples were withdrawn for enzyme assay.

DNase activity was determined by the method of Schuster[4]. Assay solutions contained 1 mgm. of calf thymus DNA (Worthington) or salmon sperm DNA (California Foundation). Blanks were subtracted for enzyme added at zero time and substrate after 30 min. at 37° C. The increases in 260 mμ readings (Δ260) were proportional to DNase and RNase concentrations up to 0·2 *O.D.* RNase was determined by the method of Dickman *et al.*[5]; some of its characteristics have been described[6]. Saline in contact with normal epidermis was found to have considerable nuclease activity (Table 1). Since there was no increase in the nuclease content of these saline washings after 45 min. contact with the skin surface, this interval was chosen for sampling.

Table 1 Nuclease Activity of Saline Washings derived from 2·54 cm.^{2} Normal Skin Surface and from Clipped Hair*

Animals	Sex	DNase Δ260/ml.			RNase Δ260/ml.		
		Initial sample	After 48 hr.	After 72 hr.	Initial sample	After 48 hr.	After 72 hr.
Guinea pigs	F	1·10	1·53	1·50	0·40	0·40	0·40
	F	0·90	1·40	1·40	0·05	0·25	0·25
	F	0·40	1·00	1·30	0·30	0·30	0·30
	M†	0·13	0·70	0·90	—	—	—
Albino rat	F	0·11	0·25	—	0·10	0·02	—
Albino rabbit	F	0·15	0·06	—	0·16	0·15	—
Guinea pig hair	F	—	1·50	—	—	0·70	—
Rat hair	F	—	0·4	—	—	0·47	—
Rabbit hair	F	—	0·25	—	—	0·70	—

DNase assay: 1 mgm. DNA, 0·1 *M tris* *p*H 7·4, 0·005 *M* magnesium sulphate and 0·1 ml. enzyme in 0·8 ml. final volume, 30 min. at 37°. Δ260 × 2 equals γ-crystalline DNase. RNase assay: 1·3 Michaelis (ref. 13) veronal-acetate buffer, *p*H 7·7; 0·2 ml. enzyme and 0·5 ml. 1 per cent dialysed yeast RNA, 15 min. at 37°. Δ260 × 0·1 equals γ-crystalline RNase.

* 1·5 gm. hair extracted in 10 ml. 0·9 per cent saline, for 48 hr. at 4° C.; 0·1 ml. aliquots for assay.

† Pigmented guinea pig.

The average enzyme activity of 1 ml. washings (4 animals) was equivalent to about 2 and 0·03γ of crystalline pancreatic DNase and RNase (Worthington), respectively (Table 1). Washing or wiping the skin surface prior to sampling removed most of it. About 24 hr. were required for surface nucleases to return to their initial level. In some instances, when the initial activity was low it rose to a high level after the second or third sampling of the area, 48 or 72 hr. later (Table 1). We have no explanation for this increase of enzyme after repeated sampling. Clipping the hair without touching the skin surface, in order to avoid trauma, results in levels comparable with closer clipping. The nucleases appear to be fairly uniformly distributed on the skin surface of the flanks. Nuclease activity can be leached out of the clipped hair (Table 1), but equally high levels are present on the depilated skin surface (Tabachnick, J., and Freed, R. M., unpublished results). Since there was no significant change in activity of the supernatant after centrifugation for 1 hr. at 100,000*g*, these enzymes are considered to be in solution in the saline washings and not associated with any cell particulate.

Skin surface DNase was inactive in the absence of magnesium ions. The optimal ionic strength (*p*H 7·0, Michaelis[13] veronal-acetate buffer), was about 0·17 in a complete medium to which sodium chloride had been added in varying concentrations. Heating the saline washings for 10 min. at 60° C. in a *p*H 4 or *p*H 7·4 buffer solution resulted in 90 per cent and 80 per cent losses of activity, respectively. As further evidence of enzymic hydrolysis of DNA we observed a change in viscosity of a 0·1 per cent substrate solution from a gel-like consistency to a watery one within minutes after addition of an aliquot of active saline solution. In addition, paper chromatograms (70 per cent *iso*propanol-ammonia) of aliquots of DNA solutions, incubated 24 hr. with saline washings or crystalline DNase, showed similar broad bands of ultra-violet absorbing oligonucleotides which had left the origin. In controls, the ultra-violet absorbing material did not leave the origin.

The *p*H activity curve of skin surface DNase is similar to that of crystalline pancreatic DNase (DNase I), the optimum being at about 7·0 with little or no activity at or below 5·3. This indicates absence of an acid DNase (DNase II) which is present in many tissues[7]. Epidermis, washed free of extracellular enzyme prior to homogenization, contains DNase with a similar *p*H optimum, suggesting that the skin surface enzyme may be of epidermal origin. Unlike DNase I of liver or kidney, which may be masked by a specific inhibitor[7], the relatively high DNase activity at neutral *p*H on the skin surface or in guinea pig epidermis points to the absence of an inhibitor. Whether an activator[8] for DNase I is present remains to be determined.

In two human subjects (the authors) no nuclease activity was detected on the normal or sweating surface of the forearm, the palm, the nape of the neck, or in saline extracts of hair. The presence of non-specific esterases on human skin has been demonstrated with histochemical methods[9]. While skin bacteria play a part in the production of free fatty acids, some of the lipolytic activity was attributed to esterases of animal origin[10]. We found no significant changes in the amount of nuclease after reducing the bacterial count of guinea pig skin surface to 0 or 5 per cent by topical application of the antibiotics, 'Surgamycin' or 'Achrosurgic' (kindly supplied by the Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York).

Steigleder[11] has referred to human skin surface as covered with an "esterase mantle", and regards human sebum and sweat as the chief carriers of the non-specific esterases. The present report suggests that other mammals, such as guinea pigs, have a nuclease coating. Since this species has no eccrine sweat glands, surface nucleases cannot originate there. In addition, in healed, β-irradiated guinea pig skin in which the dermal adnexal structures had atrophied or had been completely destroyed[12], we observed increases in surface DNase and RNase (Tabachnick, J., and Freed, R. M., unpublished results). Hence, in this animal, surface nuclease activity is probably of epidermal and/or humoral origin.

As shown in Table 1, rat and rabbit skin may also function as an excretory organ for nucleases. The question arises, Is there a selective removal of blood nucleases through the cutaneous blood vessels or are these enzymes a natural product of epidermal cell turnover and keratinization ? This technique, applied to the surface of healed, irradiated skin with atrophied or destroyed adnexal structures, may prove to be a useful tool for obtaining answers to this and other questions relating to the *in vivo* permeability of the epidermis.

We are indebted to Dr. Peter Flesch, Department of Dermatology, University of Pennsylvania, Philadelphia, for directing our attention to the plastic cup technique[2,3]. A detailed report is in preparation. The work was supported by Contract *AT* (30–1)–1727 with the U.S. Atomic Energy Commission.

Note added in proof. The low levels of nuclease activity in the initial samples (column 3, Table 1) are the result of sampling immediately after close clipping of the animal's hair. Through the generosity of Prof. P. C. Trexler, Lobund Department of Biology, Notre Dame University, we recently had the opportunity of assaying saline extracts of germ-free hair obtained from guinea pigs, rats, and rabbits, and found levels of DNase and RNase activity as high or higher than the values in Table 1.

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Transplacental Passage of Red Cells in Man

THE purpose of this communication is to report the results of an investigation into the frequency and magnitude of hæmorrhage from the fœtal to the maternal circulation in a random series of deliveries. The total separation of the maternal and fœtal circulations was a long-held biological belief, and was only first seriously questioned in 1941, when the mechanism of hæmolytic disease of the newborn was elucidated and Levine[1] showed that Rh iso-immunization occurred less often than expected in ABO incompatible matings. Because of these Rh findings, it was postulated that the antigen was carried across the placenta into the maternal circulation by intact erythrocytes.

Several investigators have since succeeded in demonstrating the passage of cellular elements across the placenta ; radioactive tagged red cells, elliptocytes and sickle cells have been injected into the maternal circulation prior to delivery, and later recovered from the fœtal circulation[2,3]. Fœtal red cells have been detected in the maternal circulation by means of differential agglutination by Chown[4] and by Zipursky *et al.*[5], using the histochemical technique developed by Kleihauer *et al.*[6].

The technique used in the present work was that of Kleihauer, with the modification that the final preparation was unstained. The principle of the method is to elute hæmoglobin A from the adult red cells by means of a citric-phosphate buffer ; hæmoglobin F remains in any fœtal red cells and these stand out as dark refractile bodies (see Fig. 1).

To establish the sensitivity of the technique, 50 male bloods were examined along with 50 specimens of blood from post-partum women, the observer being unaware of the source of the specimens. Each specimen was given a quantitative score, this being the average of the number of refractile cells counted in four 3-min. scans. No male score exceeded 0·5.

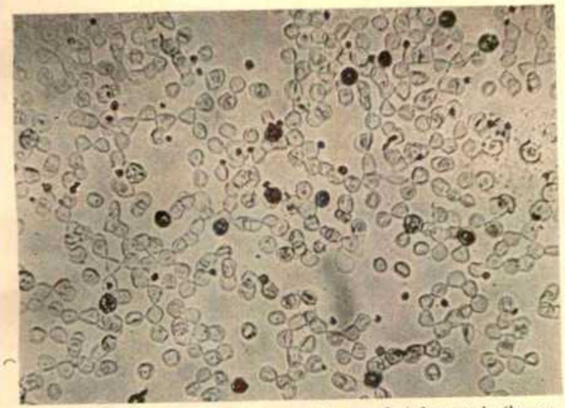

Fig. 1. Maternal blood from case of severe fœtal anæmia (hæmoglobin, 73 per cent) due to massive transplacental hæmorrhage. The fœtal red cells stand out darkly compared with the pale ghosted adult cells

Artificial mixtures of maternal and fœtal blood were next made at varying dilutions for the purpose of correlating the scores with actual amounts of fœtal blood. At a dilution of 1 : 5,000, the score was 5 ($S.D. \pm 1\cdot5$). Assuming an average maternal blood volume of 5 litres, this would represent 1 ml. of fœtal blood in the maternal circulation.

200 specimens of maternal blood were examined within 72 hr. of delivery. The average of two 3-min. counts was recorded, and scores of 2 or more were regarded as indicating definite transplacental hæmorrhage. This was detected in 12 per cent (24) of the women (Table 1).

Table 1. FŒTAL RED CELLS IN 200 SPECIMENS OF POST-PARTUM MATERNAL BLOOD. SCORES RECORDED AS WHOLE NUMBERS; FOR EXAMPLE, 2·5 IS SCORED AS 2

Fœtal red cells score	No. of specimens of post-partum blood	Fœtal red cells score	No. of specimens of post-partum blood
0	159	6	3
1	17	7	0
2	3	8	1
3	5	9	0
4	5	10–19	3
5	1	20–29	3

Scores of 10 or more were recorded on only six occasions (3 per cent), and this suggests that fœtal bleeding is rarely more than 1–2 ml. Work is in progress to find out the precise amount that is necessary to stimulate the formation of antibodies.

Though the amount noted in this random series was always less than 5 ml., we have investigated a case of marked neonatal anæmia (hæmoglobin 73 per cent) in which it was calculated that about 100 ml. of fœtal blood had entered the maternal circulation (see Fig. 1). It was shown afterwards that the mother was blood group S-negative and the fœtus S-positive, and *in vitro* it was clearly demonstrated that, on adding anti-S to the maternal blood, differential agglutination was produced (Fig. 2).

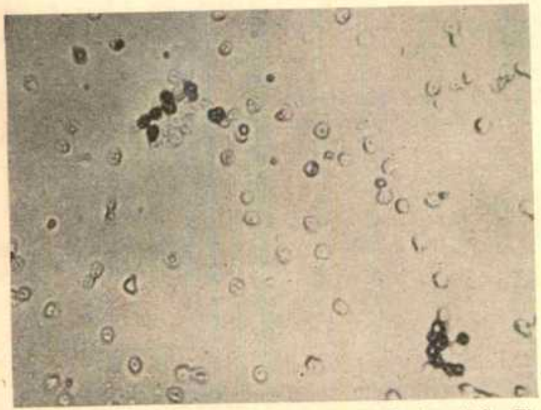

Fig. 2. Differential agglutination with anti-S at + 4° C. The small agglutinates stand out darkly (due to hæmoglobin F), thus confirming their origin from fœtal red cells

The results of the investigation have obvious relevance to the low incidence of iso-immunization in Rh-negative mothers as in the majority of cases the placenta seems to be an effective barrier against the entry of more than 1 ml. of fœtal blood. In Rh-negative patients in whom significant fœtal bleeding is detected immediately after delivery, it may be possible (as judged by the *in vitro* results) to inactivate the circulating fœtal cells by the administration of anti-D and thus prevent iso-immunization.

Using Rh-negative male volunteers, we have already shown that the administration of Rh-positive cells followed half an hour later by anti-D, results in these cells being coated with the antibody. In view of the results of Stern and Berger[7], it is hoped that this coating will prevent Rh-sensitization.

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RADIOBIOLOGY

Proliferative Cycle in the Growing Hair Follicle of the Mouse

THE intermitotic phase has been divided into three segments: the phase of synthesis of deoxyribonucleic acid (DNA) (S phase), preceded and followed by 'gaps' called G_1 and G_2, respectively[1]. The duration of these phases can be accurately measured by pulse-labelling with tritiated thymidine, followed by serial killing, preparation of autoradiographs, and determination of the fraction of mitoses labelled at various intervals between injection and death[2,3]. Several kinds of cells have been so studied; the present communication adds to the list the cells of the growing-hair follicle in the mouse.

Young adult male $CF1$ mice were used. Hair growth was initiated by plucking[4]. Tritiated thymidine (20 μc., sp. act. 1·9 c./mM) was administered intraperitoneally 10–11 days after plucking, at which time hair growth is proceeding at full speed. Animals were killed at times ranging from 10 min. to 42 hr. after labelling. Autoradiographs were prepared; 50–100 ana- and meta-phases on each slide were scored for presence or absence of label. Presence of label indicates that the cell was synthesizing DNA during the period when label was available, that is, the first few minutes following injection[5]. When the interval between injection and death is short, no meta- or ana-phases are found to be labelled, because cells in this stage at the time of death were in G_2 or prophase at the time of labelling. Longer intervals yield labelled mitoses, since the cells in mitosis at the time of death were in S phase at the time of injection. Still longer intervals again result in unlabelled mitoses, since the injection in these cases coincides with the G_1 phase, mitosis, or G_2 phase of the preceding cycle.

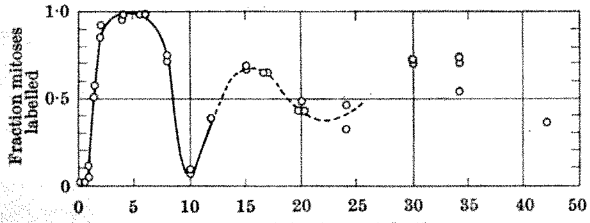

Fig. 1. Fraction of mitoses labelled as a function of the interval between injection of label and death. Each symbol represents one mouse

The results are shown in Fig. 1. The mean duration of the G_2 plus prophase is the interval between the origin and the time when 50 per cent of all meta- and ana-phases are labelled; it is 1·5 hr. The mean duration of the S phase is the width of the wave of labelled mitoses, measured between the two 50 per cent points; it is about 7 hr. The variability in the duration of the S phase can be estimated from the difference between the slopes of the ascending and descending limbs of the wave[2]: the standard deviation is about 0·9 hr. The distance between corresponding points on the first and second waves represents the generation time. However, since the second wave is not well defined, the estimation is equivocal; roughly, the generation time must be near 12 hr. Of this time, 8·5 hr. are taken up by the S and G_2 phases and prophase; allowing about 0·5 hr. for meta- and ana-phase, the remaining 3 hr. represent the mean duration of G_1. The distribution of durations of G_1 in individual cells can be roughly estimated from the shape of the second wave[3]. The data are compatible with an exponential distribution, possibly with some lag; this would imply that soon after the cells enter the G_1 phase there is a constant probability (of about 0·0055 min.$^{-1}$) that DNA synthesis will commence during the following minute.

The results of this work, together with findings in other cell systems, support a general impression that the duration of the S–G_2-mitosis sequence is fairly constant in a given species, while the duration of the G_1 phase varies widely and is chiefly responsible for differences in generation times, both within and between cell populations.

The amount of label in hair follicle cells is much less than in intestinal epithelial cells of the same animals. Maturing cells carry their label into the hair shaft, where it disappears at the same time as the nuclei. An artefact often seen is deposition of a dense layer of silver grains on the surface of hair.

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Formation of an Ommatin Pigment from Tyrosine by Ultra-violet Irradiation

In the course of an investigation of the effect of ultra-violet irradiation on the formation of melanin from tyrosine, it was found that a brief period (5 min.) of irradiation had an effect similar to that brought about by the addition of a minute quantity of DOPA; on addition of tyrosinase to the irradiated solution oxygen uptake began immediately without an induction period, and ultimately reached a value approximating that calculated for conversion of the tyrosine to melanin. As the reaction proceeded, typical melanin granules were formed.

Attempts to bring about non-enzymatic melanin formation from tyrosine by irradiation were unsuccessful. Short periods of exposure to ultra-violet light resulted in the development of a golden-yellow product; limited increases in irradiation time had the effect of deepening the golden colour; but at no time was there evidence of melanin formation, even when solutions containing the yellow product were allowed to remain at room temperature for several days. Prolonged exposure to irradiation resulted in almost complete disappearance of this colour, with an accompanying drastic increase in oxygen uptake suggesting oxidative destruction. Changes in absorption spectra brought about by increasing dosages of irradiation indicated the formation of products having greater absorbency at 245–250 mμ and at 290–340 mμ than does unirradiated tyrosine. Analysis of solutions briefly exposed to ultra-violet radiation demonstrated the presence of DOPA quinone and of 5,6-dihydroxyindole.

The yellow pigment responsible for the change in absorbance was extracted by salting out with sodium chloride and shaking with *iso*butyl alcohol; the yellowish-tan muff formed at the interface was dissolved in alkali to give a reddish-brown solution. In Fig. 1, the spectra of the extracts indicate the formation of a product with maximum absorbance in the ultra-violet at 245 mμ, with a second peak at 300–305 mμ. That this product is not a melanin is shown by curve *D*, the absorption spectrum of a melanin obtained by enzyme action on briefly irradiated tyrosine. The pigment was insoluble in *iso*butyl alcohol until brought to *p*H 2; when it was extracted into alcohol at this *p*H, it exhibited a brilliant blue fluorescence with maximum emission in the region 410–430 mμ.

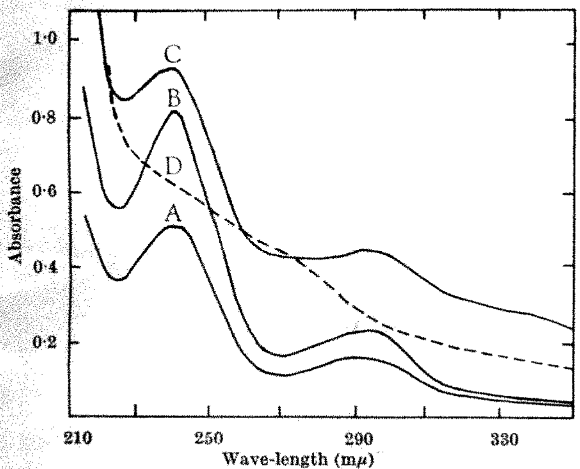

Fig. 1. Absorption spectra of pigment extracts obtained by irradiation of tyrosine for: *A*, 10; *B*, 20; *C*, 120 min.; *D*, purified melanin obtained by enzyme action on an irradiated tyrosine

In these characteristics, colour, relative insolubility, absorption spectrum in the ultra-violet region, and emission spectrum, the pigment, which is not an intermediate in the normal pathway of melanin formation, resembles the fluorescent red-yellow xanthommatin described by Butenandt *et al.*[1]. It is probable that as a result of irradiation, oxidation of the dihydroxyindole with the breaking of the 2 : 3 bond of the pyrrole ring has given rise to an *o*-amino phenol[2] which reacted with the DOPA quinone to form the ommatin pigment. This is of particular interest in relation to Fitzpatrick's[3] work on the production of pheomelanin, and as demonstrating an alternative pathway of pigment formation from tyrosine, one that is non-enzymatic.

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HISTOCHEMISTRY

Enzyme Histochemistry of the Cells in Hodgkin's Disease and Allied Disorders

The complex nomenclature applied to cellular components of the reticuloendothelial system, based on their morphological characteristics, has resulted in a variety of controversial classifications of lymphoreticular neoplasia.

Enzyme histochemistry offers valuable assistance in identifying the various cell types and their malignant counterparts.

Braunstein, Freiman and Gall[1], in a preliminary report on the distribution of enzymatic activity in malignant lymphoma, stated that in general the enzymatic activity of cells of the various histiocytic types of malignant lymphoma corresponded to that of their normal counterparts. Of particular interest was the demonstration that the cells of reticulum cell sarcoma (histiocytic lymphoma) manifested enzyme activity identical with that of non-neoplastic histiocytes. They claimed also that large anaplastic cells and Sternberg cells of Hodgkin's 'granuloma' and sarcoma also manifested a similar pattern histochemically.

Methods for the histochemical demonstration of acid phosphatase, alkaline phosphatase and non-specific esterase[2] have been applied to lymph nodes and spleen in these laboratories, as part of a similar investigation.

The results of Braunstein *et al.* were confirmed, in part, in that histiocytes, wherever they were located, showed marked activity of acid phosphatase and non-specific esterase. Cells thought to be derived from histiocytes, for example, 'epithelioid' and 'endothelioid' cells and Langhans giant cells in tuberculous lymph nodes, together with foreign-body giant cells, reacted similarly. Splenic sinus lining cells, however, were consistently negative for these enzymes and are therefore not considered to be true histiocytes[3].

No reactions for these enzymes have been observed in lymphocytes. Alkaline phosphatase was confined to capillary endothelium, to some polymorphonuclear leucocytes and to an occasional mononuclear cell.

Twenty-seven cases of malignant lymphoma and leukæmia have been investigated. These included lymphocytic lymphoma (6), histiocytic lymphoma (reticulum cell sarcoma, 5), mixed lymphocytic-histiocytic lymphoma (1), follicular lymphoma (5), Hodgkin's 'granuloma' (6), Hodgkin's sarcoma (1), lymphocytic leukæmia (2), 'monocytic' leukæmia (1), and 'leukæmic reticuloendotheliosis' (1).

Strong non-specific esterase and acid phosphatase activity was observed in histiocytes (reticulum cells), however atypical and pleomorphic, in all these conditions. Moreover, these reactions demonstrated the abundant atypical histiocytes in five cases of follicular lymphoma, thus confirming the classification of Rappaport *et al.*[4], which includes, among others, mixed lympho-histiocytic and predominantly histiocytic types.

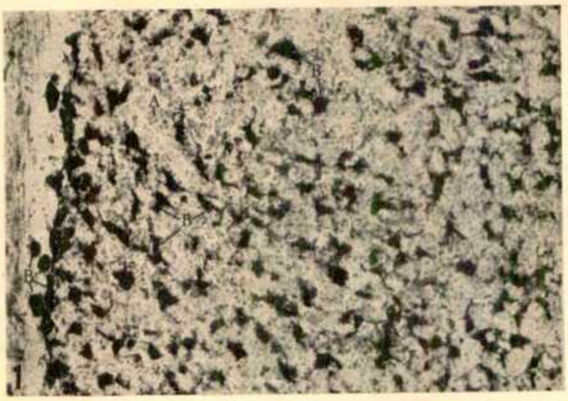
In Hodgkin's 'granuloma', however, mononuclear, binuclear (mirror-image) and multinuclear Reed–Sternberg cells were consistently negative for these enzymes, in striking contrast to the activity in adjacent reticulum cells. In one case of Hodgkin's sarcoma a minimal reaction for non-specific esterase was observed in some of the bizarre multinucleated cells.

No enzymatic activity was elicited from neoplastic lymphocytes.

The strong alkaline phosphatase of capillary endothelium demonstrated widespread proliferation of small blood vessels in all cases of Hodgkin's disease and around some foci of neoplastic tissue in other conditions. This feature is not obvious in routine paraffin sections, stained with hæmatoxylin and eosin.

In a case of 'monocytic' leukæmia, monocytoid cells infiltrating lymph nodes and spleen were also negative for these enzymes, in contrast to strong activity in surrounding histiocytes.

A case with long-standing myelophthisic anæmia, followed by the occurrence of atypical mononuclear and multinuclear cells in the bone-marrow, monocytoid cells in the peripheral blood, lymphadenopathy, hepato-splenomegaly and widespread infiltration into the skin and subcutaneous tissues, was also encountered. This type of case has been designated 'leukæmic reticuloendotheliosis'. The monocytoid cells again showed no evidence of enzymatic activity.



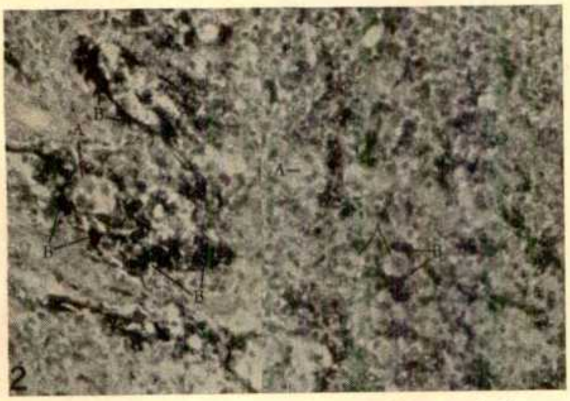


Fig. 1. Histiocytic lymphoma (reticulum cell sarcoma). Frozen section of a lymph node demonstrating non-specific esterase activity in malignant histiocytes (*B*); lymphocytes (*A*) show no activity. α-Naphthyl acetate method. (× *c*. 150)

Fig. 2. Hodgkin's disease. Composite photograph of frozen sections of lymph nodes showing non-specific esterase activity in histiocytes (*B*) but not in Reed–Sternberg cells (*A*). Mononuclear, binuclear and multinuclear cells are shown. α-Naphthyl acetate method. (× *c*. 150)

These findings indicate that, if Reed–Sternberg cells and monocytoid cells are derived from histiocytes (reticulum cells), then there has been a loss of activity of both non-specific esterase and acid phosphatase in the first two cell types. This seems most unlikely since there is no alteration in the reaction of these enzymes in malignant histiocytes. Furthermore, one would expect some cells to show a loss of activity of only one or other of the enzymes, especially in the less malignant form of Hodgkin's disease.

It seems probable, therefore, that both Reed–Sternberg and monocytoid cells are derived from cells other than histiocytes. That they may have a common origin from a primitive mesenchymal cell is not disputed, but, from the results of these enzyme studies, it appears that their relationship to the histiocyte is no closer than that of the lymphocyte to the histiocyte.

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Histochemical Analysis of some Dehydrogenase Systems in the Erythrocytes of some Lower Vertebrates

RECENTLY I have studied histochemically three dehydrogenase systems, namely, succinic phosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN) diaphorases and the DPN-dependent enzymes: lactic, malic, glutamic, β-hydroxybutyric, and ethanolic dehydrogenases, in the renal adenocarcinoma occurring in a laboratory strain of the oviparous toothcarp *Aplocheilus lineatus*[1]. In continuation of this investigation I decided to study the distribution of these enzymes, and also œstradiol-17β-dehydrogenase, in the erythrocytes of some of the lower vertebrates, namely, some teleosts, amphibians and reptilians.

These enzymes, with the exception of œstradiol-17β-dehydrogenase, were determined by the recently developed methods of Seligman, Nachlas and co-workers[2-5], based on the use of 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (nitro-BT)[3] as the chromogenic electron acceptor. The sections were incubated at 37° C. for 15 min. Some sections were also processed with Regaud's stain[6] to demonstrate mitochondria. In general, for morphological orientation parallel sections were stained with hæmatoxylin and eosin, hæmatoxylin and azophloxin or thionin.

For incubation with the œstrogens, the erythrocytes were suspended in isotonic phosphate buffer, *p*H 7·4; and afterwards the suspensions were shaken at 20° C. For the comparative determinations of enzymatic activity, quantities containing the same hæmoglobin content were used. Isolation, and qualitative and quantitative determination, of the steroids were carried out mainly by Repke's methods[7,8]. The œstradiol epimers were separated paper-chromatographically in the system chloroform-formamid.

The succinic dehydrogenase activity was low in the erythrocytes of all species investigated; it was linked to the cell membranes and the mitochondria. The DPN diaphorase activity appeared to be high, however. Also, this enzyme and the DPN-linked dehydrogenases were linked to the mitochondria and some cell membranes. The TPN diaphorase activity was much lower than the DPN diaphorase activity, and exhibited a widely dispersed and more finely intracytoplasmic distribution, which is certainly suggestive of the presence of microsomes and showed a high concentration in the cell membranes. The lactic dehydrogenase activity appeared to be rather high, the malic and β-hydroxybutyric dehydrogenase activities somewhat lower, whereas the glutamic and particularly the ethanolic dehydrogenase activity were generally low. The lactic dehydrogenase was more active than the malic dehydrogenase. As can be seen from Table 1, the enzymatic activities of the erythrocytes of the species investigated are mainly the same, although some minor differences can be established.

The œstradiol-17β-dehydrogenase occurred in the erythrocytes examined. This enzyme is capable of reducing œstrone to œstradiol-17β and of oxidizing œstradiol-17β to œstrone. An œstradiol-17β-dehydrogenase, found in ruminants, in addition to the œstradiol-17β-dehydrogenase[9], is not present in the erythrocytes of the lower vertebrates examined.

In general, the erythrocytes of the species examined correspond as regards œstradiol dehydrogenase

Table 1. Distribution of Succinic Dehydrogenase, DPN and TPN Diaphorase and Several DPN-Linked Dehydrogenases in the Erythrocytes of some Lower Vertebrates

The enzymatic activity was graded microscopically on the basis of colour reaction, 0 to 4, by inspection of the erythrocytes.

Enzymes	Teleosts										Amphibians								Reptiles					
	Anostomus anostomus Linné	*Hemigrammus caudovittatus* Ahl	*Barbus tetrazona* (Bleeker)	*Botia macracantha* (Bleeker)	*Aphyosemion coeruleum* (Boulenger)	*Xiphophorus helleri* Heckel	*Badis badis* (Hamilton)	*Aequidens pulcher* (Gill)	*Macropodus opercularis* (Linné)	*Brachygobius nunus* (Hamilton)	*Rana temporaria*	*Bufo calamita*	*Pipa pipa*	*Alytes obstetricans*	*Hyla arborea*	*Ambystoma mexicanum*	*Triturus cristatus*	*Salamandra salamandra*	*Lacerta agilis*	*Iguana iguana*	*Anolis carolinensis*	*Testudo graeca*	*Agama agama*	*Varanus niloticus*
Succinic dehydrogenase	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	1	0	1	1	1	1	1	1	0
DPN diaphorase	4	4	4	4	4	4	3	3	4	3	4	4	4	3	3	4	3	3	3	3	3	3	4	4
TPN diaphorase	2	2	2	1	2	2	1	1	2	1	2	2	2	1	1	2	1	2	1	1	1	1	2	2
DPN-linked dehydrogenases																								
Lactic dehydrogenase	4	3	4	3	3	4	3	3	4	3	3	4	4	3	3	4	3	3	3	3	4	3	4	4
Malic dehydrogenase	2	2	2	2	2	2	1	1	2	1	2	2	1	1	1	2	1	1	1	2	1	1	2	2
Glutamic dehydrogenase	1	1	1	1	1	1	0	0	1	1	0	1	1	0	1	1	1	1	0	0	1	0	1	1
β-Hydroxybutyric dehydrogenase	2	1	2	1	2	2	2	1	2	1	2	2	2	1	1	2	1	2	1	1	1	2	2	2
Ethanolic dehydrogenase	1	0	0	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	0	0	1	0	1	0

activity with those in man, rabbit and guinea pig[9] and imperfectly with those in ruminants[10,11].

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Non-specific Esterase Activity in Histiocytes

Histiocytes in subcutaneous connective tissue are characterized by having marked phagocytic activity. We have reported previously that histiocytes increase their enzymatic activities in proportion to the degree of transformation of these cells under various irritations[1].

Recently, we have examined histochemically non-specific esterase activity in a dorsal subcutaneous spread from rabbits intensely stained with trypan blue. The dose of trypan blue was 10 c.c. of a 1 per cent solution injected intravenously once daily for seven days. It was found that histiocytes having trypan blue granules in their cytoplasm exhibited strong non-specific esterase reaction in discrete areas corresponding to the trypan blue granules. In contrast, fixed histiocytes, without phagocytosed particles, in a spread of normal subcutaneous tissue, showed strong diffuse enzymatic activity in their cytoplasm. It is not clear whether this non-specific esterase activity exists inside the trypan blue granules or around them. However, it is unquestionable that, when trypan blue is being ingested by the digestive vacuoles of the histiocyte and concentrated into granules within them, the non-specific esterase distributed in the protoplasm becomes organized and collects in the region of the trypan blue granules.

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PATHOLOGY

Evidence for Multiple Cæruloplasmin Components in Human Serum

More than one component of human cæruloplasmin has been reported by several investigators[1]. Morell and Scheinberg[1] have observed 4, possibly 5, components of cæruloplasmin by fractionating a pool composed of 9,109 individual sera. In such a large pool, infrequent unusual components would be easy to lose. Furthermore, the complex fractionation procedure provides opportunity for artefactual alteration of specific proteins. The resolving power of vertical starch-gel electrophoresis[2] combined with direct chemical and enzymic analysis on the gel provides a simple and elegant means of examining the problem in individual sera. Using this technique we have found 5 electrophoretically distinct proteins having one or more characteristic properties of cæruloplasmin.

Sera were obtained from 43 pregnant women (in the third trimester of pregnancy or within a few days *post partum*), 17 patients with various diseases (Table 1) and 5 normal individuals. A voltage gradient of 5 V./cm. was maintained for periods of 16–20 hr. at room temperature. Half of each starch-gel block was stained with amido black 10*B*[2]; the other half was stained with *p*-phenylenediamine dihydrochloride (PPD), employing a modification of the method of Morell and Scheinberg[1]. Filter paper strips were saturated with a 1·0 *M* acetate buffer (*p*H 5·0) which contained 0·5 per cent PPD. These strips were pressed on the cut surface of the block for 5 min. and

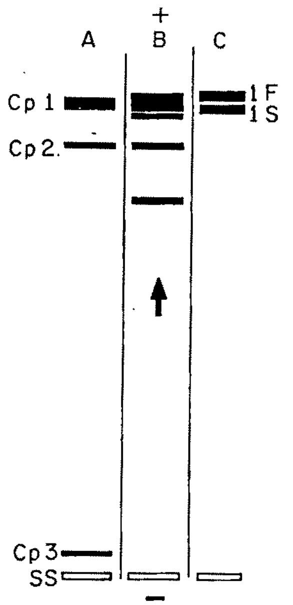

Fig. 1. Schematic diagram of the results of vertical starch-gel electrophoresis of serum of a pregnant human (*A*), cæruloplasmin concentrate, K.A.A.B.I. *DWZ* 71 (*B*) and 2 sera with atypical cæruloplasmins (*C*). *Cp* 1, Principal cæruloplasmin component; *Cp* 2 and *Cp* 3, probable and presumptive minor cæruloplasmin components respectively; 1*F*, major PPD oxidase component with fast mobility in serum of normal pregnant human; 1*S*, major PPD oxidase component with slow mobility in serum of patient with the muco-cutaneous syndrome

then removed. The block was allowed to stand at room temperature for the next 6 hr., during which time a deep purple colour developed in the region of the fast α-2-globulins. This principal component (*Cp* 1 of Table 1 and Fig. 1) was found in all specimens except one from a patient with Wilson's disease. Two other patients with this disorder had markedly diminished but detectable amounts of this material having a mobility exactly like that of the principal component of normal individuals. A principal component having a slightly faster mobility (*Cp* 1*F*) was found in the serum of one normal pregnant woman; one with a slightly slower mobility (*Cp* 1*S*) was found in the serum of a patient with the muco-cutaneous syndrome[3].

In the area associated with the transferrins, a second violet band of products of PPD oxidation (*Cp* 2) was found in specimens from all pregnant subjects, from one normal female, from a patient with carcinoma of the lung and from a patient with prostatic carcinoma who had been receiving stilbœstrol for 5 years. The second band in all cases was considerably less intense than the main component. A second examination was needed to demonstrate its presence in the sera of a few pregnant subjects.

Table 1

Group	No. of subjects	No. with		
		Cp 1	*Cp* 2	*Cp* 3
Normal subjects	5	5	1	3
Pregnant subjects	43	43*	43	21
Subjects with disease states:				
Wilson's disease	3	2	0	—
Bronchogenic carcinoma	3	3	1	—
Huntington's chorea	2	2	0	—
Chronic bronchitis	1	1	0	—
Malignant lymphoma	1	1	0	—
Multiple myeloma	1	1	0	—
Prostatic carcinoma	1	1	1	—
Active tuberculosis	1	1	0	—
Schizophrenia	2	2	0	—
Personality disorder	1	1	0	—
Muco-cutaneous syndrome	1	1†	0	—
Total	65	64	46	24 (out of 24 studied)

* One specimen had atypical (fast) mobility.
† Atypical; (slow) mobility.

A very faint third component (*Cp* 3) appeared immediately in front of the sample insertion slot in all sera for which it was examined (3 normal and 21 pregnant subjects).

A commercially prepared cæruloplasmin concentrate of human *post-partum* serum (lot *DWZ* 71 from K.A.A.B.I. of Stockholm) showed a very intensely stained principal component covering a broad zone in the fast α-2-region, apparently comparable to *Cp* 1 described above, and a second band with the same mobility as that of *Cp* 2 described above. Two much fainter bands were also found: one between the principal component and the second band and another with considerably slower electrophoretic mobility than the second band.

Several blocks were stained by filter paper strips saturated with 1·0 *M* acetate buffer (*p*H 5·0) which contained 6 m*M* concentrations of serotonin creatinine sulphate, L-adrenaline or L-nor-adrenaline bitartrate. These strips were pressed on the cut surfaces of the starch blocks and left there for 15 min. Coloured products developed during the next 24 hr. in the α-2-globulin region (*Cp* 1) of samples from normal and pregnant individuals and in the K.A.A.B.I. cæruloplasmin concentrate. Staining of *Cp* 2 in the region of the transferrins was visible only in the cæruloplasmin concentrate.

Blocks were stained for copper by placing them in a saturated ethanolic solution of rubeanic acid for 24 hr., after which time greenish-black bands were seen at the sites of *Cp* 1 and *Cp* 2. Visual estimates of the PPD staining intensity suggest that the concentration of *Cp* 3 is of the order of 1/1,000th that of *Cp* 1; its failure to react with rubeanic acid may therefore be due to its low concentration.

The *Cp* 1 component has the principal characteristic properties of cæruloplasmin: it has a natural blue colour, contains copper, and oxidizes PPD, 5-hydroxytryptamine, adrenaline and nor-adrenaline[4,5]. *Cp* 2 contains copper and is visibly capable of oxidizing PPD and the other substrates; this is probable evidence that this protein is a cæruloplasmin. *Cp* 3 oxidizes visibly PPD; this is presumptive evidence that this protein is a cæruloplasmin. *Cp* 1*F* and *Cp* 1*S* have not as yet been examined in detail with respect to properties other than PPD oxidation.

The occurrence of the *Cp* 2 in pregnant women and in a male receiving stilbœstrol suggests that œstrogens influence the appearance of this protein in detectable amounts. In all these cases, *Cp* 1 was visibly increased in concentration as compared with that found in normal sera; this is in accordance with the known ability of œstrogens to influence cæruloplasmin as measured by other techniques[4]. The appearance of *Cp* 2 appears to be determined by some factor other than concentration, since it could not be found in normal sera which had been concentrated five-fold and ten-fold, raising the concentration of the *Cp* 1 component to the equivalent or excess of that seen in the serum of pregnant subjects (serum was placed in a dialysis casing which was suspended in air in front of an electric fan).

The atypical components, *Cp* 1*F* and *Cp* 1*S*, are being investigated further by means of family studies.

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Note added in proof: *Cp* 1*S* has a normal mobility when the haptoglobin (type 1–1) is completely removed from this zone by saturation with hæmoglobin. Blood relatives of this patient all have the normal *Cp* 1. A study of four generations of the family in which *Cp* 1*F* is segregating indicates inheritance of the trait as a dominant character. *Cp* 1*F* is differentiated more readily from *Cp* 1 at *p*H 9·5 (0·0125 *M* borate buffer).

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BIOLOGY

Effect of 5-Bromuracil on Crown Gall Tumour Induction

Crown gall tumours in plants are initiated by the action of virulent strains of *Agrobacterium tumefaciens* (Smith and Town) Conn on susceptible plant cells. Once these cells have been altered to tumour cells, the presence of the bacteria is no longer required for their continuing, autonomous growth[1]. The unknown agent or method by which this alteration of properly conditioned cells to tumour cells is accomplished is not known[2]. There have been a number of recent claims that this agent is a nucleic acid, specifically a deoxyribonucleic acid (DNA)[3,4] or that DNA plays some accessory part in tumour induction[5]. Some of these claims have been subject to critical examination and have been shown to rest on inadequate evidence[6]. The most recent claim that the transforming agent may be a DNA rests on the work of Bopp[4], who reported that the pyrimidine analogue 5-bromuracil does not inhibit the growth of *A. tumefaciens*, the wound-healing reaction of the host plant, or the growth of induced tumours, but does inhibit the initiation of tumours. This communication is concerned with an evaluation of these findings.

Four different samples of 5-bromuracil were obtained from commercial laboratories. Sample 1 was purchased from California Biochemicals, Los Angeles, California; sample 2 was purchased from Mann Research Laboratories, New York; sample 3 was a sample used by Dr. Bopp in his experiments and kindly sent to us; it was manufactured by Flüka A. G., Buchs, Switzerland; while sample 4 was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

The effect of these samples at a concentration of 200 μgm./ml. on the generation time of the bacteria is shown in Table 2. In these experiments the bacteria were grown in a defined medium[7] for 24 hr., then diluted 100,000 times into special flasks equipped with tubes for photometry. The medium containing 5-bromuracil and the control medium were both filter-sterilized through *HA* Millipore filters (Millipore Filter Corp., Bedford, Mass.), because it was found that autoclaving destroyed the inhibitory principle in sample 4. Not enough of sample 3 was available to test the effects of autoclaving on its growth-retarding component. The flasks were shaken about 150 times per minute on a motor-driven shaker in an incubator kept at 27° C. Turbidimetric readings were made with a Coleman, Jr., spectrophotometer at 410 mμ.

The effect of 5-bromuracil on tumour induction was tested by inoculating the midrib of young *Kalanchoe daigremontiana* (Hamet and Perrier) leaves with the highly virulent strain *B*6 of *A. tumefaciens* and placing one drop of a solution containing 200 μgm./ml. 5 bromuracil on the inoculation site on the day of inoculation and on 3 successive days. Water was applied to the control inoculations. About two weeks later the infected sites were scored independently by each of us. The average for 32 inoculations is given in Table 1. These results clearly indicate that samples 1, 2, and 3 did not interfere with tumour induction, whereas sample 4 had a definito inhibitory effect.

Table 1. THE EFFECT OF 5-BROMURACIL ON BACTERIAL GROWTH AND TUMOUR INDUCTION

Treatment	Bacterial growth (in terms of generation time)	Average tumour score*
Control (water)	2·5	1·5
Sample 1, 200 μgm./ml.	2·5	1·6
Sample 2, 200 μgm./ml.	2·5	1·6
Sample 3, 200 μgm./ml.	3·0	1·6
Sample 4, 200 μgm./ml.	4·0	0·4

* Scoring: 0 = no reaction, 0·5 = questionable reaction, 1 = definite reaction, and 2 = strong reaction.

Chromatographic analysis of the four samples by ascending paper chromatography on Whatman No. 1 paper was carried out in two separate solvent systems. In an *n*-butanol/water (43 : 7) system, samples 1, 2, and 3 showed an ultra-violet absorbing spot at $R_F \sim$ 0·35; no other spots were detectable. In an *iso*-propanol/hydrochloride (13 : 7) system, samples 1, 2, and 3 showed ultra-violet absorption only at $R_F \sim$ 0·60; sample 2 had a slight 'tail' behind it. Sample 4 did not show any ultra-violet absorbing or other ultra-violet detectable spots at any point past the origin in either of these two solvent systems. Samples 1 and 2 were white, sample 3 was light pink, and sample 4 had a pinkish tinge. Elemental analysis of these samples was carried out by Mr. S. T. Bella of the Rockefeller Institute. The results of these analyses are shown in Table 2 and confirm the chromatographic evidence of the impurity of sample 4.

Table 2. ELEMENTAL ANALYSIS OF FOUR SAMPLES OF 5-BROMURACIL

Sample	Carbon	Hydrogen	Nitrogen
1	25·18	1·64	15·38
2	25·41	1·45	14·89
3	25·50	1·51	*
4	16·39	1·75	15·43
Theoretical	25·14	1·58	14·67

* Insufficient sample available for nitrogen determination.

These results indicate that there is associated with certain commercially available samples of 5-bromuracil a heat-labile impurity which does not absorb in the ultra-violet. This impurity inhibits bacterial growth and thus cannot be considered a specific inhibitor of tumour induction. We suggest that the tumour inhibition observed by Bopp reflects the presence of an impurity in his samples. Such an impurity, although inhibitory to the bacteria, would not have been detected by means of the bacteriological techniques which he employed.

Samples of comparatively pure 5-bromuracil failed to inhibit tumour induction, thus seriously questioning the validity of the data which were interpreted to indicate that 5-bromuracil specifically inhibits the formation of an effective tumour-inducing agent and that such an agent might be a DNA.

We acknowledge the interest and support of Dr. Armin C. Braun during this investigation.

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Seasonal Acclimatization of Oxygen Consumption to Temperature in a Tropical Poikilotherm, the Earthworm, *Megascolex mauritii*

SEASONAL acclimatization of oxygen consumption to temperature has been demonstrated in poikilotherms by many authors, and its profound ecological significance is widely recognized[1-5]. But most such investigations are restricted mainly to marine organisms of cold and temperate zones. Comparatively little attention has been paid to such seasonal compensatory changes in oxygen consumption in tropical, freshwater and amphibious animals and much less in the terrestrial forms. Except for the work of Vernberg[6] on intertidal crabs, nothing has been reported of the seasonal fluctuations of respiration in any tropical poikilotherm.

The results of this work show the existence of a homoiostatic mechanism in oxygen consumption, compensating for the seasonal temperature changes in the tropical earthworm, *Megascolex mauritii*.

The oxygen consumption of these worms was measured under water at 15°, 20°, 25°, 30°, 35° and 40° C., by Winkler's method[7]. The determinations were made during the winter (January) and the summer (June) of 1959 and 1960. Measurements at 40° C. in June 1959, and those at 15° C. in June 1960, could not be made. The results for the year 1960 are presented in Fig. 1. The regression lines for 1959 summer samples are also included for comparison.

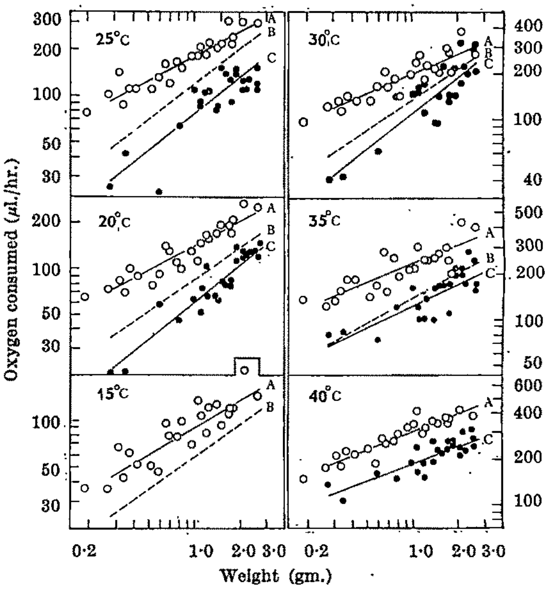

Fig. 1. Oxygen consumption in *Megascolex mauritii* as a function of size and temperature during winter and summer. Each point represents an individual measurement; double logarithmic grid; *A* (open circles), winter animals of January 1960; *B*, summer animals of June 1959; *C* (closed circles), summer animals of June 1960

Acclimatization of oxygen consumption to seasonal change in temperature occurs in the tropical earthworm *Megascolex mauritii*, as may be seen from the fact that, at all the temperatures investigated, the metabolism of the winter animals (lines *A* in Fig. 1) was higher than that of the summer samples (lines *B* and *C*). The difference in the slopes of the regression curves of winter and summer animals indicates that the effect of weight on oxygen consumption changes with season. In other words, the degree of acclimatization to temperature varies with weight. The degree of acclimatization may be greater in smaller worms, which generally consume oxygen at a higher rate than larger worms. This difference, between smaller and larger worms, in the degree of acclimatization to seasonal temperature changes, explains the flat and steep regression curves of oxygen consumption of winter and summer populations, respectively. Another fact that is clear from Fig. 1 is that the June 1959 population (lines *B*) utilized more oxygen than that of the June 1960 (lines *C*).

Roberts[8], working on population samples of *Pachygrapsus crassipes*, could not detect any seasonal thermal acclimatization of respiration. Recently, it was shown by Vernberg[6] that no seasonal variation in oxygen consumption was to be found in the tropical form, *Uca rapax*, whereas a marked seasonal acclimatization was exhibited by the temperate form, *Uca pugnax*. This absence of seasonal acclimatization to temperature in the tropical crab, he thought, might be due to small annual temperature fluctuations. He also felt that the results of Roberts on *P. crassipes* might be due to low annual fluctuations in temperature. But the high and low mean monthly temperatures recorded by Roberts were about 12° C. in January and 22° C. in August. The high and low mean temperatures recorded during my investigation were 35° C. in May 1959, 36·5° C. in May 1960, and 25·5° C. in December 1959. Though the difference in the high and low temperatures is nearly the same in both cases, it is interesting to note that good metabolic acclimatization to seasonal change was observed in this tropical form.

The difference in oxygen consumption between the two summer populations of June 1959 (lines *B*) and June 1960 (lines *C*) can be correlated with the history of temperature during the two summer seasons (Table 1). The temperature during summer 1960 was not only higher (36·5° C.) than in 1959 (35° C.) but also was maintained for a longer duration. It is noteworthy that the degree of thermal acclimatization of oxygen consumption in summer populations depends on the intensity as well as the duration of high temperature during that particular season.

Table 1. STATEMENT OF MEAN MONTHLY TEMPERATURE (IN ° C.) DURING WINTER AND SUMMER OF 1959 AND 1960

Year	Winter		Summer			
	Dec.	Jan.	Mar.	April	May	June
1959	—	—	31·5	33·5	35	35
1960	25·5	26	31·5	35	36·5	36

Similar work, during the same period (January and June 1960), on another tropical earthworm, *Octochætus* sp., also showed a good seasonal acclimatization of respiration to temperature and this acclimatization was more evident in the smaller worms.

A complete report will be published elsewhere.

I wish to express my gratitude to Prof. Kandula Pampapathi-Rao, head of the Department of Zoology, Sri Venkateswara University, Tirupati, for his interest and guidance throughout. Part of this work was carried out during the tenure of a junior research fellowship of the Council of Scientific and Industrial Research, New Delhi.

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A Hermit Crab New to Britain

OF recent years the hermit crab *Clibanarius misanthropus* (Risso) has been reported from stations successively farther north along the coast of the Bay of Biscay[1]. It seems probable that this represents a true northerly advance of this example of an essentially tropical genus, for it is unlikely to have been overlooked since its almost exclusively intertidal habit and its brilliant colours attract the attention. By 1957 it had been reported to the north of Brest, but it does not seem yet to have been found along the north French coast: in particular, the collectors of the Station Biologique at Roscoff have not found it. Indeed, Crisp and Fischer-Piette[1] advance reasons for supposing that it is unlikely to spread along this coast. On the other hand, the drift of surface water which occurs in some years from Finistère towards Land's End or towards the Scilly Islands might be expected to carry larvæ to the British shores. Despite regular surveys of the shores of the Scilly Islands, our colleagues have not found this species there; but during the early months of 1960 large numbers were found on the shore at Mousehole on the southern coast of Cornwall (lat. 56° 6′ N., long. 5° 31′ W.), near Land's End. It is significant that the water at the western end of the English Channel in March and April 1959 (when the larvæ of *C. misanthropus* would be planktonic) had an unusually high salinity, suggesting the presence of Biscay water flowing into the Channel.

C. misanthropus is a hermit crab with subequal claws and hence quite unlike any of the native species which have unequal claws. It seems likely that it was not present on this shore before the winter of 1959–60, to judge by the reports of amateur collectors in this region, nor has it been found on any of a number of beaches and shores in the neighbourhood. During the winter it was the commonest hermit crab at about mid-tide level on this one shore, as many as 48 being found in one small rock-pool, together with no more than 2 specimens of other species; in the summer it has been confined mainly to pools lower down on the shore, around low-water neap tide mark, but is still abundant. Most of them inhabit shells of *Littorina* sp., while a few are in shells of *Nucella*. In this they contrast with the French specimens, which were mainly found in *Gibbula* or *Monodonta* shells. During the winter all the animals collected were of nearly the same size; but in September 1960 a number of much smaller crabs were present in the population, suggesting that they had bred successfully. It will be interesting to observe if they succeed in permanently colonizing British shores.

It has been suggested[1] that *Pagurus fasciatus* Bell is identical with this species. Bell[2] described and named *P. fasciatus*, without ever having seen a specimen, from a coloured drawing supplied by a Mr. Cocks of Falmouth, where, presumably, he collected his specimen. We feel that this identification cannot be correct, but the description is too vague for any certainty. There is just a slight possibility, therefore, that *Clibanarius misanthropus* has been taken before on the south coast of Cornwall, more than a hundred years ago.

We wish to thank Dr. A. F. Turk for his interest.

Note added in proof. During spring, 1961, a new year-class has appeared at Mousehole and specimens of this same (new) year class have been collected at Wembury, a similar habitat on the southern coast of Devon, 105 km. east of Mousehole. Since Wembury (lat. 50° 19′ N., long. 4° 5′ W.) is probably the commonest collecting ground of all for workers and students at the Plymouth Laboratory, it seems exceedingly unlikely that *Clibanarius* should have been present in earlier years. It thus seems that it is continuing its northward and westward spread.

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A Bathyal-abyssal Hermit Crab with a Calcified Asymmetrical Abdomen

IN the highly productive part of the Pacific Ocean, off Central America, the Danish *Galathea* Deep-Sea Expedition in 1952 made an exceptionally rich haul with a large herring otter trawl at a depth of 3,570 m. This haul yielded *inter alia* the first known species of *Neopilina* and a total of 126 species and about 2,100 specimens of benthic animals.

The 10 species of decapod crustaceans comprised 18 specimens of a pagurid, *Probeebei mirabilis*[1]. This genus and species was described by Boone[2,3] as "a primitive macruran crustacean". First and foremost, because of the calcified abdomen the author did not recognize it as a pagurid, and apart from a reference to it in the *Zoological Record* of 1926 it has since been almost totally neglected, for example, in Bronn, by Melin[4], Gordan[5], and others. Within the Paguridea it belongs to the Pagurinae Latr. (= Eupagurinae Ortm.). Boone's family Probeebeidae[3] is thus invalid.

The holotype was the only specimen collected by the *Arcturus* Expedition in 1925, south of Cocos Island in the Gulf of Panama at 1,145 m. depth. It belongs to the youngest post-larval stage which was represented in the *Galathea* material, and is 20 mm. long.

The largest of the adult specimens measures 65 mm. The carapace is somewhat broadened and furnished with many short spines and a long rostrum. The abdomen is moderately large, completely calcified dorsally, almost entirely symmetrical in the male and somewhat asymmetrical in the female, the left part of each segment being a little longer than the right and with only the left pleopods developed. The telson is symmetrical in both sexes. The right cheliped is slightly larger than the left, and peræopods II and III are very elongated as in *Tylaspis*, the hitherto deepest recorded hermit crab[6]. The male has the first pair of pleopods preserved, the female pleopods 2–5.

Phylogenetically the genus is most closely related to other genera with paired pleopods in the male and with a corresponding number of pairs of gills (eleven): *Parapagurus*, *Sympagurus*, *Tylaspis*, and *Tomopagurus*. Apart from the latter, which occurs at a depth of 132 m. off the Barbados, the remaining genera are bathyal or abyssal.

A number of instances are known of hermit crabs having evolved means other than a gastropod shell for protection of the abdomen, probably first and foremost because of lack of sufficient shell material. Primarily in the deep sea, several species have taken advantage of *Dentalium* shells, pieces of wood or bamboo, polychæte tubes, etc. In *Tylaspis*[6] and especially *Ostraconotus*[7], the abdomen has become reduced and is probably unprotected, although it is soft-skinned and can only, to a lesser degree, be folded under the cephalothorax. In these two genera, in practically all the species with an almost straight protective object, and in the three instances mentioned below, the animals have acquired a secondarily symmetrical or almost symmetrical abdomen; but their origin from asymmetrical forms in gastropod shells is always discernible by the presence of unpaired, left-hand pleopods, at any rate in the females. Furthest advanced along this line are those forms which have regained the calcified abdomen of their ancestors (the primitive, quite symmetrical Pylochelidae and the Thalassinidea), namely: (1) the stone crabs (Lithodidae); (2) the robber crab (*Birgus latro*); (3) *Probeebei*, which all originate from the Paguridae but which have evolved from systematically very differing stocks within the family[8]. The Lithodidae are fully 'carcinized'[9], with the comparatively small abdomen firmly pressed against the cephalothorax, with a very crab-like carapace, and with peræopods IV as long as II and III.

Birgus and *Probeebei* represent parallel lines of evolution in environments—dry land and abyssal depths—both of which are extremes in comparison with the warm shallow water in which the pagurids primarily evolved. In both cases the secondary calcification of the abdomen must be due to lack of sufficient shell material. The calcification is further advanced in *Probeebei*; but in several other respects the evolution to a freely roaming animal has caused a considerably greater change of the adult *Birgus* than of *Probeebei*: peræopod IV is comparatively much larger and can be used for walking; not only the male but also the female abdomen are practically symmetrical (apart from the lack of right pleopods), and the uropods have become almost completely reduced; also in the adaptation to the life on dry land (shape of eye-stalks, antennulæ and antennæ, presence of lungs, behaviour, etc.[10]), *Birgus* is much more advanced than the species of the closely related genus *Coenobita*, which are likewise land-dwelling.

On most islands where *Birgus* occurs it outgrows the available land snails soon after having left the sea, but the metamorphosis into the free-living stage can be considerably postponed experimentally[11]. It is to be hoped that future investigations in the area of distribution of *Probeebei mirabilis* will reveal the early post-larval development of this species, which may prove as interesting as that of *Birgus latro*.

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Adaptive Euryhalinity in the Cyprinodont *Lebistes reticulatus* Peters

Lebistes reticulatus has always been considered as a strictly freshwater fish[1] (that is, it can tolerate only a narrow range of salt concentration in the water). In an evacuation canal, which opens in the sea, at Hann (north of Dakar), we were astonished to find numerous individuals of this species, even in spots where the concentration of sea-water was rather high. Experiments in the laboratory led us to the following conclusions:

(1) The upper limit of salinity tolerated by this species, at 20–25° C., when transferred directly from fresh water, is about 50 per cent sea-water. At such a concentration, mortality is very low (7 per cent). At a concentration of 85 per cent sea-water or higher, all fish perished within 2–3 days.

(2) Fish kept in 50 per cent or 75 per cent sea-water, for 1 wk., were transferred to 100 per cent sea-water, without any mortality resulting. During the following two months these fish were regularly observed; they seemed well and healthy, and their behaviour was apparently normal.

(3) Several series of fish were transferred successively to baths containing higher and higher concentrations of sea-water (25, 50, 75 and finally 100 per cent sea-water). They were kept for 1 wk. in each bath; the resulting mortality was nil. This seems to prove that we are dealing with a phenomenon of adaptation rather than selection.

(4) Fish adapted to sea-water were transferred directly to fresh water; no mortality resulted. This means that individuals adapted to sea-water have no need of a new adaptation to fresh water.

(5) The adaptation to sea-water is temporary, since individuals adapted to sea-water all died when transferred to fresh water and returned to sea-water within a week.

(6) A special design permitted us to adapt individuals to more and more concentrated media, by gradually increasing the sea-water content in the aquarium in which the fish were put, in such a manner that the fresh water was replaced by sea-water, within 28 or 36 hr. When fresh-water replacement was accomplished within 28 hr., all fish without exception died during the 24 hr. following. When the replacement was accomplished within 36 hr., only three individuals (out of fifty fish) died. This means that the minimum time for adaptation to sea-water is about 36 hr.

(7) Several series of 25 individuals each were adapted to sea-water during at least 3 wk.; they were transferred to fresh water and then returned again to sea-water, 24, 36, 48 or 72 hr. after. The results obtained were as follows:

Time (hr., in fresh water)	No. dead
24	none
36	4 individuals
46	8 individuals
72	20 individuals

This means that individuals begin to lose their adaptation to sea-water when kept in fresh water for about 36 hr., that is, the minimum time for adaptation to sea-water is approximately equivalent to that during which the fish keep their adaptation to sea-water.

(8) Having noticed during the previous experiments that males usually die before the females, 100 adult individuals of both sexes were transferred directly to pure sea-water. Five and a half hours after, fish began to die (especially males). Six and a half hours after they began to die, all males were already dead, but only 55 females were quite dead. The remaining females died within the following 12 hr.

We are aware of the probable existence of the factor, namely, weight of the individuals in the mortality, since the youngest adult female is usually heavier than the biggest male. However, we think that this factor is not exclusive. For the same sex (male), the histogram has shown that mortality was not a function of the weight, but represented by a Gaussian curve.

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Effect of Temperature on *Apis indica* Workers

Recent work on the effect of environmental temperature on the European honeybee, *Apis mellifera*[1-3], has been partially repeated with two colonies of *Apis indica* brought from Ceylon by Mr. L. A. S. Perera.

Groups of 100 *A. indica* were kept in cages for three days with sugar syrup (2 parts sugar : 1 part water) and water at temperatures from 20° to 40° C., three groups being kept at each temperature; the amount of food and water consumed, the temperatures at the centres of the groups, and the number of bees which died, were recorded. The results are given in Table 1. Comparable results for *A. mellifera* are in brackets.

Table 1

Environmental temperature (° C.)	Mean temperature of groups (°C.)	Mean consumption (mm.^{3}) per bee per day		Mean No. dead at end of day 1
		Sugar syrup	Water	
20	24·0 (24·9)	17·1 (31·7)	0·1 (0)	8·9 (4·0)
25	29·3 (29·6)	17·2 (21·3)	0·1 (0·3)	5·5 (1·0)
30	31·4 (30·8)	14·3 (11·5)	0·1 (0·5)	4·0 (4·0)
35	35·7 (35·0)	11·6 (18·6)	1·2 (10·1)	1·0 (3·7)
40	39·6 (39·2)	1·9 (5·5)	21·8 (16·7)	15·0 (13·8)

Groups of *A. indica* at 20° and 25° C. took similar amounts of food and were both about 4 deg. C. above environmental temperature but, in general, the lower the environmental temperature the greater the difference between it and the group temperature, and the greater the food consumption. Thus *A. indica* like *A. mellifera* responds to low temperature by increased food consumption, indicating increased metabolism, and increased production of heat. At 40° C. both species took little food but large quantities of water, reflecting a high rate of losing water. The mortality of both species was similar at the higher temperatures, but was greater for *A. indica* at the lower temperatures.

To find the temperature at which the two species enter chill coma single *A. indica* and *A. mellifera* workers were taken from their hive entrances and placed at low temperatures. Table 2 shows the numbers that moved and were still after exposure for 1 hr. All those that were still, moved again when the temperature was raised.

Table 2

Environmental temperature (° C.)	*A. indica*		*A. mellifera*	
	Moved	Still	Moved	Still
8	0	12	0	12
9	0	17	3	15
10	1	30	20	10
11	22	22	33	10
12	15	12	21	3
13	21	4	26	0
14	6	0	6	0

Unfortunately, the *A. indica* colonies died before much information could be obtained on the effect of previous acclimatization on chill coma temperatures. However, after acclimatization at 35° C. for 24 hr. only 6 out of 21 *A. indica* moved at 11° or 12° C., whereas 12 out of 18 *A. mellifera* moved. Thus the species differed in behaviour ($P < 0 \cdot 05$) even when acclimatized to the same temperature. Both appeared to have a higher chill coma temperature after being kept at temperatures higher than they would have experienced at their hive entrances.

Mellanby[4] found that *Cimex lectularius* (from temperate regions) was more resistant to starvation and desiccation, and could breed more successfully at low temperatures than *C. rotundatus* (from tropical regions) but found no circumstance in which *C. rotundatus* was markedly superior. Our results indicate that *A. mellifera* is more adapted to temperate climates than *A. indica*, but do not show that *A. indica* has any corresponding adaptation to the tropics.

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Effect of Ringing Cotton Stems on Content of Reducing Sugars and Insoluble Nitrogen and on Degree of Infection with *Xanthomonas malvacearum* (E.F.S.) Dowson

Hughes and Fowler[1] reported that the glucose-level of the leaves of synthesized varieties containing black-arm resistance genes B_2 or $B_2 + B_3$ was higher than that of the susceptible parent type during the first three months after sowing. Bird and Joham[2] found that girdling the main stem of a susceptible variety caused cotton plants to become more resistant to the same disease. In the present investigation the effect of ringing the main stem of cotton plants on the level of reducing sugars and insoluble nitrogen in the leaves, and its relation to infection with the angular leaf spot disease, were examined.

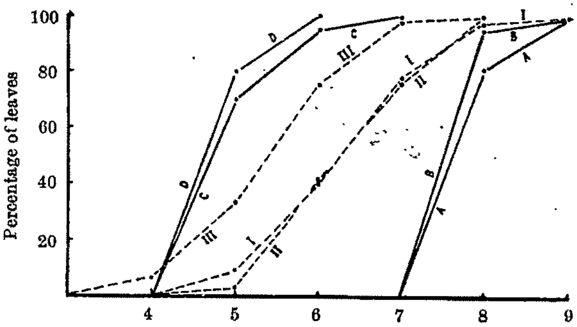

Fig. 2. Cumulative percentage of leaves showing different grades of infection (for explanation of letters and figures, see Table 1). ●——●, 1957 series; ● – – – ●, 1958 series

Plants of susceptible Domains Sakel cotton (*Gossypium barbadense* L.) were grown singly in 18-l. tins, topped when about 4 months old to induce thicker foliage, and inoculated about 4 wk. later. Two series of experiments were carried out. In the first, namely, 1957 series, inoculation was by rubbing the under-surface of the leaves with the crude suspension of *Xanthomonas malvacearum* prepared from naturally infected leaves of cotton. Twenty to thirty leaves, borne on four plants, were inoculated for each treatment (Table 1). In the second series (1958 series), inoculation was by spraying a thick water suspension of a recent isolate of *X. malvacearum* on to the leaves of the same variety of cotton. Eight plants were so inoculated in each treatment and 80–100 leaves were used for assessing the degree of infection about 4 wk. later in accordance with the grading system adopted in ref. 3. The grades were based primarily on the size and shape of the spots, with 1 representing full immunity and 12 full susceptibility.

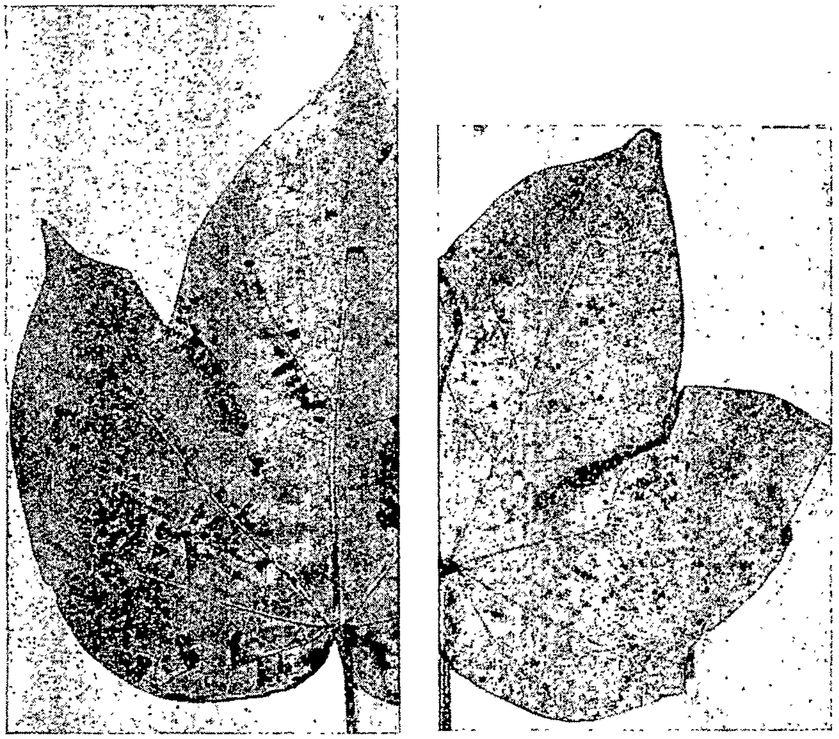
(*a*) (*b*)

Fig. 1. Angular leaf spots on Domains Sakel cotton (1957 series); (*a*) plants not ringed, and (*b*) plants inoculated 10 days after ringing the stem

Representative samples were taken from each plant of the 1958 series for determination of reducing sugars and insoluble nitrogen contents. These samples were chosen of approximately the same size and age, and were cut just before spraying the bacterial suspension at 10–12 a.m. Maskell and Narain's modification (unpublished) of the method in ref. 4 was

Table 1. RINGING AND INOCULATION TREATMENTS OF 1957 AND 1958 SERIES TOGETHER WITH REDUCING SUGARS AND INSOLUBLE NITROGEN CONTENTS OF THE SECOND SERIES

1957 series			1958 series				
Treatment	Ringed on	Inoculated on	Treatment	Ringed on	Inoculated on	Insoluble nitrogen gm./100 gm. dry wt.*	Reducing sugars mgm. gluc./100 gm. fr. wt.†
A	—	Oct. 12	I	—	Nov. 10	3·24	86·9
B	Oct. 12	Oct. 12	II	Nov. 10	Nov. 10	3·55	109·7
C	Oct. 12	Oct. 22	III	Oct. 30	Nov. 10	3·02	208·7
D	Oct. 12	Oct. 27					

* Means of 8 samples; $F = 1·56$, not significant at 5 per cent level.

† Means of 8 samples; $F = 3·64$, significant at 5 per cent level, for difference to achieve significance between treatments at $P_{0·05} = 28·0$.

adopted for determining reducing sugars. Insoluble nitrogen was determined using a modification by Hassan[5] of the method in ref. 6.

The results of the 1957 series of inoculation experiments showed a marked increase in resistance to angular leaf spot disease 10–15 days after ringing the stems of cotton plants (Fig. 1). Plants ringed and inoculated on the same day showed no appreciable difference in the degree of infection as compared with the control (not ringed). The results of the 1958 series showed a similar trend, but were less marked (Fig. 2), probably due to differences in the method of inoculation adopted in each series. The increase in resistance coincided with a significant rise in the level of reducing sugars in the ringed plants (Table 1). Insoluble nitrogen contents, on the other hand, did not differ significantly in the three treatments. However, there was a tendency toward a lower level of insoluble nitrogen in ringed plants, which was manifested further by a pale green colour of the leaves.

As shown[7], carbohydrates increase in the leaves of stem-ringed plants while the nitrogen-level remains more or less the same. The above results show that this trend causes the plants to become more resistant to angular leaf spot disease. They show, furthermore, that a high level of reducing sugars should prevail at the time of infection. Thus, unless ringed plants are allowed a sufficient time to accumulate sugars in the leaves before inoculation, ringing would exert little or no influence on the degree of infection. The insoluble nitrogen, on the other hand, does not seem to have an effect on the degree of infection. This is in accordance with Hassan's results (unpublished).

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ENTOMOLOGY

Use of an Artificial Spermatophore in the Study of Activation of Spermatozoa and Development of Spermatophores in the Cockchafer

I HAVE already described[1] the development of the reproductive organs in the male of the cockchafer (*Melolontha melolontha* L.) and the origin, development, and function of the spermatophore. The spermatophore is formed in the copulatory sac of the male while it is inserted in the bursa copulatrix of the female. The contents of the genital duct (bundles of inactivated spermatozoa, cyst cells) and the secretion of the accessory glands (the hyaline component staining green with Masson's trichrome, a granular component staining red, and basophil droplets staining blue-violet) are expelled into the copulatory sac. Within three hours of copulation, the spermatophore is encased in a protein membrane and deposited in the bursa copulatrix.

The components forming the spermatophore are at first randomly mixed. During copulation and following the deposition of the spermatophore in the bursa copulatrix, characteristic changes take place. The components of the secretion of the accessory glands are differentiated into islands of hyaline and granular substances. The spermatozoa are activated, separate from the bundles, and form clusters. They digest the hyaline substance. By this digestion numerous basophil drops begin to form. The activated spermatozoa, by means of chemotaxis, then enter the receptaculum seminis. The entire course of development has been studied by serial sections.

The suggestion of using an artificial spermatophore originated in the attempt to discover if the mechanism initiating the reactions in the spermatophore was the simple mixing of the spermatophore components or the influence of the ejaculatory duct during copulation or of the bursa copulatrix. The artificial spermatophore is prepared as follows. The contents of the dissected ejaculatory duct and the accessory glands of the male are mixed on a slide approximately in the ratio of 1 : 2 (this ratio corresponds to the ratio in natural conditions). The drop is mixed lightly and covered with a cover-slip. The mixture must be spread without any air bubbles under the entire cover-slip, the edges of which are then sealed with 'Vaseline'. It is necessary to carry out the work quickly. The entire development can then be examined by phase-contrast microscopy.

The components of the artificial spermatophore are distributed randomly when it is first made (Fig. 1). After 30 min. the differentiation of the components of the accessory glands begins. 1–2 hr. later the individual spermatozoa are activated and they move about in the hyaline substance (Fig. 2). After 2–3 hr. the spermatozoa are completely activated and concentrated in large clusters. From 6 to 24 hr. the hyaline substance gradually decreases. After 24 hr. (18° C.) when the hyaline substance is used up, movement of the spermatozoa ceases.

Artificial spermatophores not only clearly show that all the changes are entirely independent of external factors, but also make it possible to observe the entire course of development of the spermatophore *in vitro*.

When the artificial spermatophore was subjected to flowing oxygen, air and nitrogen, development of the spermatophore was found to be quickest and activation most complete in nitrogen ; air was not so

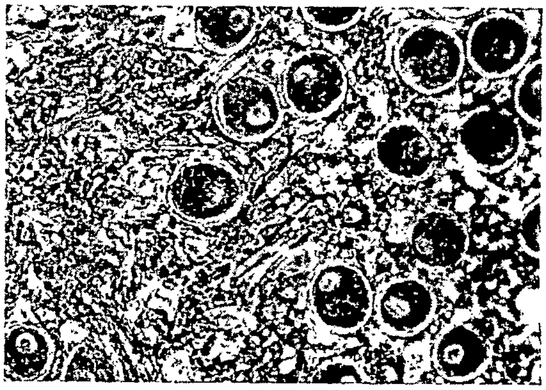

Fig. 1. Artificial spermatophore immediately following its preparation. The components of the secretion of the accessory gland and genital duct are randomly distributed. Phase contrast. (× 210)

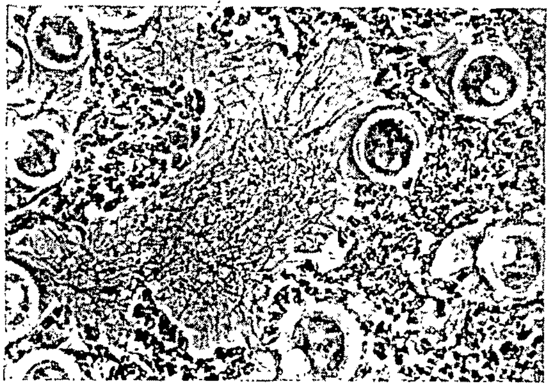

Fig. 2. Artificial spermatophore after 1–2 hr. The components of the secretion of the accessory glands are being differentiated. Individual, activated spermatozoa appear in the hyaline substance. Phase contrast. (× 210)

effective. Although a quick activation of spermatozoa takes place in oxygen, there is, however, no differentiation of the secretions of the accessory glands in oxygen, so that the spermatozoa die in about 1 hr. The mechanism of the development of the spermatophore is, therefore, adapted to the conditions in the bursa copulatrix where there is a lack of oxygen.

Experiments have shown that although oxygen itself may initiate the activation of spermatozoa, it does not do so, however, under natural conditions. Both in the artificial and natural spermatophore, the activation of spermatozoa is initiated by the hyaline substance (in making artificial spermatophores a slight activation often takes place due to contact with air). 10–15 min. after sealing the cover-slip, movement ceases. Complete activation with subsequent clustering may be observed only after differentiation of the secretion of the accessory glands.

The differentiation of the secretion is caused both by the cyst cells and by the bundles of inactivated spermatozoa, evidently due to the change in the surface tension in the spermatophore mass.

The method of making artificial spermatophores, on account of its simplicity, may be used not only in the work on special problems of the development of the spermatophore in insects, but also in some general biological problems, mainly those of activation, inactivation and nutrition of spermatozoa.

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Prague.

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Termination of Pupal Diapause in the Cinnabar Moth and the Reproductive Capacity of the Resulting Females

Many thousands of pupæ of the cinnabar moth, *Tyria jacobaeae* L., from southern England, and a smaller consignment from central Italy, were introduced into Australia for the control of the noxious weed, ragwort (*Senecio jacobaea* L.), in southern Victoria. In England this moth has a strictly univoltine life-cycle[1] that includes an obligatory pupal diapause[2] lasting for an average of 270 days. Because of this diapause[3], difficulties were expected in synchronizing emergence of the moths with the Australian summer—for this would require diapause to be terminated in less than 150 days or extended to at least 450 days.

The pupæ were stored at 1–3° C. between layers of moist sphagnum. It was hoped that diapause could be terminated by chilling pupæ and later exposing them to higher temperatures. All pupæ were so treated except one small batch of the Italian consignment which was stored at room temperature (range 5–15° C.).

At the beginning of the Australian summer, most of the pupæ were transferred to an insectary where temperatures ranged from 15° to 35° C., but only 6·1 per cent of the English and none of the Italian pupæ produced moths. Strangely enough, 50 per cent of the Italian pupæ kept at room temperature emerged. However, very few of the progeny of these Italian moths completed the larval stage, whereas those from adults of English origin were reasonably successful. Dissections made at the end of summer showed that most of the English pupæ were still alive. The remainder were returned to a cold room and stored until the following year when, after 500 days in diapause, they were again subjected to temperatures in the range of 15–35° C. This second exposure produced an 8·6 per cent emergence.

Three groups of pupæ were set aside to study the effect of temperature and duration of exposure on emergence of adults (see Table 1). Group *B* was held continuously in the cold room at 1–3° C. for 270 days. Group *A* was similarly chilled for 50 days, and then exposed to temperatures within the range 20–48° C. for 150 days. Group *C* was chilled for 90 days, and then held at 15–35° C. for 150 days. Both *A* and *C* were then returned to the cold room. When the pupæ had been in diapause for 300 days, 2–6 batches, each of 25 pupæ, were exposed to 20° C. Samples were drawn from each experimental group at intervals of three weeks. The emergences that resulted are summarized in Table 1. The results for Group *A* suggest that abnormally high temperatures alternating

Table 1. Emergence of Moths after Exposure to Different Temperature Régimes

Groups	No. of replicates	Total No. of pupæ	Details of treatment*		Duration of pupal stage (days) 302	323	344	365	386	407	428	449	470	491	512	533	554	575	Total percentage emerged
A	14	350	50 days at 1–3° C. 150 „ „ 20–48° C. 135+ „ „ 1–3° C. remainder at 20° C.	Mean percentage emerged				44	60	38	56	40	34	62					48
B	52	1,300	270+ days at 1–3° C. without interruption remainder at 20° C.		81	72	51	50	37	36	38	21	8	3	5	4	2		31
C	84	2,100	90 days at 1–3° C. 150 „ „ 15–35° C. 30+ „ „ 1–3° C. remainder at 20° C.		53	47	49	55	57	62	62	54	46	50	41	40	25	21	58

* The pupæ were in transit for 32 days before the treatments named were commenced.

Table 2. Reproductive Capacity of the Cinnabar Moth

Stock	No. of females tested	No. of diapause (days)	Time of emergence	Percentage of females ovipositing	No. of eggs laid	No. of eggs hatched per female
English	40	150	Jan. 1955	6·2	75	10
Italian	20	150	Jan. 1955	8·5	94·4	29·9
English	100	270	May 1955	8·6	95·7	60·1
English	100	360	Aug. 1956	7·9	81·5	37·6
English	100	420	Oct. 1956	7·8	60·2	15·8
English	100	500	Jan. 1957	8·0	58·9	8·1
Australian	50	270	Dec. 1957	7·8	155·1	74·7

with cold treatment produce an erratic although tolerably high emergence of adults. Prolonged cold treatment (group *B*) was apparently detrimental to the pupæ, as the gradually decreasing values suggest. This was also reflected in the statistical analysis, where emergence from group *B* was significantly different from that of group *A* or *C*. In group *C*, receiving alternately cold and warm conditions, relatively high emergence (40 per cent) was recorded after 533 days, that is, after the insects had spent almost twice the normal period in diapause. The sharp contrast in the results from the English and Italian consignments suggests that they may have comprised two different strains of insects. The English strain proved itself to be the more suitable for the climate of Gippsland, Victoria.

Three factors influencing the reproductive capacities of the moths that emerged under various treatments are summarized in Table 2. All three factors—percentage females ovipositing, number of eggs laid and number of eggs hatched per female—were optimal or near optimal when pupal diapause extended for the normal period of 270 days. In the case of the English stock, however, this corresponded to emergence during the Australian winter. A diapause period of 150 days (that is, emergence in the first Australian summer) resulted in less-favourable reproductive capacity, as also did such longer diapause periods as 360, 420 or 500 days. Although females of the Italian stock laid more eggs than those of the English stock, and their viability was also appreciably higher, very few of their progeny completed larval development. The differences in fecundity associated with the duration of diapause were found to be significant.

To sum up, although termination of diapause in *T. jacobaeae* was more successful when this stage was prolonged rather than shortened, the fecundity of the moths produced was adversely affected by long periods of diapause.

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BACTERIOLOGY

Interspecific Induction of Transformation of Bacteria with Respect to Phage-susceptibility

Several examples of bacterial transformation have been reported since the discovery of the phenomenon by Griffith[1]. Among the properties found to be transformed, besides synthesis of the capsular polysaccharide antigens, are drug-resistance and enzymic capacities[2]. In work concerned with the host-specificity of a phage (*S-a*) attacking an amylase-forming bacterium, *Bacillus subtilis* var. *amyloliquefacus*[3], we have tried to transform a non-susceptible organism into a susceptible one. This communication describes the successful results of such an experiment.

First, a trial was made to obtain a susceptible organism through mutation of non-susceptible strains of the *subtilis* group, but with little success, because of the difficulty of discriminating between the wild form and any mutants which might have been produced. A coloured organism, *Bacillus megaterium roseus*, was used in the next trial, in view of the ease in overcoming this difficulty. In fact, several colourless mutants were obtained by the application of a cell-free extract prepared from the susceptible organism. Then tests were made to examine these mutants for their susceptibility to the phage under investigation, which led to the discovery among them of one susceptible mutant. Experimental results will be described briefly now.

The transforming agent was prepared from the cells of the susceptible organism (*Bacillus subtilis* var. *amyloliquefacus*) in the logarithmic phase of its growth (4–5 hr. after inoculation). The bacterium was incubated in a nutrient broth medium in a reciprocal shaker at 37° C. Bacterial cells were gathered by centrifugation, washed three times in 0·9 per cent physiological saline and resuspended in about 10 ml. of the same saline. The cells were disrupted with a sonic oscillator operating at 9–10 kc./s. for 15 min. at 5° C. Cell fragments were collected by centrifugation (10,000*g* 20 min.), the supernatant filtered through a Seitz filter and used as transforming agent after a test for absolute sterility. On the other hand, the cells of *Bacillus megaterium roseus* in the logarithmic phase of growth were incubated at 37° C. in a broth medium with the addition of the transforming agent (final concentration, about 20 μgm. DNA per ml., as determined by the method of Webb and Levy)[4]. At intervals, platings (broth agar) were made to detect the formation of mutants, using several dilutions of aliquots taken from the culture. After 16–18 hr. of incubation, the occurrence of transformation was actually detected, as revealed by the appearance, on later plating, of discoloured mutant colonies at a frequency of approximately one in 10^3–10^4 genuine, pink-coloured colonies. Some of these mutants were pale-yellow in colour, some colourless. No change in morphological characteristics (size and shape) of the cell was discovered in either of them. Each colony of the mutants, as discerned by the naked eye, was separately tested for its susceptibility to phage *S-a* by plating the cells with the addition of appropriate amounts of the phage. With most of the mutants, the result was negative, but there was a colourless strain which showed evident plaque-formation in the test. Multiplication of the phage afterwards was also confirmed by several successive transplantations of the plaques on the colourless mutant.

No back-mutation has been detected, offspring obtained by subculturing the mutant strain, when tested, always exhibiting the acquired characteristics of the mutant, white colour of the colony and susceptibility to phage.

The formation of mutant, either coloured or colourless, was found to be reproducible, its frequency being augmented to some extent by increasing the concentration of the transforming agent. On adding still larger amounts of the inducing agent, however, a state of saturation was obtained, corresponding to a frequency of one mutant colony per 10^3 normal, pink-coloured colonies.

These results are best accounted for by assuming a transformation of the originally phage-resistant, pink-coloured strain of *Bacillus megaterium roseus*, into a phage-susceptible, colourless mutant.

The role of the bacterial extract in inducing the transformation in question is indicated evidently by the fact that no single example of formation of susceptible mutant has ever been observed without the addition of the extract. The colourless mutants appearing, although far less frequently, as a result of spontaneous mutation (that is, without any addition of transforming agent) were also tested for phage-susceptibility but always with negative results. This transforming agent was also found not to be replaceable with extracts prepared in a similar way but from other phage-resistant strains of bacteria (for example, *Bacillus mesentericus vulgatus* Piorkowisky and *Bacillus subtilis NRRL* 558). As to the actual nature of the transforming principle contained in the effective extract, any decisive account will be premature. In view of the finding, however, that the inducing activity of the extract is abolished by treating it with deoxyribonuclease, there is a possibility that a specific deoxyribonucleate is involved (prolonged treatment with other hydrolysing enzymes, such as ribonuclease and trypsin, had no effect on the activity).

In conclusion, it may be stated that the facts reported above will be of some importance in disclosing the possibility of interspecific induction of transformation in bacteria mediated by a soluble transforming principle.

The present work was initiated by the advice given by Prof. A. Takamiya, University of Tokyo, to whom our thanks are due.

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Chitinoclastic Activity in the Luminous Bacteria

There would appear to have been no reports in the literature on luminous bacteria of chitinoclastic activity in this group[1] nor, during attempts to obtain chitinoclastic bacteria from marine environments, have luminous bacteria been isolated[2,3]. Indeed, in a recent publication[4] it is stated that members of the genus *Photobacterium* "do not or are not known to hydrolyse . . . chitin". It is, consequently, of importance to report observations made some time ago on the chitinoclastic activity of a variety of luminous bacteria, all the species available at present having been examined.

Chitin was prepared from crab shells by the method of Benton[5], and following precipitation from sulphuric acid by potassium hydroxide and washing it was added to a basal medium consisting of glucose 0·1 per cent, peptone 0·1 per cent, dipotassium hydrogen phosphate 0·005 per cent and agar 1·5 per cent in sea-water, to give a milky opacity. The cultures were streaked on the surface of this medium, incubated at 20° C. for up to 14 days, and examined for the production of a zone of clearing of the chitin around the bacterial growth.

Table 1. Chitinoclastic Activity of Various Species of Luminous Bacteria

Species	No. of strains examined	Source	No. of strains exhibiting chitinoclastic activity
Photobacterium splendidum	1	Delft collection	1
Ph. sepiae	1	Delft collection	1
Vibrio albensis	1	Delft collection	1
Ph. harveyi	1	Delft collection	1
Ph. harveyi	1	Prof. Johnson, Princeton	1
Ph. pierantonii	1	Delft collection	1
Ph. fischeri	1	Prof. Johnson, Princeton	1
Ph. phosphoreum	1	Delft collection	1
Ph. phosphoreum	1	Prof. Johnson, Princeton	1
Ph. phosphoreum	10	Fish intestinal contents, North Sea	8
Ph. phosphoreum	3	Fish intestinal contents, Dr. Georgala, Cape Town	3
Ph. phosphoreum	12	Marine animals, Drs. Iijima, Yosaki, Japan	11
Unidentified, similar to *Ph. fischero*	29	Fish intestinal contents, North Sea	21
Unidentified possibly new species	1	Fish intestinal contents, North Sea	1

Table 1 shows the strains examined, their source, and their ability to digest chitin under the experimental conditions used. The cultures recorded as negative were not re-examined nor was incubation continued beyond 14 days. It would appear that chitinoclastic activity is widespread among the luminous bacteria, even the freshwater species, *Vibrio albensis*, exhibiting it, and it is, consequently, perhaps not unreasonable to suggest that chitinoclastic activity, to a greater or lesser degree, may be a constant characteristic of this group of bacteria.

The part that the luminous bacteria may play in the degradation of chitin in Nature is obscure. Certain members have been found to be almost invariably present in and frequently forming a large part of the bacterial flora of the intestines of a variety of fish caught in the Arctic, North Sea and around the British Isles. Crustacea may form a not inconsiderable portion of the diet of such fish, and it has been suggested that symbiotic bacteria may aid animals in the digestion of chitin[2]. It is probable, however, that much of the chitin in the oceans finds its way to the bottom deposits and there is little, if any, information concerning the occurrence of luminous bacteria in this environment.

The work described in this communication was prepared as part of the programme of the Department of Scientific and Industrial Research.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Tuesday, June 6

Operational Research Society, and the British Institute of Management (at the Connaught Rooms, Great Queen Street, London, W.C.2), at 9.30 a.m.—Conference on "Operational Research".

University of London (in the Clinical Lecture Theatre, Middlesex Hospital Medical School, Mortimer Street, London, W.1), at 4.15 p.m.—Sir William Ogg: "Soils, Crop Nutrition and Health"* (Sanderson Wells Lecture).

University College (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. G. P. Lewis: "Bradykinin, its Chemistry, Pharmacology and Role in Physiological and Pathological Reactions".*

University of London (at the Institute of Archæology, Inner Circle, Regent's Park, London, N.W.1), at 5.45 p.m.—Prof. F. E. Zeuner: "Archæological Problems of the Canary Islands".*

Wednesday, June 7

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. P. Wildy: "Architecture of Viruses".*

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. G. R. Coope: "A Northern Insect Fauna from a Terrace of the River Avon at Fladbury, Worcestershire": Dr. H. J. Sullivan: "Distribution of Miospores through Coals and Shales of the Coal Measures Sequence Exposed in Wernddu Claypit, Caerphilly, South Wales".

Thursday, June 8

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. J. Bradley: "The Rapid Measurement of Intensity with an Oscillating Fabry-Perot Spectrometer: Isotope Abundance in Mercury": Mr. A. H. Cook: "Precise Measurements of the Density of Mercury at 20° C. II: Content Method".

Royal Institution of Naval Architects (at 10 Upper Belgrave Street, London, S.W.1), at 4.45 p.m.—Mr. J. Clarkson: "The Behaviour of Deck Stiffening under Concentrated Loads" and "Uniform Pressure Tests on Plates with Edges Free to Slide Inwards".

Mineralogical Society (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

Institute of Physics and the Physical Society, Acoustics Group (in the Physics Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 5.30 p.m.—Annual General Meeting, followed by Dr. W. L. Nyborg (University of Vermont): "Biophysical Applications of Non-linear Acoustics".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Worker in Glaciology—Dr. Hal Lister, Lecturer in Polar Studies, King's College, Newcastle-upon-Tyne 1 (June 8).

Assistant Lecturer in Experimental Physics, to collaborate in research in high energy nuclear physics using the accelerators of C.E.R.N. and the Rutherford Laboratory—The Secretary to Council, Westfield College (University of London), Hampstead, London, N.W.3 (June 9).

Assistant Lecturer and a Lecturer in Physics—The Registrar, The University, Nottingham (June 10).

Lecturer or Assistant in Geology—The Secretary of University Court, The University, Glasgow (June 10).

Research Assistant in Mathematical Statistics in the Department of Applied Mathematics—The Registrar, The University, Liverpool (June 10).

Scientific Officer (graduate with first- or second-class honours in mathematics with statistics, or a diploma in statistics) in the Statistics Section, for duties mainly concerned with the design and analysis of field experiments—The Secretary, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (June 10).

Research Assistant (with a degree qualification in physics, and preferably an interest in high vacuum techniques and/or electronics) in the Department of Physics and Mathematics, College of Technology, Liverpool, to participate in a research programme dealing with electron impact and corrosion—The Director of Education, City of Liverpool Education Committee, 14 Sir Thomas Street, Liverpool 1 (June 12).

Demonstrator (with a good honours degree in biology or zoology and preferably with experience in practical genetics) in the Biology Department—The Registrar, University College of North Staffordshire, Keele, Staffs (June 15).

Assistant Lecturer in the Geology Department, to teach mineralogy and encourage research in this field—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 16).

Geochemist (with a special interest in spectrography and preferably previous experience of the geochemistry of sedimentary rocks) in the Department of Geology—The Registrar, The University, Sheffield (June 16).

Lecturer in Biochemistry—The Secretary of University Court, The University, Glasgow (June 16).

Assistant Lecturer (Grade III) in Botany—The Registrar, The University, Bristol 8 (June 17).

Professor of Botany—The Secretary and Bursar, University College, Earlsfort Terrace, Dublin, Republic of Ireland (June 17).

Research Assistant (graduate in chemistry or biochemistry) in Chemistry, to work in the Department of Chemistry of the Faculty of Science under the direction of Dr. G. R. Barker on the chemistry and biochemistry of nucleic acids—The Registrar, The University, Manchester 13 (June 17).

Research Assistant in the Department of Mathematics and Physics, to take part in research work on the microwave properties of certain magnetic oxides and to undertake a certain amount of teaching—The Registrar, College of Technology, Park Road, Portsmouth (June 17).

Research Associate (Applied Mathematician) in the Department of Theoretical Mechanics (Faculty of Engineering)—The Registrar, The University, Bristol 8 (June 17).

Virologist, Experimental Officer (male, with a degree in botany or agriculture and experience of plant virus techniques), to co-operate in research aimed at developing virus resistance in the potato—The Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian (June 17).

Lecturer (Grade II) in the Department of Mechanical Engineering—The Registrar and Secretary, The University, Bristol (June 19).

Assistant Lecturer in Biochemistry in the Department of Agricultural Biochemistry—The Registrar, University College of Wales, Aberystwyth (June 24).

Lecturer in Pharmacology in the Department of Pharmacology and Therapeutics—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (June 24).

Research Assistant (man or woman graduate of any university) for freshwater phytoplankton investigations—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (June 24).

Lecturer (with a knowledge of selection and breeding of Nigerian grasses) in Plant Breeding in the Department of Agriculture, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (June 25).

Lecturer or Assistant Lecturer in Physiology—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (June 30).

Lecturers (2) in Chemistry at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, W.C.1 (New Zealand and London, June 30).

Lecturer or Assistant Lecturer in Physical Metallurgy—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (July 1).

Research Students (with the degree of Bachelor or Master of a British University other than Manchester) in Science—The Registrar, The University, Manchester 13 (July 1).

Senior Lecturer (experienced in clinical biochemistry as well as in administration and capable of directing the clinical pathology service of a general hospital of approximately six hundred beds) in Clinical Pathology at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, July 7).

Senior Lecturer or Lecturer in Scientific Thought in the School of Philosophy, University of New South Wales—Agent-General for New South Wales, 56–57 Strand, London, W.C.2 (Australia and London, July 14).

Assistant Professor in Biophysics and an Assistant Professor in Meteorology—G. M. Shrum, Physics Department, University of British Columbia, Vancouver 8, B.C., Canada.

Distillers Company Research Student in the Department of Chemical Engineering, for work leading to a higher degree on the kinetics of the gaseous oxidation of aromatic hydrocarbons—The Secretary, University College, Gower Street, London, W.C.1.

Graduate (with a good first degree in chemistry, physics or chemical engineering) in the Chemical Engineering Department, to undertake research for a higher degree on some aspects of flame propagation—Dr. J. H. Burgoyne, Chemical Engineering Department, Imperial College of Science and Technology, London, S.W.7.

Head of the Department of Parasitology, The Secretary, Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh 9.

Head (with high academic qualifications in mathematics and teaching and research or industrial experience) of the Department of Mathematics—The Principal, Huddersfield College of Technology, Huddersfield.

Lecturer in Mathematics: and an Assistant, Grade B, in Mathematics—The Registrar (Department *F*), Bradford Institute of Technology, Bradford 7.

Low Temperature Physicist (Assistant Professor, Associate Professor or Professor) in the Low Temperature Laboratory—G. M. Shrum, Physics Department, University of British Columbia, Vancouver, B.C., Canada.

Postdoctoral Fellow, for work in the field of photochemical reactions in the alkali halides—Dr. P. W. M. Jacobs, Chemistry Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

Postdoctoral Research Fellow, for research in gas kinetics—Dr. H. M. Frey, Chemistry Department, The University, Southampton.

Reader (with high academic qualifications and considerable teaching and research experience in any field of botany or zoology) in Biology—The Registrar (Department *F*), Bradford Institute of Technology, Bradford 7.

Scientific Officer or Senior Scientific Officer (honours graduate in chemistry or biochemistry) in the Physiology Department, to study the isolation and properties of various enzymes from the mammary gland and milk—The Secretary, The National Institute for Research in Dairying (University of Reading), Shinfield, near Reading, quoting Ref. 61/16.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Neurophysiological Aspects of Hallucinations and Illusory Experience. By W. Grey Walter. (Fourteenth Frederic W. H. Myers Memorial Lecture, 1960.) Pp. 24. (London: Society for Psychical Research, 1960.) 2*s*. net. [203

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 61: The Care and Management of Breeding Bulls. Pp. 7. Leaflet No. 70: Ringworm. Pp. 2. (Belfast: Ministry of Agriculture, 1961.) [203

The United Steel Companies Limited. Review of Progress: a Report for Employees. Pp. 44. (Sheffield: The United Steel Companies Limited, 1961.) [203

Department of Scientific and Industrial Research. Instruments in the Factory. Pp. 12. (London: Department of Scientific and Industrial Research, 1961.) [203

International Aid for Economic Development—Possibilities and Limitations. By Dr. V. K. R. V. Rao. (Eighteenth Montague Burton Lecture on International Relations.) Pp. 29. (Leeds: The University, 1960.) 6*d*. [203

Annual Report of the National Oceanographic Council, 1 April, 1959–31 March, 1960. Pp. v+42+7 plates. (Cambridge: At the University Press, 1960.) 5*s*. net. [203

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Educational Television: a Survey and a Plan. By John Wellens. Pp. 44+8 plates. (Guilsborough, near Northampton: John Wellens (ITT), Ltd., The Sun House, 1961.) 5*s*. [203

The Science of Continuously Operating Systems. By Prof. K. G. Denbigh. (Inaugural Lecture, 1 November, 1960). Pp. 18. (London: Imperial College of Science and Technology, 1960. Distributed by The Modern Book Co., Ltd., 19 Praed Street, W.2.) 5*s*. [203

Central Youth Employment Executive. Choice of Careers, No. 103: Art and Design. Pp. 56. (London: H.M. Stationery Office, 1960.) 2*s*. net. [203

Science and the Future of Britain. (A Labour Party *Looking Ahead* Pamphlet.) Pp. ii+46. (London: The Labour Party, 1961.) 2*s*. [203

General Register Office. The Registrar General's Statistical Review of England and Wales for the year 1959. Part 2: Tables, Population. Pp. x+181. (London: H.M. Stationery Office, 1960.) 11*s*. 6*d*. net. [203

Forestry Commission. Forest Record No. 44: A Creosoting Plant at Thetford Chase. By D. Small. Pp. 28. 2*s*. 6*d*. net. Forest Record No. 46: Group Dying of Conifers. By J. S. Murray and C. W. T. Young. Pp. 19+12 figures. 3*s*. net. (London: H.M. Stationery Office, 1960 and 1961.) [203

Other Countries

Berichte des Deutschen Wetterdienstes. Nr. 71 (Band 10): Die Meteorologischen Verhältnisse auf der Flugstrecke Deutschland—Südamerika. Von Herbert Regula. Pp. 26. 5.25 D.M. Nr. 72 (Band 10): Wissenschaftliche Beobachtungen auf dem Nordostland von Spitzbergen 1944–1945. Von Wilhelm Dege. Pp. 134. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1960.) [133

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CO-OPERATION IN THE EXPANSION OF HIGHER EDUCATION

THE strength of a modern economy depends on its industrial vigour, and this in turn on technology, or the continuous application of scientific knowledge. All are based on the creative capacity of man and on his education. Accordingly, an adequate supply of scientists, engineers and technicians is a major factor in economic growth, and recognition of this led the Organization of European Economic Co-operation to establish in 1958 its Office for Scientific and Technical Personnel, with the main task of assisting member countries to increase their scientific and technical man-power, in quality as well as in quantity. Major aspects of the programme of this Office include forecasting future needs for scientific and technical man-power; determining the investment required in education and training facilities to meet those needs; modernizing methods of teaching and curricula in mathematics and science subjects in the schools; training and re-training teachers; and facilitating international exchange of information and personnel in this field.

When the Office was established in 1958, few member countries had adequate statistics on their position with regard to man-power, and still fewer on future requirements. The statistical procedures for producing such data had no international standard, nor were there standards for academic qualifications or international equivalents. As a first step, therefore, the Office arranged a conference on "Techniques for Forecasting Future Requirements of Scientific and Technical Personnel" at The Hague in November 1959, at which a series of papers was presented on such subjects as economic growth and the man-power factor; educational planning in the light of economic requirements; the role of statistics in forecasting the demand for, and supply of, scientific and technical personnel; and intellectual resources and the growth of higher education.

These papers and brief summaries of the ensuing discussion have now been published under the title *Forecasting Manpower Needs for the Age of Science**. The Office clearly proposes to work on very similar lines to those of the U.S. National Science Foundation, and it needs no more than a glance at such an article as "More Universities" in *The Westminster Bank Review* of February to appreciate how closely the programmes of both bodies bear on current problems in Britain. It is obvious, too, from recent addresses of university authorities, such as that of Sir Charles Morris, vice-chancellor of the University of Leeds, at a meeting of the University Court last November, or that of Dr. B. V. Bowden at the annual meeting of the Court of Governors of the Manchester College of Science and Technology in the same month, how keenly they are aware of the importance of these factors. Dr. Bowden, for example, stressed particularly the importance of adequate expansion in engineering and urged re-consideration of the establishment of a technological university on a large enough scale. At Leeds the questions of numbers. standards and balance of studies, alike in relation to general education and to the demands of the community for particular categories of qualified men and women, are obviously much in mind.

The article in *The Westminster Bank Review* is concerned more especially with the inquiry now being undertaken by the Robbins Committee, and surveys very fairly the situation which confronts Britain in both university and technical education with the growth of secondary education. It indicates very clearly some of the decisions which will have to be made at an early date—decisions which should be based on facts that are ascertainable, not on prejudice or tradition. Apart from the question of how large is the supply of students potentially capable of profiting by higher education, whether or not at a university, and taking account both of the 'bulge' and of the nation's needs for trained man-power, including overseas needs, the article stresses the vital importance of nourishing among as many young people as possible the ability to think for themselves and to question what Galbraith has termed broadly 'the conventional wisdom'. This does not imply that such education should necessarily be given at a university, though it is true that many students who fail to obtain a good degree have benefited from the broadening influence of university education, and that it is wrong that anyone of proved ability should be excluded from a university purely for lack of space.

The difficulties of university expansion are not to be under-estimated, or attributed simply to a reluctance of many university authorities to lower high academic standards, or to existing concepts of the optimum size for a university, any more than it should be assumed that an academic education is the best type of higher education for every promising student. Much more thought could well be given here to the function of the colleges of technology; and the question of balance, particularly in relation to the technical colleges, is extremely important. There are problems of staff, of the siting of new institutions, and of finance, and it is imperative that, so far as possible, these decisions should be based on careful analysis of the facts, and on critical and creative analysis by the Robbins Committee.

Whether, therefore, we agree or not with the view put forward at the conference at The Hague already mentioned by Dr. Alexander King, director of the Office for Scientific and Technical Personnel (Organization for European Economic Co-operation), that education is essentially a long-term process which requires planning on a careful assessment of the broad pattern of needs, and that forecasting of man-power needs is thus an essential pre-requisite to the elaboration of sound educational programmes, some such forecasting must have a place in planning the expansion of higher education of all types. This is just as true in such a country as the United Kingdom as it is in the lesser-developed countries. University expansion must be planned in relation to the potential needs, even although it must be planned in relation to available resources of staff, buildings and equipment and finance. In fact, as Mr. Erik Jb. Schmidt recognizes in his paper on economic growth and the man-power factor, the physical limits to a rapid development of technical education are likely to be greater obstacles than the economic limits, and this is especially true of staff.

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* Organization for European Economic Co-operation: Office for Scientific and Technical Personnel. *Forecasting Manpower Needs for the Age of Science.* Pp. 141. (Paris: Organization for European Economic Co-operation; London: H.M. Stationery Office, 1960.) 7.50 N.F.; 10*s.* 6*d.*; 2 dollars.

Mr. Schmidt visualizes a definite vertical build-up of the educational system in any country from the elementary to the most-advanced scientific stages, with appropriate balance between the several professional and vocational sectors, varying from country to country. He stresses the need for flexibility, and suggests that even with the present imperfect knowledge of the most appropriate structure, much could be done to improve the effectiveness of existing systems. Better vocational counselling, the differentiation of education and the development of various types of education in a system of graduated steps, shortening the period of education by eliminating obsolete material in courses and using modern methods of instruction and more differentiated use of teachers are all means by which existing teachers could be used more efficiently and the demands made both on them and on students minimized.

Mr. P. H. Coombs in his paper seeks to indicate the assumptions which could form a basis for educational planning, the changes required and the basic steps which should be taken. Obviously the first step is a clear analysis and appraisal of the existing educational system; but there was a wide difference of opinion at the Conference as to the practicability of defining the educational aims and goals most appropriate for the period covering the next ten years. The Danish delegate thought that reliable long-range forecasts were scarcely possible, whereas Prof. M. Vermot Gauchy went much further than Dr. H. Goldstein or Dr. S. Moberg, who merely discussed the role of statistics in forecasting supply and demand or indicated methods and techniques used in Sweden for forecasting specialized man-power requirements, in that he presented an elaborate statistical technique for measuring a myriad of future needs within each individual economic sector. Nevertheless, all three appear by implication to accept the practicability of Mr. Coombs's third and fourth steps: determination of the economic resources and internal changes required to achieve these goals, and of the adequacy of institutional and political mechanisms and processes for educational planning and decision-making, for mobilizing educational resources and for executing agreed plans.

Mr. Coombs lays much stress on the contribution of the economist to the planning of education, and much more work of the type that Mr. Vaizey has undertaken in "The Costs of Education" is certainly needed. Nevertheless, the report does not ignore the inherent conflict between the planning and guidance of man-power on one hand and the freedom of choice in a free society on the other. Moreover, Prof. P. de Wolff's paper on intellectual resources and the growth of higher education, which leads to the general conclusion that available reserves seem to allow for a considerable expansion of participation in higher education,. points also to the importance of international co-operation in improving and unifying the methods for obtaining comparable data in this field. Prof. de Wolff also agrees with Dr. Goldstein that there is a sound basis for identifying talent among comparable social groups but stresses the considerable influence of environmental factors.

Generally, it may be said that the Conference agreed that as yet no established and unique methodology exists for forecasting in respect of scientific and technical personnel, and it would be unwise to regard forecasts as a rigid and infallible guide to policy. Forecasts of demand should indeed be increasingly comprehensive, but long-term forecasts require supplementing by short-term checks, and overall analyses and projections require supplementing by detailed examination, industry by industry, and by type of qualification. Apart from the attention given to the reserves of intellectual potential, stress was laid on the opportunities for increasing the efficiency of educational systems in utilizing talent, and this aspect must become of increasing importance as the cost of education continues to rise. Further, it is interesting to note not only the agreement at the Conference as to the value and opportunity for international co-operation in methodology in this field but also the recognition of the special problems of the under-developed countries in the supply of scientists, engineers and technicians, particularly in respect of special needs for investment in education within the framework of national economic development. As the writer in *The Westminster Bank Review* remarks, more than 6,000 places at British universities are at present taken by Commonwealth students, and a further 4,000 places by other foreign students. Despite the growth of universities in Colonial and Commonwealth territories, the general improvement in educational standards throughout Africa and Asia will lead increasing numbers of Commonwealth students to seek to finish their education at British universities and colleges of technology.

It would be folly to disregard this demand or to fail to provide for it as generously as possible in plans for the expansion of higher education in Britain. The existence of this need, however, only illustrates further the need for close integration of the economic, scientific and technological and the human aspects of the many problems involved, and the danger of taking sectional decisions at almost any stage or level. Again, the report of the Conference of the Office for Scientific and Technical Personnel well emphasizes the need for flexibility and for further inquiry in many directions. The need for experiment is no less urgent and should be remembered at all levels in planning the expansion of existing or the development of new universities, in the field of technological education, and in the inquiries for which the reconstituted Central Advisory Council for Education in England, under Lord Amory's chairmanship, is responsible.

It is perhaps particularly desirable that such considerations should be given due weight at conferences like that on specialization in schools convened early in March by the Education Group and the Manchester Branch of the Institute of Physics and the Physical Society, at which Lord James of Rusholme uttered strong words against forcing the choice between arts and science too early, and again in considering the reconstruction of technical education. Something more than better links between schools and technical colleges is required. The proposals in the White Paper on Technical Education (see *Nature*, March 4, p. 701) aimed at broadening the education received by the students, adapting the system more closely to the needs of industry, increasing the variety of courses offered and reducing the existing wastage should receive unqualified welcome; but these will not remove the need for much more creative and constructive thought about the relations of technical and technological education, and the relations of both to the schools and the universities. Moreover, while the national and economic aspects of these interrelated problems are rightly kept in the forefront, the human aspects are no less important, and it is unfortunate that only too often these tend to receive the least-discriminating treatment, or to be made the play-ball of party political prejudice. One very important reason for welcoming the co-operation between industry and educational institutions and authorities of all types, which the White Paper on Technical Education emphasizes, is the contribution it could make to eliminating this tendency.

A DIVERSITY OF THEOLOGICAL SPECULATIONS

Immortal Longings

By Stephen Findlay. Pp. 192. (London: Victor Gollancz, Ltd., 1961.) 21*s*. net.

The Firmament of Time

By Dr. Loren Eiseley. Pp. viii+183. (London: Victor Gollancz, Ltd., 1961.) 16*s*. net.

WHEN confronted with a good book with which he fundamentally disagrees, the first duty of a reviewer is to direct attention to its merits, and in the case of Mr. Findlay's volume they are considerable. *Immortal Longings* is a very well-written and well-informed study of the case for human immortality and the survival of bodily death. It surveys the familiar ground of metaphysical and theological beliefs, arguments and theories with admirable lucidity and concludes with a brilliant summary of the present situation in psychical research. I can at least cordially agree with Mr. Findlay in his opinion that philosophers and scientists would do well to pay more attention than they are wont to 'parapsychology'. The upshot of Mr. Findlay's critical study is almost completely negative. At the end 'immortal longings' remain no more than longings and personal immortality a faint possibility. To justify a position almost the exact opposite of Mr. Findlay's would need a book as long as his, and here I must note only some objections to his treatment of the subject. He rightly sees that the Christain belief in immortality depends on belief in God and in revelation. It is a pity that his chapter which touches on this basic theme is inadequate. A destructive criticism of the traditional theistic 'proofs' in their traditional form is not enough, and even this meagre ration of argument is imperfect. Can one, for example, fairly state the teleological argument without some reference to the evolution of mind? A process which issues in the capacity to understand itself, the evolution of the theory of evolution, has, one might think, at least some *prima facie* appearance of purpose; and, Can Kant be so far ignored as to omit all reference to the moral argument? Theistic philosophy has survived Hume's scepticism and moved on, just as science has survived his radical scepticism of causation and necessary connexion and moved on.

The negative conclusion about God and revelation vitiates Mr. Findlay's discussion of the resurrection of Christ. Those who first believed it accepted it as a culminating point in a revelatory process, apart from which it would have no significance, and the situation is the same to-day. The long section on empirical evidence for survival, though, as already remarked, excellently done, seems to me to be dominated by the presupposition that any hypothesis, however complex and conjectural, is to be preferred to that of survival, so that sometimes we seem to be invited to explain the incomprehensible by the inconceivable. This is particularly notable in the interesting discussion of the 'cross-correspondence' data. Almost at the end of his book Mr. Findlay introduces a new and disturbing factor—the mind-body relation. "The whole trend of modern neurology", he writes, "is towards regarding mind and brain as the subjective and objective aspects of the same thing." One could wish that he had elaborated this dictum by explaining what possible meaning it can have. What is this 'thing' which has two such different aspects and is itself presumably neither the one nor the other? This late appearance of the 'unknowable' is confusing for it raises the question whether perhaps it may be immortal!

Dr. Eiseley's book is a very different matter, though, in a wide sense, it bears on the same subject —the nature and destiny of man. It does not incite us, however, to argue or criticize but rather to listen to a prophetic word, to enter into the vision of a man of science contemplating the whole range of life and its development. The titles of the chapters are sufficient indication of the scope and trend of the author's thought: "How the World Became Natural"; "How Death Became Natural"; "How Life Became Natural"; "How Man Became Natural"; "How Human is Man?"; "How Natural is 'Natural'?". The book is in fact a prolonged meditation on the meaning of Nature. The dangers and terrors of the present and the future are not concealed; but the conviction emerges that there is that in man which transcends Nature, and, in Kierkegaard's words, "through the eternal we can conquer the future". Anyone who is depressed in spirit by reading Mr. Findlay's account of the prospects of immortality might find through reading Dr. Eiseley's *The Firmament of Time* a renewal of hope. These two books, diverse as they are in spirit and method, have one common character—they are both written with such grace and mastery of English prose that it is a pleasure to read them. W. R. MATTHEWS

ADVANCES IN ANALYTICAL CHEMISTRY

Advances in Analytical Chemistry and Instrumentation

Vol. 1. Edited by Charles N. Reilley. Pp. vii+445. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 90*s*.

THIS is the first of a series of volumes, to be published at annual intervals, the purpose of which is to select new ideas and instrumental techniques in analytical chemistry and subject them to a detailed, critical review by acknowledged practical experts.

The advisory board controlling this publication is composed of eighteen chemists of such eminence that the success of the series could unequivocally be predicted purely from a consideration of the personalities involved. The excellence of this initial volume serves, however, to confirm any such predilection in the mind of a reviewer.

E. H. Swift and F. C. Anson have contributed a welcome and authoritative chapter on the use of Iwanow's thioacetamide as a substitute for the traditional, but toxicologically objectionable, hydrogen sulphide reagent in general analysis. From kinetic hydrolysis studies, the best conditions for the employment of thioacetamide are established for acid and alkaline media. Detailed practical examples are quoted for the separation of many cations using this unique reagent.

C. A. Horton discusses trends in the macro- and micro-determination of fluorine. This is an element of considerable commercial interest, and it also is important in the fields of water treatment (dental caries) and archæological dating. The review is detailed and up to date, and includes discussion of Prof. Belcher's new reagent, cerium 1,2-dehydroxyanthroquinonyl-3-methylamine-N-N-diacetic acid.

W. Schoniger presents a chapter on organic micro-analysis. The oxygen-flask micro-combustion method is discussed side by side with the more classical micro-chemical methods. The chapter begins with some most useful advice on weighing procedures.

Gas chromatography attracts wide interest because of its delicacy and specificity in micro-analysis. R. B. Seligman and F. L. Gager have contributed an excellent paper on modern detector systems, with particular comment on the great sensitivity of the newer ionization devices. R. F. Godda presents a similarly detailed treatment of the successful application of near infra-red spectrometry in the 1–3μ region. This paper provides a series of valuable tabular information on absorption spectra in this unique range, together with useful comment on the application of various commercial infra-red spectrophotometers.

Approximately a quarter of this first volume is devoted to a critical review by H. Flaschka and A. J. Barnard of the analytical usages of the tetraphenylboron reagent, which for almost a decade has been employed extensively for the gravimetric determination of potassium. The authors describe the applications of tetraphenylboron to the other alkali metals in titrimetric determinations both of volumetric and electrometric type, and also its uses in spectrophotometry and turbidimetry. The applications of tetraphenylboron in the identification and estimation of organic compounds are also described. W. Reinmuth contributes a review chapter on the theory of electrode processes, and the charge transfer phenomena associated with reversible and irreversible systems. Problems associated with electrode materials, salt effects, surface active species, etc., are all discussed critically and the article provides an extensive bibliography of papers on this most complex subject.

This series presents in a compact form the authoritative views of acknowledged experts on techniques and theoretical advances in the physical and analytical fields, and will undoubtedly be welcomed by the research worker and the academic student alike.

D. T. LEWIS

FAST-NEUTRON HANDBOOK

Fast Neutron Physics

Edited by J. B. Marion and J. L. Fowler. Part 1: Techniques. (Interscience Monographs and Texts in Physics and Astronomy, Vol. 4.) Pp. xiv+983. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 218*s*.

FAST neutron physics, in the present context, concerns the study of interactions of atomic nuclei with neutrons of energies between a few keV. and about 50 MeV. The past decade has seen a rapid growth of interest in this field. The stimulation is in large part due to the increasing demands of reactor designers. There is also, however, a better appreciation of the value to the theoretician of data from nuclear reactions which involve neutrons, since the interpretation of such data does not require the inclusion of complicating Coulomb effects. In such a rapidly growing field the description of the latest techniques has been confined mostly to research reports, and little effort has been spent on trying to summarize and evaluate developments. A core of basic technology has, however, been built up, and the present book attempts to summarize this and to provide a standard reference work for the field. The book is the first of two volumes; the second, which is still to be published, will deal with the experimental results and their interpretation by present-day nuclear theories.

This first volume is subtitled *Techniques*, and is concerned primarily with the technical problems of the production, detection and shielding of fast neutrons. It consists of twenty-four articles by authorities in their particular field. The scope of the work can be appreciated by considering the four sections under which the articles are grouped. Section 1 concerns neutron sources, both radioactive sources and the most important neutron-producing reactions. Section 2, "Recoil Detection Methods", has articles on detection by photographic plates and by cloud chambers as well as by proportional and scintillation recoil spectrometers. Section 3 considers other neutron detectors, including 'long counters', solid and gaseous scintillators, helium-3 neutron-spectrometers and fission detectors. Finally, Section 4 treats a number of special topics such as time-of-flight techniques, neutron-flux measurements, collimation and shielding of neutrons and health hazards. In many of the articles information is assembled which has previously been scattered through the literature, or merely passed informally between laboratories; notable in this regard are the articles on flat response counters, on neutron collimation and shielding, and on neutron-producing targets. The work is still reasonably up to date in spite of the fact that it was written in 1958—this shows that a clear need existed for a reference book of this type. The usefulness of the book is enhanced by excellent bibliographies, and these, together with the wide range of topics that it covers, will make it of great value to all research workers in the fast-neutron field.

To end with some mild criticism, the book shows evidence of a lack of balance in assembling the material. A particular example of this is that there are no tables of the emitted neutron energy *vs.* the bombarding particle energy for three commonly used neutron-producing reactions, D(d,n)^{3}He, T(p,n)^{3}He and T(d,n)^{4}He, whereas such tables are included for several rather uncommon reactions such as ^{51}V(p,n)^{51}Cr.

Furthermore, I was left with the impression that many of the articles could have been shortened appreciably without affecting their scope. One can understand the problem that the editors must have had in combining so many parts into one comprehensive whole, but it might have been better if they had exerted their authority somewhat more firmly regarding the length of the contributions. The result of their failure to do this has been that the book is priced well beyond the means of most physicists and will be relegated to the library shelf instead of the experimenter's desk.

J. H. Montague

MATHEMATICS FOR THE BIOLOGIST

An Introduction to the Mathematics of Medicine and Biology

By J. G. Defares and I. N. Sneddon. Pp. xii+663+6 plates. (Amsterdam: North-Holland Publishing Company, 1960.) 85*s*.

THIS is a comprehensive introduction to mathematics for the aspiring biologist and clinician. On second thoughts 'perspiring' would be the better word. The authors are painstakingly meticulous in the correctness of their approach, and some pages read almost like a university text-book in analysis. The reader for whom the book is intended is supposed to have ceased the active study of mathematics several years ago, and it is not difficult to imagine his reactions when confronted with the definition of a field on page 4, and what is virtually a Dedekind section on page 5. However, after learning about convergence, permutations, the binomial theorem and partial fractions, on page 47 the reader is being told in elaborate detail how to plot a point on a graph. This unevenness is explained by the interests of the two authors. One is a mathematician, the other a physiologist, and it is usually evident which chapter has been written by which.

The authors guide the readers from limits and derivatives, through the standard techniques of integration and partial differentiation to differential equations, partial as well as ordinary. On the way, he will learn about logarithms, gamma functions, Laplace transforms and Fourier series, so it will be seen that the scope is indeed comprehensive.

Throughout, the theory is illustrated by a wealth of practical examples. They tend to be mainly drawn from physiology, but one excellent feature of them is the emphasis placed on the construction of a suitable model. Throughout these examples, however, all points found from practical experiments lie exactly on a straight line or other appropriate curve. No indication is given as to what the biologist is to do when this is not the case, as will so often happen.

The authors deliberately exclude the theory of statistics from their scope; but one would have hoped for a little guidance in dealing with this situation in a book intended for biologists, whose results are usually so very variable.

There are one or two mistakes in the examples and in the solutions of the problems. Example 13 in Chapter 4 has a bad slip, and in example 4 of Chapter 6, x should tend to zero. I could make no sense out of paragraph 10 in Chapter 10, and there are some serious misprints in equation 2 on p. 290. The six plates seem useless and their exclusion might possibly reduce the rather high cost. The printing and lay-out are excellent throughout.

To sum up, then, this book is not likely to convince the biologist that mathematics is easy or exciting. As a reference book, it may be extremely useful, but as a text-book, it is much too difficult and laborious for the reader for whom it is intended.

J. F. Scott

RECENT ADVANCES IN ENTOMOLOGY

Annual Review of Entomology, Vol. 6

Edited by Edward A. Steinhaus and Ray F. Smith. Pp. vii+470. (Palo Alto, Calif.: Annual Reviews, Inc., 1961. Published in Co-operation with the Entomological Society of America.) 7 dollars.

IN the six years since this *Review* was started it has already made itself indispensable. It fulfils its declared object exactly, which was to invite specialists to provide authoritative and scholarly reviews of the literature in their particular specialties. In each volume, the editors have presented a list of titles that ranges over every aspect of entomology. The papers are all of a high level of authority, and are supported by very complete bibliographies, so that each takes the reader at once into the heart of the subject.

Volume 6 contains the following articles: "Darwin's Contributions to Entomology", by J. E. and C. L. Remington; "Insect Nutrition", by H. L. House; "Nutritional Factors in Insect Resistance to Chemicals", by H. T. Gordon; "Principles of Insect Cold-hardiness", by R. W. Salt; "The Biochemistry of Insect Hemolymph", by G. R. Wyatt; "The Role of Mitochondria in Respiratory Metabolism of Flight Muscle", by B. Sacktor; "Diurnal Rhythms", by Janet Harker; "The Theoretical and Practical Study of Natural Insect Populations", by O. W. Richards; "Principles of Insect Predation", by C. S. Holling; "Biological Control of Pest Insects in Europe", by J. M. Franz; "Sampling Crop Pests and their Hosts", by A. H. Strickland; "Biology of Chiggers", by Manabu Sasa; "The Phenomenon of Industrial Melanism in Lepidoptera", by H. B. D. Kettlewell; "A Review of the Phylogeny of Mites", by T. A. Woolley; "Palæoentomology", by O. Martynova; "The Analysis of Insect Embryogenesis", by S. J. Counce; "The Chemistry of Organic Insecticides", by T. R. Fukuto; "Mode of Action of Insecticides", by C. C. Roan and T. L. Hopkins; "Ecological Aspects of Plant Virus Transmissions", by W. Carter; "Mechanism of Transmission of Viruses by Mosquitoes", by R. W. Chamberlain and W. D. Sudia; "Arthropod Vectors as Reservoirs of Microbial Disease Agents", by C. B. Philip and W. Burgdorfer; and "Transmission of Filarioid Nematodes", by F. Hawking and M. Worms. In addition there are cumulative indexes to the volumes 2–6 (Why not 1–5 ?).

Not all, however, are equally rewarding to the non-specialist. While most authors interpret the word 'review' to mean a general survey of a field of study, which any interested person might use as a starting point, a few, including one or two in this volume, have moved to the extreme that the editors originally said they wished to avoid: ". . . a mere concatenation of bibliographical summaries or abstracts. . .". An article that can be understood only by people already working in that field would be better placed in a specialized journal.

Apart from these that are a breathless catalogue of new facts, a few others are spoiled by being written in a language that purports to be English, but has to be mentally translated before it can be understood. "Furthermore, lifelong infection of true hibernating mosquitoes may explain, at least in part, the overwintering of virus in colder temperate regions where continuous transmission is interrupted." Why not say: "In colder temperate regions mosquitoes stop biting during the winter, but those that hibernate may act as a winter reservoir for the virus" ? H. B. D. Kettlewell on "Industrial Melanism in Lepidoptera" is a model of clarity: "The canopy of all trees is washed by rain and the pollution is carried down the branches, and finally the trunk".

HAROLD OLDROYD

IN SEARCH OF RACE RELATIONS

White Man

A Study of the Attitudes of Africans to Europeans in Ghana before Independence. By Dr. Gustav Jahoda. Pp. xii + 144. (London and New York: Oxford University Press, 1961. Issued under the auspices of the Institute of Race Relations.) 21*s*. net.

IN the confused and confusing field of studies called 'race relations', the general reader and the research student will welcome a book such as this one. Dr. Jahoda has succeeded in making clear the limits of his study pursued in Ghana just before independence, the methods by which he carried it out, and the conclusions he drew from it. This is a notably difficult type of study, by a European, of African attitudes to the white races. Its scientific basis is that of a psychologist who is willing to use not one but several techniques, and to discuss how far they are applicable and wherein lie their limitations.

The chapters can be arranged in three main groups: the history of White contacts with Ghana (chapter 1); the main groups of informants, school-children and adults, and the methods used to ascertain and record their views (chapters 2–6); and the final two chapters (7 and 8) in which Dr. Jahoda makes an analysis of the results of the study. He says more than once that he felt himself cramped by the limitations of the space allowed, and this is particularly evident in the final two chapters, where a fuller discussion, both of the methods of study and the results, would have been advantageous.

Among the many studies of political and economic development now being made in Africa, this field of 'attitudes' is new, neglected and important. There are two distinct yet related aspects of this problem: the attitudes of Africans to Europeans, and that of Europeans to Africans. Studies have been going on at the University College of Central Africa in both these aspects. There is obviously room in all the African countries for a development of these 'attitudes' studies on a scientific basis, which will be recognized by Africans and Europeans as being valid, objective and yielding results which can be applied to contemporary situations. It is therefore imperative to speed up the process by which African research students can receive the kind of advanced training which will enable them to work as fully equipped colleagues with Europeans. These are not studies to be rushed into in order to provide quick solutions for political and economic relationships. They require the detached, unemotional research which can validate its own methods and justify and explain its results, however unexpected or unpalatable they may appear to Africans or to Europeans.

Dr. Jahoda has provided us in this book with several ideas which form the main theoretical leads in his own work and provide suggestions for further development in subsequent studies. One of these concepts is that of the stereotype held by the members of one race about the members of another, and he emphasizes that this stereotype when acquired is "notoriously resistant to change". This complex of attitudes towards the members of another race is the result of a historical time process, and hence there is an element of "time lag" in the formation and expression of attitudes. This is probably the most outstanding contribution in the present study, and is well worth searching out in all the chapters of the book.

Another factor, illustrated in Chapters 2–5, is the difference in the attitudes of Africans with varying degrees of Western education or none at all. Hence there is no one 'African attitude', and these educationally differentiated attitudes also vary in relation to the stages of political and economic development.

Thirdly, and this concept calls for anthropological as well as psychological investigation, the Africans in this Ghana study distinguished between the British expatriates with whom they had personal contacts in the country, and the British institutions and culture which had been brought to Ghana over a long historical period. This raises in the field of race-relations studies the fundamental question of how far stereotypes and attitudes depend on personal contacts, and how far on identification with or revolt against an imported culture.

There is no index, but there is a detailed synopsis of all the chapters and a useful selected bibliography.

MARGARET READ

Lectures on Ergodic Theory

By Prof. Paul R. Halmos. Pp. vii+100. (New York: Chelsea Publishing Company.) 2.95 dollars.

THIS book is a re-issue of one previously available under the imprint of the Mathematical Society of Japan. It consists of the notes on which Prof. Halmos based a series of lectures given at the University of Chicago in 1955, and, although it makes no claim to being an exhaustive survey, it is yet rich in content.

The lectures are, of course, addressed to pure mathematicians. Although ergodic theory had its origin in Boltzmann's development of the kinetic theory of gases, the subject, as extended by mathematicians, now encompasses a much wider field. Thus only the first few chapters, which deal with recurrence, mean and pointwise convergence, ergodicity (in the sense of metrical transitivity) and mixing theorems, have relevance to the problem of irreversibility as it appears in physics.

The discussion in these initial chapters is in terms of transformations on a measure space; afterwards, algebraic concepts are introduced and topics then considered include conjugacy and equivalence with particular reference to transformations which possess a discrete spectrum, weak and uniform topologies for automorphisms, the topological size of sets of strongly mixing, weakly mixing and ergodic transformations, and the existence of a sigma-finite invariant measure equivalent to a given measure. A list of unsolved problems is appended.

The author's style displays its customary clarity, and the lucid presentation, enhanced by informal asides, augurs well for the author's declared aim of rekindling interest in this branch of mathematics.

I. E. FARQUHAR

Impact

The Theory and Physical Behaviour of Colliding Solids. By Prof. Werner Goldsmith. Pp. xv+379. (London: Edward Arnold (Publishers), Ltd., 1960.) 90*s*. net.

PROF. GOLDSMITH has produced a reference book which will be welcomed by those working on problems of the impact of solids, since much of the subject-matter has hitherto been available only in published papers. The book embraces a wide range of topics, and contains much factual information in the form of tables and graphs. The treatment is concise, and in many of the theoretical solutions only an outline of the main steps is given, with appropriate references to published papers.

After a survey of the classical theory of the impact of rigid bodies, the author considers vibration and wave motion in elastic bodies, and discusses problems involving the collision of elastic rods, and the impact of a rigid mass on a rod or beam. The book continues with a discussion of contact phenomena, and the effect of these phenomena in elastic problems is investigated. Impact problems involving plastic flow are considered, with a brief discussion of the hydrodynamic theory, and a more extended treatment of theories of plastic wave-propagation in rods, beams, and plates. A chapter on impact experiments includes a description of experimental techniques, together with results of many experiments involving elastic and plastic deformations. The book concludes with a chapter on the dynamic properties of materials, and the techniques used in their measurement. There is also an extensive bibliography of nearly six hundred titles.

R. P. N. JONES

Analytical Techniques for Non-Linear Control Systems

By Prof. John C. West. (Electrical Engineering Series.) Pp. 223. (London: English Universities Press, Ltd., 1960.) 25*s*. net.

THIS book describes successively the application of three techniques, namely the phase-plane and describing function techniques, and the use of random inputs, to the analysis of non-linear control systems.

In an approach basically similar to that used in a previous book by the same author on linear servomechanisms, the application to specific simple systems is introduced at an early stage in the description of each technique. Application to more complex systems is then described and the analysis developed from these examples to more general considerations concerning the technique. The same non-linear systems are analysed by each technique and a comparison of results made which forms a particularly interesting connecting link between the three separate groups of chapters.

The style is clear and concise, and the author does not allow mathematical complication to obscure the fundamental principles he describes. This, together with the basic approach of progressing from the particular to the general at each stage, leads to a text which may be easily read as an introduction to the subject, but which at the same time takes the reader further than would an introductory work. Indeed, the material in parts reaches, albeit briefly, almost as far as the present development of the subject permits.

This book fills a definite gap in the existing literature in the field of control mechanisms and is a useful addition to it.

E. M. DEELEY

Physics of Semiconductors

By A. F. Ioffe. Pp. xi+436. (London: Infosearch, Ltd., 1960. Distributed by Cleaver-Hume Press, Ltd., 1960.) 84*s*.

THIS is an English translation of the Russian book published in 1957 which was reviewed in *Nature* (Dec. 7, 1957). Some amendments and additions have been made in the preparation of the translation; the bibliography has been extended and a subject index has been added. A twenty-page section has been added on materials for thermoelements which provides an excellent discussion of impurity effects and solid solutions, and of the attainment of the required high electrical conductivity, high thermoelectric power and low thermal conductivity. The ten pages of "Conclusions" reflect the late author's particular interest in the phenomenon of low carrier mobility in certain materials and the dilemma posed by apparently very short mean free paths.

The book indicates the state of semiconductor physics up to 1957, and the treatment throughout is aimed at giving physical insight into fundamental processes without excessively detailed analytical discussion. It is felt that the book is successful in attaining its intended objectives and can prove stimulating in certain areas of contemporary semiconductor research.

R. E. BURGESS

CENTENARY OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY

By DR. JAMES R. KILLIAN, jun.

Chairman of the Corporation, Massachusetts Institute of Technology,
formerly Special Assistant for Science and Technology to the
President of the United States

ONE cannot live in the atmosphere of a great research and educational institution, renewed year after year by youth, with its hopes and energies, and by students, old and young, with their dreams and discoveries, without acquiring a faith in the power of intelligence to achieve a future of peace and order among all peoples.

This faith was strengthened during the recent celebration of the centenary of the Massachusetts Institute of Technology, when some of the world's most eminent intellectual leaders met at the Institute to exchange ideas on how greater good may be sought through greater knowledge. The Institute was honoured by their presence, and we believe the occasion proved a fruitful one for them.

More than 150 men and women from many professional fields in 35 countries participated in a conference on the prospects and implications of scientific and engineering education for the world. Later there were provocative discussions by such thinkers as Aldous Huxley, Prof. J. Robert Oppenheimer, Sir John Cockcroft, Prof. Chen Ning Yang, the Right Hon. Philip J. Noel-Baker, Prof. George W. Beadle and Prof. Peter B. Medawar, on what the future holds in science, the arts, industry and arms control. The Right Hon. Harold Macmillan and Mr. Dean Rusk, Secretary of State, gave notable addresses which cast new light on the complexities of international affairs.

No attempt will be made here to summarize the many points of view that were expressed in the discus-

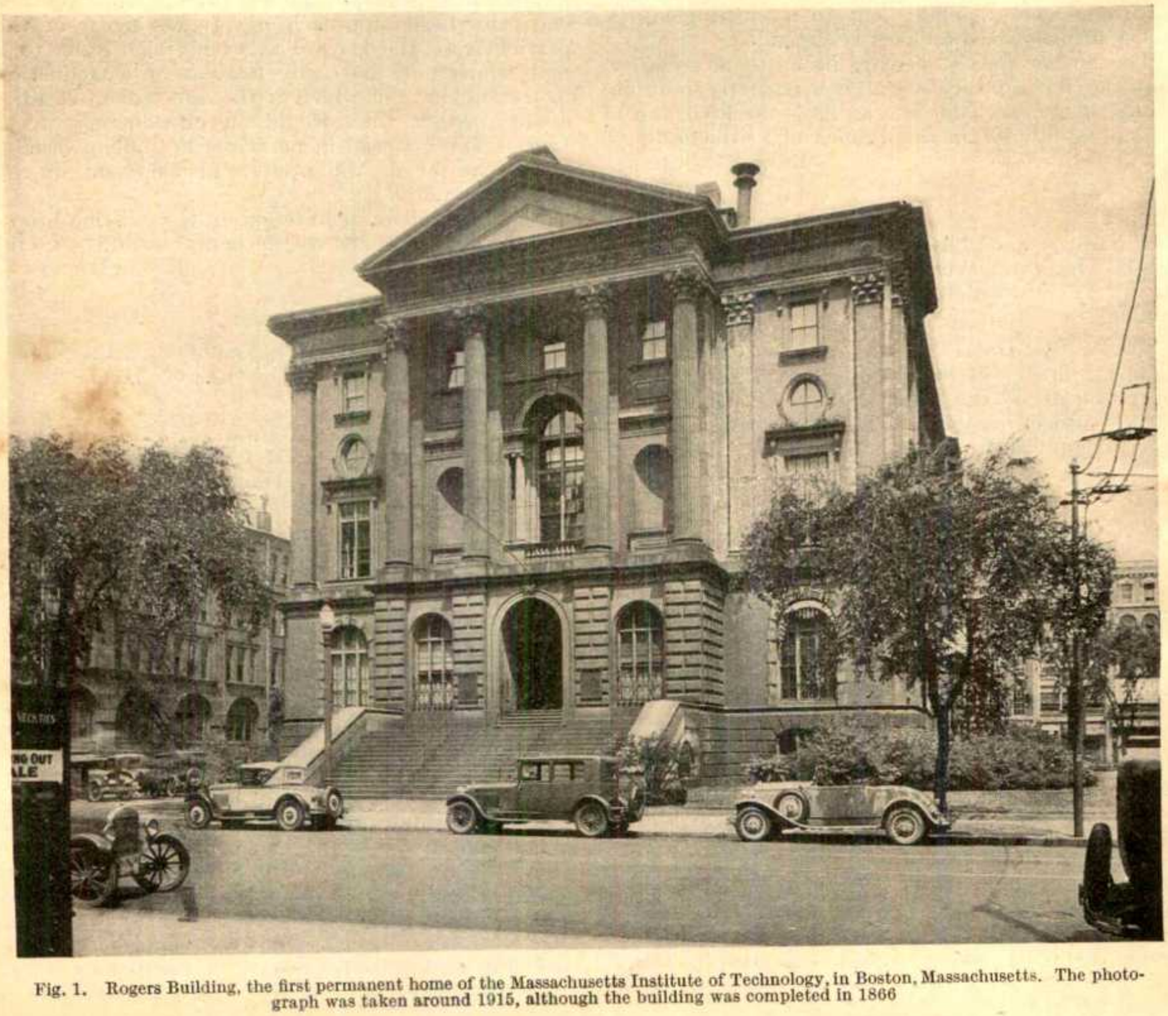

Fig. 1. Rogers Building, the first permanent home of the Massachusetts Institute of Technology, in Boston, Massachusetts. The photograph was taken around 1915, although the building was completed in 1866

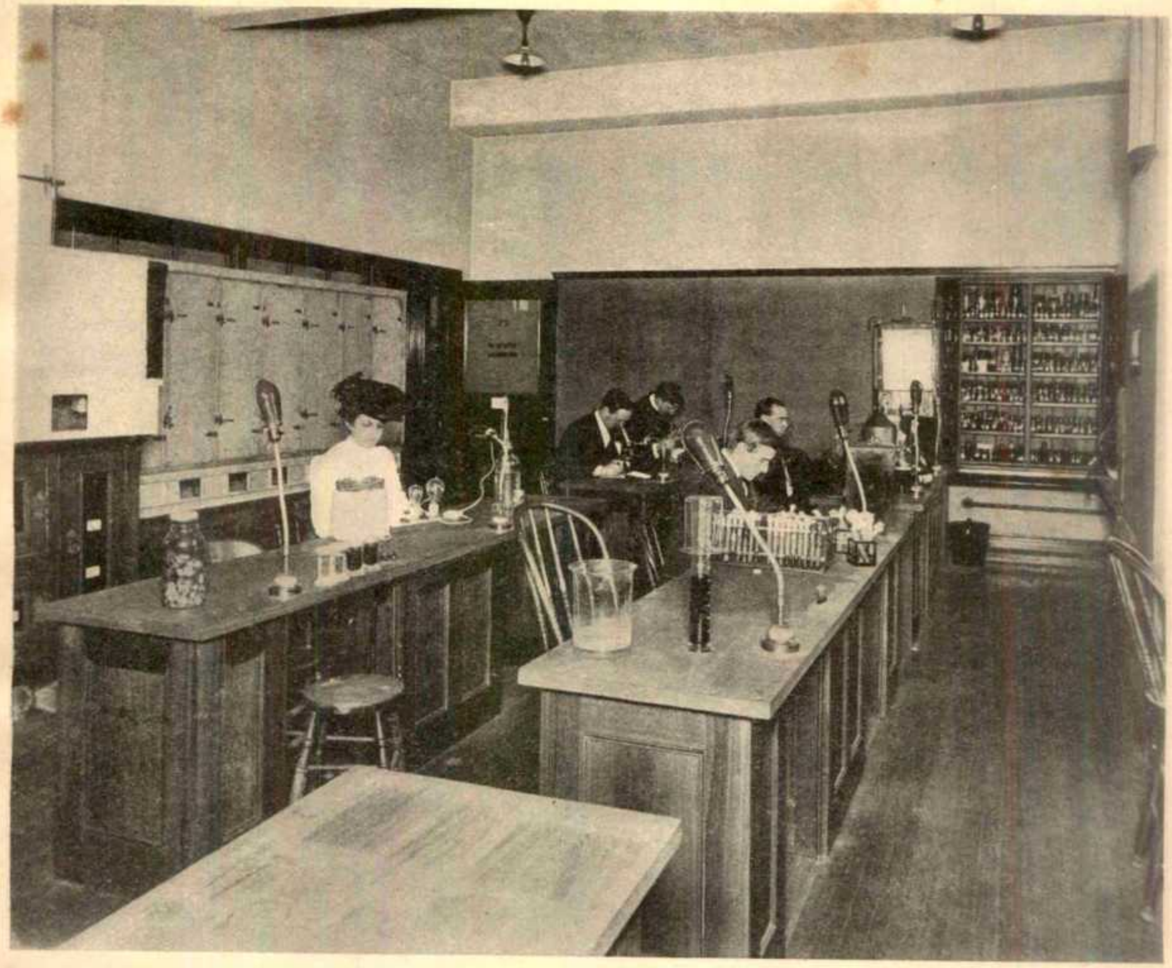

Fig. 2. Photograph of a research laboratory taken when the Institute was in Boston (1895). The students are of graduate-level and are performing experiments in electrolysis and the study of cultures in chemistry

sions. There was no intention that clear-cut conclusions should be sought, and no final solutions of the problems which press on the world from every side were offered. Yet, there was an atmosphere of hope, of awareness of our magnificent opportunity to imagine a better human community, and, through a new blending of the sciences, the social sciences and the humanities, to devise the new strategies required to move toward a better human community.

The expectations and intentions of to-day are closer than one might suppose to those which a hundred years ago led to the founding of the Institute. By the end of the second decade of the nineteenth century, the economic expansion of the United States, with the growth of industry and agriculture, was calling forth new public attitudes toward education and toward science and technology. There were strong forces leading to the popularization of knowledge, including a growing awareness by people from all walks of life that science could provide a better life.

This understanding brought into being lyceums and mechanics' institutes, the forerunners of a new pattern of education, the means for the application of scientific principles to the practical arts, and the development of a new and closer relationship between the scholar and the citizen.

Concurrently, there was a growing demand for universal, free, public education. It was in 1837 that Horace Mann, the eloquent apostle of "well-appointed and efficient means for the universal education of the people", became the first full-time secretary of Massachusetts' new State Board of Education, and in this post led a transformation of the State's schools and set a new pattern of public education for the nation.

In the midst of this national ferment a Virginian, William Barton Rogers, who was to found the Massachusetts Institute of Technology, began to form ideas for a new kind of educational institution to respond to the needs of a growing industrial society.

Rogers had held the chairs of natural philosophy and chemistry in William and Mary College, and of natural philosophy in the University of Virginia. He had served as head of the Virginia State Geological Survey, an experience that broadened his understanding of the social and economic importance of science.

He recognized that science should no longer remain the esoteric concern of a few learned and cultivated men. It was in the public interest that it be the concern of all. The economic expansion of the country, the gradual opening of the West, the growth

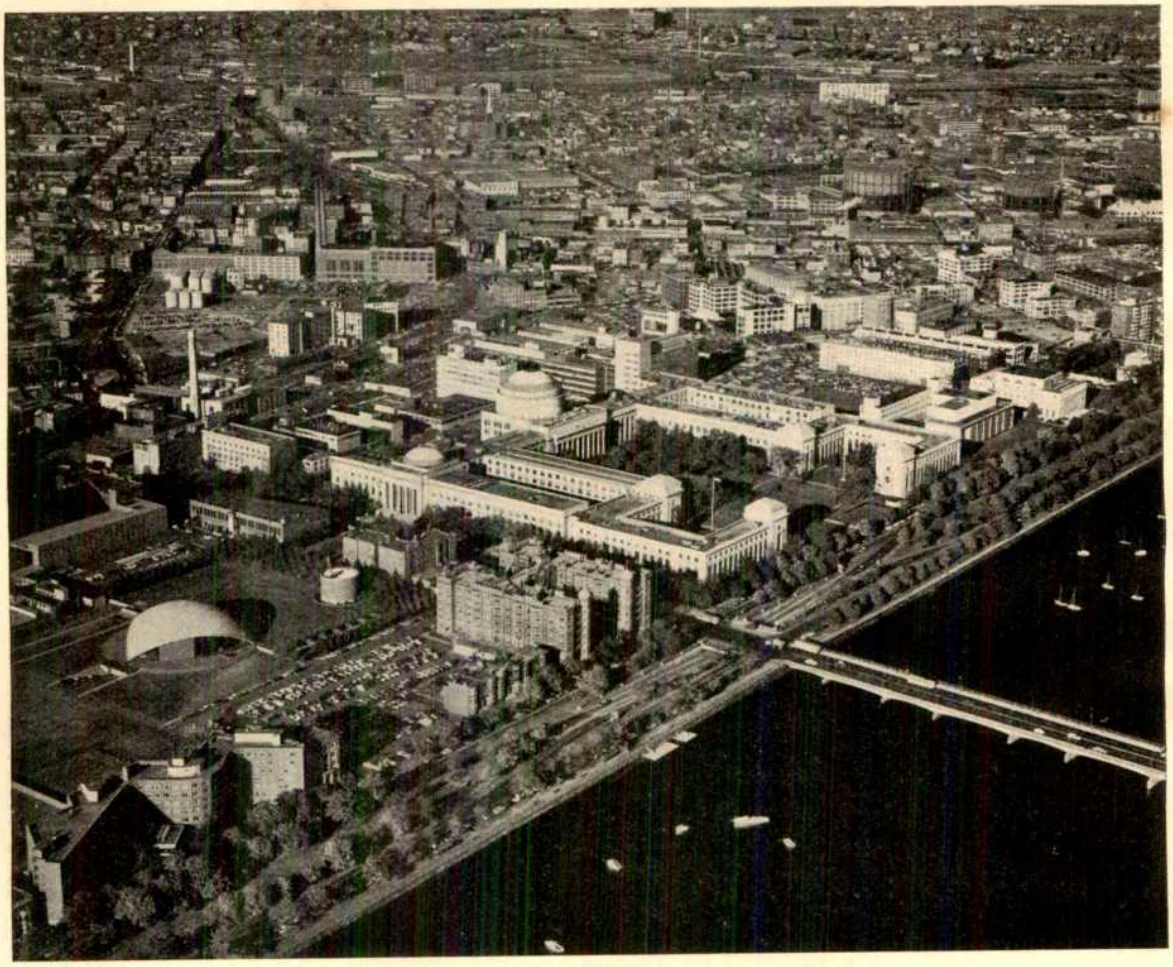

Fig. 3. Aerial view of the Massachusetts Institute of Technology. Kresge Auditorium and the Institute Chapel (the triangular and cylindrical shaped buildings) are on the left. From left to right, immediately facing the Charles River, are Baker House Dormitory, Graduate House Dormitory, the Great Court with the Main Building of the Institute, Hayden Memorial Library, and Walker Memorial Student Union. (*Photo: Lawrence Lowry*)

of industries, navigation, transportation, mining, and agriculture, increased the need for a deeper knowledge of the resources of the nation.

What treasures were buried in its rocks, hidden in its forests, lying beneath its waters? How could Nature be controlled to offer better opportunities for manufacturing, transportation, agriculture, and, most important, men? The existing educational institutions were inadequate in attitude, facilities and personnel to meet these demands.

College education was still centred on the study of the classics. Natural science was considered of relatively little importance in comparison. The colleges, as a rule, still believed in 'training the mind', for which purpose Cæsar, Plutarch and Euclid were considered unsurpassed. Engineering as a professional study was almost non-existent in the university system.

A few schools, notably Yale and Williams, had hesitantly and half-heartedly attempted to teach the application of scientific principles to the productive arts. Rensselaer Institute under Amos Eaton and the United States Military Academy under Sylvester Thayer had already pioneered in the training of science teachers and civil engineers, respectively.

Even so, the United States was definitely lagging in technological education relative to Europe, where engineering had long been recognized as a profession. The École Polytechnique in Paris, the Bergakademie at Freiburg, the Technische Hochschule in Zurich—all emphasized a highly intellectual, scientific and analytical approach to education for the technological services demanded by State and industry.

Since his move to the University of Virginia, Rogers had become immersed in college administration. In 1844 he was chosen chairman of the Faculty, a post equivalent to that of the university's chief executive. This position strengthened Rogers's administrative and organizational skills, while affording him full opportunity to ponder Jefferson's educational ideas stressing the use of science and technology for the betterment of society.

His experience led Rogers to question seriously the future of science in the current curricula of existing colleges. Dearth of facilities, improperly trained personnel, and above all the lack of understanding by educators seemed to foretell a dim future for education in science and engineering. It became apparent to Rogers that only through the formation of a new school, free from the vested interests of traditional education, devoted specifically to polytechnic education, could science and its practical application be placed in its proper perspective in the general system of American education. Such circumstances led Rogers in 1846 to prepare this famous "Plan of a Polytechnic School".

Virginia, he concluded, was not then the place to start such innovations. Rogers turned to Boston as the site for consummation of his ideas on polytechnic education. This city, with its flourishing arts and sciences, the centre of factory- and machine-conscious New England, and philanthropically minded families, was a logical place to promote and establish a school devoted to technology.

The proposal attracted nation-wide interest, although it was not until 1861 that Rogers saw its culmination when the "Act to Incorporate the Massachusetts Institute of Technology" was passed by the Massachusetts Legislature "for the purpose of instituting and maintaining a society of arts, a museum of arts, and a school of industrial science, and aiding generally by suitable means the advancement, development and practical application of science in connexion with the arts, agriculture, manufacture, and commerce".

The forerunner of a new type of educational institution, this was fully tuned to the changing technological character of the nation. It was to serve as the model for many other schools throughout the world. The work of Rogers undoubtedly did much to pave the way for the Morrill Act of 1862, which led to the establishment of America's unique system of land-grant colleges.

In his plan for a new kind of university, Rogers stressed four fundamental principles:

First, he emphasized the importance of being useful. He had no sympathy with the prevailing view that the practical professions lacked dignity, or that utility corrupted liberal education. He was one of the earliest advocates of what J. B. Conant has described as the philosophy of the modern American university, "a philosophy hostile to the supremacy of a few traditional vocations, a philosophy moving toward the social equality of all useful labor".

Next Rogers stressed the educational gain derived from building a college programme around a professional objective. He recognized that the discipline, the thoroughness, and the motivation inherent in a professional programme have educational values. He refused to admit of any inevitable conflict between specialism and liberal education.

Rogers's next principle, that of learning by doing, he expressed through the laboratory method of instruction. While this idea was not original with him, he gave it its first systematic application in the

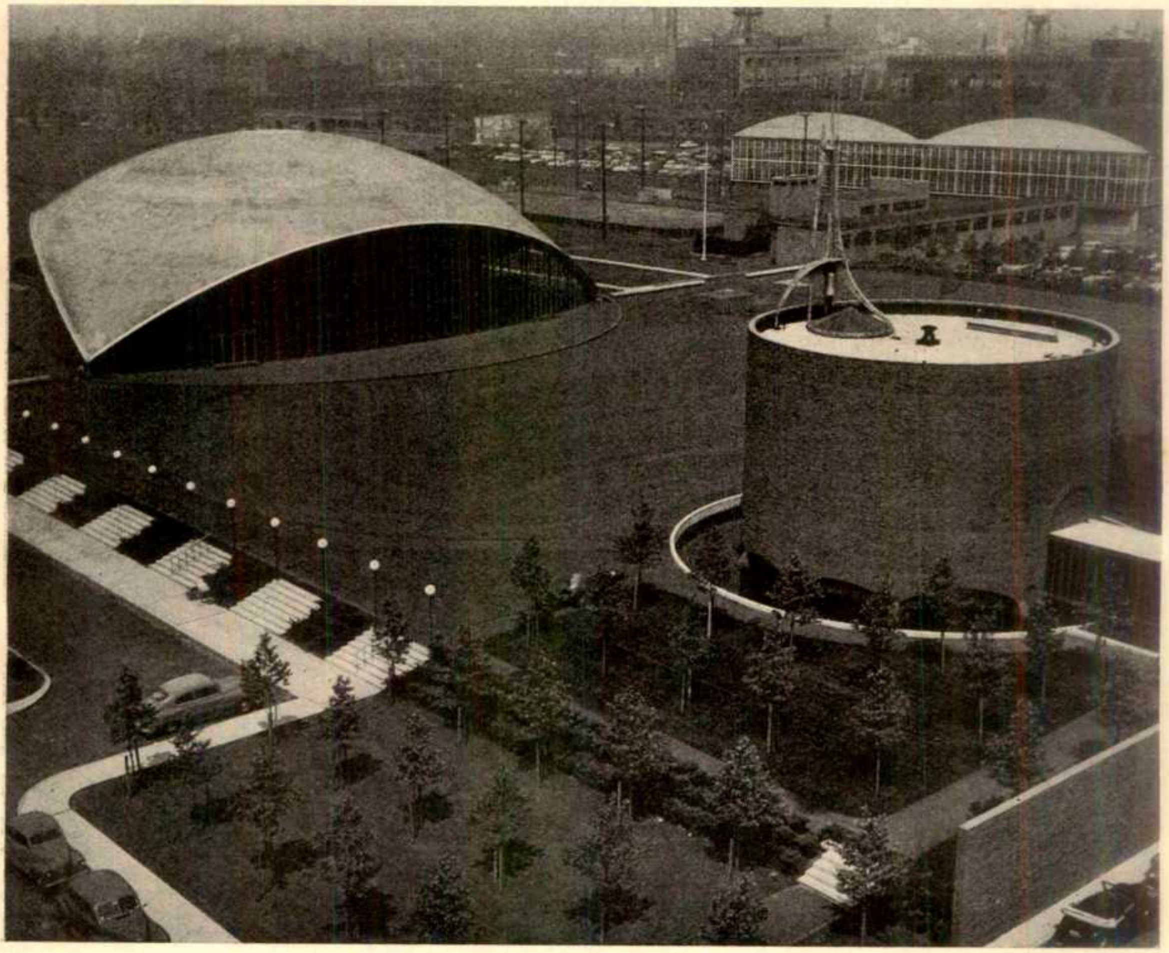

Fig. 4. Kresge Auditorium (left) and the Institute Chapel (right) were designed by Eero Saarinen and are focal points of the Massachusetts Institute of Technology community

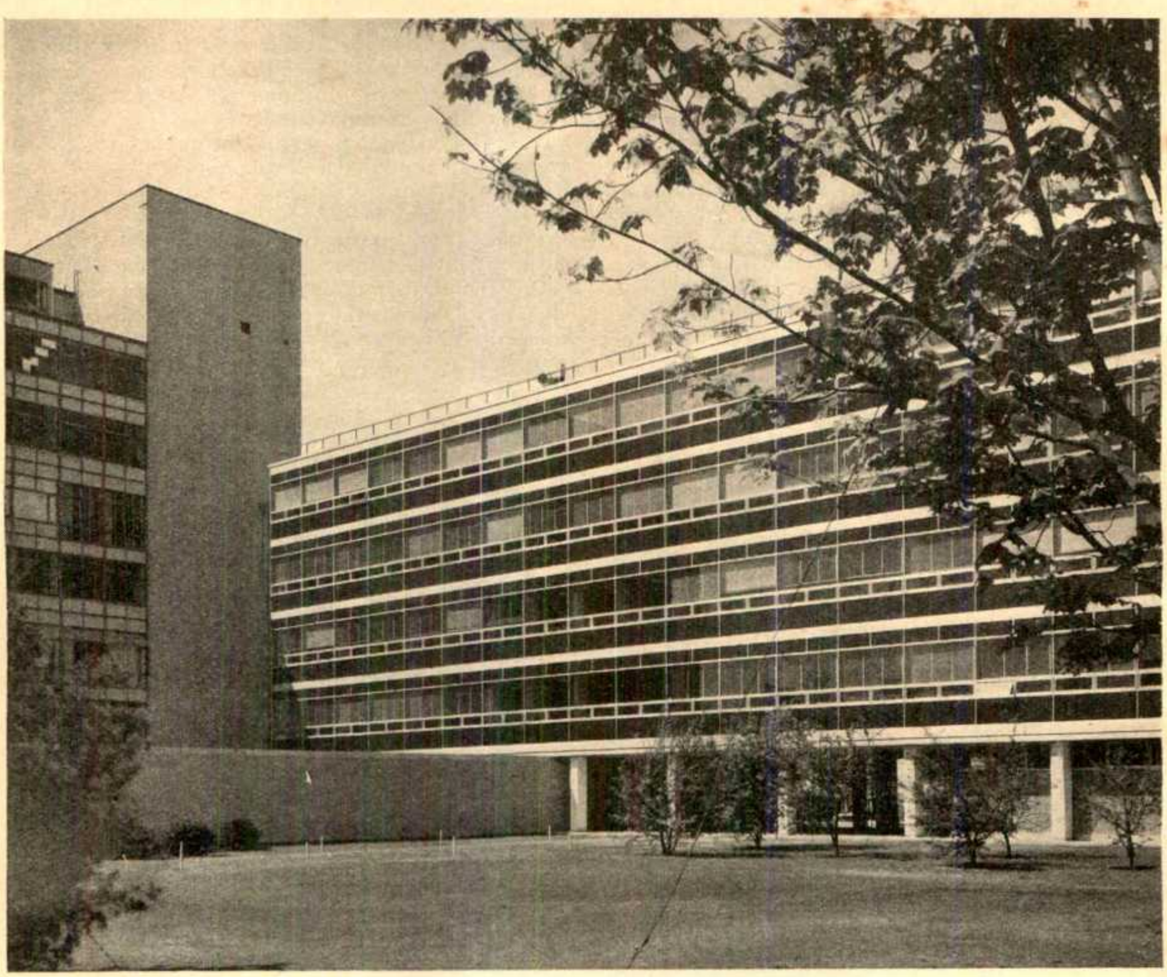

Fig. 5. The Karl T. Compton Memorial Laboratories, which house the Computation Center and the Research Laboratory of Electronics at the Massachusetts Institute of Technology

United States. In his educational thinking, Rogers always stressed method; in a time of scepticism, he had faith in the scientific method. He foresaw the far-reaching effects on higher education of the spirit and method of research, the concept which holds that learning thrives best in an atmosphere of creativeness and not in one limited to imitativeness and antiquarianism. American universities were later to assimilate this idea from the German universities; but Rogers's idea was indigenous.

Next, Rogers was emphatic in his belief that learning principles is more important than learning facts. "We believe", he said, "that the most truly practical education, even in an industrial point of view, is one founded on a thorough knowledge of scientific laws and principles, and which unites with close observation and exact reasoning", and, note this, "a large general cultivation".

He wanted the humanities taught along with engineering and science. He anticipated the need, met by only a few institutions even to-day, of a new kind of institution with its centre of gravity in the sciences instead of the classical studies, but with the humanities and social sciences fully represented and enthusiastically supported as full partners.

In his original prospectus of 1846 Rogers had the boldness and foresight to dream of a destiny for his institution. "I doubt not", Rogers wrote, "that such a . . . school would finally expand into a great institution . . ."!

Rogers laid down the grand design, and successive presidents carried the institution steadily forward toward the quality of institution Rogers had doubted not would be achieved. Francis Amasa Walker, distinguished economist and Civil War general, gave validity to the design and brought the Institute to national prominence.

Richard Cockburn Maclaurin, a Scotsman from New Zealand, who was educated as both lawyer and physicist at the University of Cambridge, attracted to the Massachusetts Institute of Technology financial resources that for the first time began to be commensurate with the Institute's responsibilities and moved the Institute to its present more ample home in Cambridge, Mass. Karl Taylor Compton provided a new thrust by strengthening the sciences and expanding graduate study and research.

Where does the Institute stand at the end of its first hundred years in relation to the aims and hopes of its founder ? How has the innovation served the nation ? What is the record ?

The Massachusetts Institute of Technology has been an educational pioneer since its founding. It virtually created the modern profession of chemical engineering and was the first technological institution to recognize and provide for economics as an important element in the education of the engineer. The Institute was the first school to establish courses in aeronautical engineering, architectural engineering, chemical engineering, food technology, industrial biology, marine engineering, and naval architecture. It established the first collegiate programme in architecture.

The Institute's contributions to education continue to-day to find new expression over a wide range. One striking current example is the work of the Physical Science Study Committee, devoted to creating a new, complete physics course for secondary schools.

The Institute is at present taking the leadership in upgrading and modernizing the education of engineers. Its Electrical Engineering Department has led the way with a drastic overhaul of its curriculum, which embodies a more fundamental approach to electrical engineering—an approach which has prompted acclaim in industry as well as in education. This spirit is now finding rapid acceptance through the School of Engineering.

The Massachusetts Institute of Technology awards 13·6 per cent of the nation's total output of doctorates in engineering and 9·5 per cent of all Master's degrees in engineering. The number of engineering doctorates awarded by the Institute is more than double the number awarded by the second-ranking institution. In this critical area of education (only about 700 engineering doctorates per annum are at present awarded in the entire nation), the Institute has led the country in the number of doctorates awarded in almost all the professional fields of engineering.

The Institute now awards approximately 7 per cent of all the U.S. doctorates in physics. On no other single campus in the United States have so many doctorates in physics been awarded.

The Institute has gained exceptional strength from the quality and cosmopolitan character of its student body. Its 3,600 undergraduates and 2,700 graduate students are a highly select group, and in their origins they probably represent a wider geographical spread than any other student body in America. Typically, Massachusetts Institute of Technology freshmen rank in the top 10 per cent of their high-school graduating classes; the first-year class of 899 students in 1960 was drawn from 677 secondary schools distributed through almost every State and in many foreign countries.

In the graduate school more than 500 American and foreign universities are represented among the student body.

Harvard and Massachusetts Institute of Technology stand first and second respectively, and far ahead of most other institutions, in the number of National Merit Scholarship recipients enrolled. They also lead the country in the number of students receiving General Motors Scholarships and National Science Foundation Fellowships who elect to study in these two institutions.

In its service to the nation, the Institute has played a part that has demonstrated it to be one of the most significant sources of national security and strength in the United States. In recent years it has created and managed the Lincoln Laboratory, out of which has come the *SAGE* system of Air Defense and the concept and design of the *DEW* Line, the early warning line which has been vital to the deterrent power of our strategic striking force.

The Instrumentation Laboratory has pioneered in the development of inertial guidance for missiles, space-vehicles, aircraft, and submarines. Out of this Laboratory have come designs, components and systems which have resulted in industrial production running into hundreds of millions of dollars: for example, the guidance systems used in the *Polaris*, *Thor* and *Titan* missiles. *Thor*, with its 1,500-mile range, was the nation's first operational intermediate-range ballistic missile.

The Research Laboratory of Electronics, with its wide-ranging programme of basic research, has been a well-spring for new concepts and new technology important both to advancement of defence and industrial technology.

From its beginning a hundred years ago, the Institute's resources have thus been brought powerfully to bear on the development of America's industrial strength. The development of every technically based industry has been quickened by research done at the Institute or by its graduates serving in industrial research, engineering, or management capacities.

Massachusetts Institute of Technology graduates have helped to create our modern urban environment and its unequalled array of transport systems and public work. They have brought a command of the methods of science, engineering and management to the defence of the nation, to the achievement of a higher standard of living, and to the advancement of public and private health.

In addition to its contributions to science and engineering, the Institute has been, from the beginning, a centre of management education, a contribution that now finds formal expression in its young and vigorous School of Industrial Management. Industry's growing dependence on engineers in management is confirmed by a recent study which revealed that almost half the Institute's graduates in engineering eventually occupied positions in management, many within five years of their graduation.

Through leadership positions in American industry, the Institute's graduates have contributed to the strength and prosperity of the nation. Some 1,500 alumni of the Institute are presidents of private companies and institutions in the United States; about 1,200 are vice-presidents. The Institute heads the list of educational institutions the graduates of which (more than 1,400) appear in *Who's Who in Engineering*—a number almost double that of the next ranking institution.

At present the Massachusetts Institute of Technology probably has the most comprehensive and varied research programme in the physical sciences and technology to be found in any one place in the world. It has at present under way 900 separate research projects. There is scarcely any aspect of American science and technology that is not touched—and advanced—by its creative activity. Thus, the Institute stands to-day as a generative force, with the opportunity before it to achieve a new order of service to America, through both research and education.

Major among the developments of the past two decades has been the strengthening of the humanities and social studies. The Center for International Studies has been established, the School of Humanities and Social Science has been formed to give formal focus and recognition to programmes in these fields. Political science has been added as a degree-granting programme.

The Massachusetts Institute of Technology has moved steadily, as Rogers clearly hoped it would, toward becoming a university polarized around science.

RECENT ADVANCES IN THE BIOCHEMISTRY OF GLYCOGEN AND STARCH*

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GLYCOGEN, and the components of starch, the largely unbranched amylose and the highly branched, glycogen-like amylopectin, were the first polysaccharides to be synthesized enzymically *in vitro*. Several factors helped in this achievement and in the subsequent rapid progress in the nineteen-forties. The substrate, α-D-glucose 1-phosphate, can be readily obtained in large quantity; the progress of synthesis of the chain-forming α-1,4-linkage is very easy to measure; and the enzymic synthesis of the branch points is accompanied by characteristic changes in the iodine-staining properties of the polysaccharides and their susceptibility to degradation by exo-enzymes. Ten years ago the enzymic synthesis and degradation of these polysaccharides could be reviewed with the feeling that further progress would consist only in adding detail to the already firm outlines.

However, the nineteen-fifties saw the widespread discoveries of the sugar nucleotides and their involvement in carbohydrate biosynthesis. Glycogen and starch have been caught up in these discoveries and the whole conception of their biosynthesis *in vivo* from glucose 1-phosphate is now held to be incorrect. The new evidence points to both polymers originating from uridine diphosphate glucose, polymerized by 'glycogen synthetase'[1] or a corresponding plant enzyme, referred to here as 'amylose synthetase', first reported last September[2]. Phosphorylase is thought to be an agent only of degradation. Other discoveries have been scarcely less disturbing to the older hypotheses. For example, rabbit muscle-phosphorylase *a*, the first glycogen-metabolizing enzyme to be crystallized, is not the predominant form in resting muscle[3]. Instead it is phosphorylase *b*, which dimerizes to the *a* form by an adenosine triphosphate-dependent reaction during the *in vitro* manipulations of isolation[4]. Because phosphorylase *b* requires adenosine monophosphate for activity while *a* does not, a reconsideration of the interrelation of *in vivo* processes must follow. The reverse conversion of phosphorylase *a* into *b* by *PR*-enzyme has also been re-examined. *PR*-enzyme had been thought to be a factor removing a prosthetic group (hence the initials), but after the discovery of the halving in molecular weight when rabbit-muscle *a* enzyme turns into *b* (ref. 5), the initials could be held to apply to phosphorylase rupture. We now know that they stand equally for phosphate removal[6]:

$$\text{phosphorylase } a \xrightarrow{PR\text{-enzyme}} 2 \text{ phosphorylase } b + 4PO_4^{3-}$$

Other enzymes which have been re-scrutinized are those splitting the branch linkages of glycogen and amylopectin. The plant enzyme (*R*-enzyme), attacking amylopectin, has proved to be two enzymes, one specific for the natural macromolecule and the other attacking only the small branched oligosaccharides formed during α-amylolysis of amylopectin[7]. Correspondingly, the activity of the animal enzyme, amylo-1,6-glucosidase, is due to two components[8], but in this case both activities, those of a hydrolase and of a transglycosylase, are necessary to complete the debranching process.

The transglycosylase is one of a vast group of enzymes discovered in the past ten years, the existence of which went unsuspected for lack of suitable means of detection. Paper chromatography provided that means and was instrumental in the discovery in potato juice of an enzyme the specificity of which has not yet been matched by that of any mammalian enzyme. This catalyst, *D*-enzyme[9], redistributes α-1,4-bonds in starch-type polymers. Maltotriose is the smallest molecule on which it exerts any rapid action. It is first converted into maltopentaose and glucose, for the enzyme prefers to transfer glucose residues severally rather than singly. There is no hydrolysis and therefore no change in the average size of the molecules. The enzyme represents a major activity in potato juice and is presumably implicated in starch metabolism. When the action is coupled with that of hexokinase and adenosine triphosphate, to convert glucose into the 6-phosphate, the enzyme is driven to form long, amylose-like chains from maltodextrins and merits consideration as yet another rival to phosphorylase for starch synthesis[10]. Enzymes which likewise redistribute α-1,4-glucosidic bonds but appear to act readily on maltose are also known now in plants[11] and animals[12,14]. Separate but similar catalysts have been discovered in potato (*T*-enzyme)[13] and rabbit muscle[14]. These also transfer α-glucose from maltose but reposition the sugar at C-6, not at C-4, forming the trisaccharide panose. The chemistry of this action is identical with that of the polysaccharide-branching enzymes, *Q*-enzyme in plants, amylo-1,4→1,6-transglycosylase in animals. *T*-enzyme is separable from *Q*-enzyme, so just as with *R*-enzyme (see above) it seems that separate systems are necessary for the same reaction according as to whether the substrate is an oligosaccharide or a polysaccharide. In fact, of the enzymes which it has been customary to involve in the cycle of glycogen–starch synthesis and degradation, only the branching enzymes seem to have retained their places unchallenged. This situation may, however, simply reflect the comparatively scant attention these enzymes have received.

Biosynthesis of Glycogen and Starch

The chain-forming process. The synthetic pathway to glycogen based on uridine diphosphate glucose (Fig. 1) was first reported in 1957, by Leloir and Cardini[1], as taking place in rat liver. Since then, the enzyme has been found in muscle tissue of pigeons[15], humans[16], rabbits[17] and rats[18], in rabbit brain[19], yeast[20] and in beans[2,20a], potatoes and sweet corn[20a]. The early observations of the insolubility of the animal enzyme are probably the result of adsorption on glycogen in the microsomes, and this finding led Leloir *et al.*[2,20a] successfully to search for the enzyme in starch grains. The enzyme preparation reported is in fact a suspension of starch.

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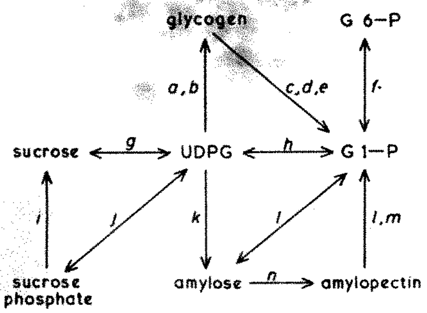

Fig. 1. Synthesis and degradation of glycogen, starch and sucrose. G 6-P, glucose 6-phosphate; UDPG, uridine diphosphate glucose; G 1-P, α-glucose 1-phosphate; *a*, glycogen synthetase; *b*, amylo-1,4 → 1,6-transglucosylase; *c*, phosphorylase; *d*, amylo-1,6-glucosidase; *e*, amylo-1,4 → 1,4-transglucosylase; *f*, phosphoglucomutase; *g*, sucrose synthetase; *h*, UDPG pyrophosphorylase; *i*, sucrose phosphatase; *j*, sucrose phosphate synthetase; *k*, amylose synthetase; *l*, phosphorylase; *m*, *R*-enzyme; *n*, *Q*-enzyme

The finding recalls Stacey's[21] suggestion that the starch grain might contain the enzymes of synthesis.

The discovery of the nucleotide pathway to glycogen explained several observations inconsistent with the idea of phosphorylase as the provider of glycogen[22]. For example, when epinephrine or glucagon are used to stimulate phosphorylase, glycogen disappears. Also the ratio of inorganic phosphate: glucose 1-phosphate in animal tissue is totally unfavourable for glycogen synthesis by phosphorylase. Glycogen synthetase action does not depend on the level of inorganic phosphate, and the conversion of uridine diphosphate glucose is essentially complete[1,15,18].

Glycogen synthetase needs a primer, and its specific requirements are similar to those of muscle phosphorylase. Thus glycogen is the most efficient primer but loses its activity after degradation by α-amylase. Polysaccharides with lower degrees of branching than glycogen are less effective and linear material does not prime[17,18,20]. The problem is therefore posed with glycogen synthetase, as with muscle phosphorylase[23], of the reason why, in order to synthesize α-1,4-bonds, the enzymes require a primer containing α-1,6-bonds and of high molecular weight. Potato phosphorylase[24] and amylose synthetase[20a] have no such scruples. Work with glycogen synthetase presents experimental problems due to the instability of the enzyme and the exotic nature of the glucose-donating substrate. So far the records of 'glycogen' synthesis in fact amount to lengthening of the unit chains of the added primer by only a few glucose residues. A very curious feature of glycogen synthetase is the dependence of the activity of the animal and yeast enzymes on glucose 6-phosphate. The rat-liver enzyme[1], for example, is activated up to 15-fold by the ester, and the 6-phosphates of fructose, glucosamine and galactose can replace the glucose phosphate. The glucose moiety of the phosphate is not, however, incorporated into the synthetic polysaccharide[18], and it remains uncertain whether the effect of sugar phosphate is simply a protection of enzymic activity or something more intimate[1]. The effect has not been mentioned for the bean enzyme[2,20a], but one wonders whether there is any connexion with the still unexplained occurrence of 6-phosphate residues in starch, mainly in the amylopectin component.

Branching enzymes. There is a very close correspondence between glycogen metabolism in animals and starch synthesis in plants. In fact every enzyme implicated in glycogen metabolism has its plant counterpart. Such differences as are found concern specificity, as between *D*-enzyme and its animal analogue, mentioned earlier. These differences are of two types, one concerned with group transfer and the other with the differing shapes of the plant and animal macromolecules. Thus, in regard to the latter, glycogens having 'normal' values of average unit chain-length are not attacked by potato phosphorylase, *D*-enzyme or *R*-enzyme[25]. Only when the glycogen has been fragmented, thereby opening the compact structure, do these enzymes attack it. Yet potato phosphorylase[26] has a molecular weight only 40 per cent of that of rabbit-muscle phosphorylase *a* (ref. 5). A similar comparison can be made between the plant and animal branching enzymes. Potato *Q*-enzyme synthesizes amylopectin from amylose, and will not introduce further branches into amylopectin[27]. The branching enzyme from yeast, which stores glycogen, acts on amylose to produce amylopectin but continues the branching process until the degree of branching is that of glycogen[28], about twice that in amylopectin.

Degradative Enzymes

Phosphorolysis and debranching. The plant and animal phosphorylases are exo-enzymes and in the presence of inorganic phosphate they phosphorolyse amylopectin and glycogen, respectively, until the points of branching are approached. The limits of approach by potato phosphorylase have not been measured, but Cori and Larner[29] defined those of muscle phosphorylase after finding that the residual glycogen dextrin (φ-dextrin) yielded glucose on treatment with amylo-1,6-glucosidase. This suggested a structure in which side-chains (*A* chains) had been degraded to a single 1,6-linked α-glucose residue. The outer length of each chain carrying this residue (*B* chains) was thought to be 5–7 glucose units. Walker and Whelan[8], however, found no such 1,6-bonded single glucose residues in the φ-dextrins of glycogen or amylopectin. They concluded that both the attenuated *A* chain and the outer portion of the *B* chain were each four units long. Their explanation of the appearance of free glucose during debranching is that a transglycosylase repositions glucose residues in the φ-dextrin to form the Cori–Larner-type molecule, then attacked by the glucosidase. A preparation of the glucosidase was in fact found to contain a transglycosylase which repositioned the α-1,4-bonds of maltose and maltotriose, but Illingworth *et al.*[29a] report the lack of this enzyme in a purified preparation. Differences of group transfer also occur between the plant and animal debranching enzymes in that the latter seems capable only of detaching single 1,6-linked glucose residues[29] whereas *R*-enzyme only removes such side-chains when they are two units or longer[30].

α- and β-Amylases. The actions of the α- and β-amylases, endo- and exo-enzymes respectively, are now well understood. The enzymes are generally considered to be entirely specific for the hydrolysis of the α-1,4-glucosidic linkage. Certainly they do not hydrolyse the α-1,6-branch linkages but caution in rigidly defining the specificity is prompted by the recent report[31] that a 'specific' β-1,3-glucosidase also hydrolyses β-1,4-glucosidic bonds when these are proximate to a β-1,3-bond. Also β-amylase liberates both nigerose (α-1,3-diglucose) and maltose from Floridean starch[32].

The limits of action of the amylases on linear and branched amylaceous polysaccharides have been defined. The smallest natural substrate for either enzyme is maltotriose[33,34], hydrolysed to glucose and maltose, but it is maltotetraose which is hydrolysed at rates comparable with those of polysaccharides. The branch linkages offer partial and absolute protection to adjacent 1,4-bonds. In its endwise progression from the non-reducing chain ends of amylopectin and glycogen, β-amylase is unable to hydrolyse the last α-1,4-bond before a 6-substituted glucose unit[35,36]. The fragmenting α-amylases can carve the 6-substituted glucoses away from the polysaccharide, but not in the form of the 1,6-linked disaccharide isomaltose. One or more 4-substituted glucose residues remain in the fragments (α-limit dextrins). Thus the smallest α-limit dextrin formed by malt α-amylase is panose (4-α-isomaltosylglucose), by human-salivary, pig-pancreatic and *Aspergillus oryzae* α-amylases it is the tetrasaccharide 4-α-panosylglucose, whereas *Bacillus subtilis* α-amylase yields a pentasaccharide, tentatively assigned a structure in which α-maltose is joined to C-6 of the central glucose residue in maltotriose[34].

Glycogen-Storage Diseases

A practical outcome of work in a largely academic field of research has been the explanation of the inherited disorders characterized by abnormal deposits of glycogen in the storage organs. The subject has been reviewed very recently[22] and will be mentioned here only briefly. Of the six types of disease listed by Stetten and Stetten[22], four are associated specifically with the impairment of a particular enzyme of the glycogen cycle. In the two other types no specific enzymic lesion has yet been detected. When the deficiency resides in glucose 6-phosphatase (Von Gierke's disease) or phosphorylase (type VI) the glycogen in the diseased tissue has a normal structure, but has undersized outer chains; when the debranching enzyme is deficient (type III) or a low degree of branching in the type IV disease, assumed to be due to lack of branching enzyme.

If glycogen synthetase and phosphorylase are to be thought of as opposing forces then a lack of the latter should lead to excessive amounts of glycogen. This indeed happens in type VI disease, known in human muscle and liver. The normal structure of the resulting glycogen implies that the branching enzyme is in a concentration sufficient to cope with the extra glucose polymerized. It was observed very early in work with phosphorylase that synthesis from glucose 1-phosphate with crude rabbit-muscle extracts led to amylose-like material[23], that is, the chain-lengthening and chain-branching enzymes were in an entirely unfavourable ratio for glycogen formation. Glycogen synthetase activity in rabbit muscle is, however, less than 1 per cent of that of phosphorylase and the balance of synthetase with branching enzyme must therefore be more favourable for glycogen synthesis. The activity of synthetase in rat muscle is known to be sufficient to account for the amounts of glycogen being synthesized[18,37], but the level of glucose 6-phosphate in rat liver, necessary to activate the enzyme (see above), is so low as to limit the rate of synthetase action[1]. The excessive relative activity of muscle phosphorylase can be seen as yet another reason for relegating the enzyme to a place in the catabolism of glycogen, saving only that the supply of adenylic acid could regulate the action of phosphorylase *b*.

Tests of activity of enzymes in heart and liver from cases of type II disease failed to reveal an enzyme deficiency which could account for the excess of glycogen[38]. The results are, however, disturbing in revealing a total absence of glycogen synthetase in four, and a marked deficiency in two, of the six organs examined. The results are obviously incompatible with the excess of glycogen found in the tissues and at face value point to the existence of yet another pathway to glycogen.

Enzyme Structure

Many of the different amylases and phosphorylases have been crystallized and some facets of their structures are emerging. The most significant recent advance in the α-amylase field has been their recognition as metallo-enzymes[39]. All contain at least one atomic proportion of calcium, essential both for the activity of the enzyme and to preserve its integrity against the depredations of proteases. Possibly the calcium maintains the amylase molecule in a configuration unfavourable for proteolysis. *B. subtilis* α-amylase also contains an atom of zinc, uniting two protein moieties, both of which are active in the presence and absence of zinc. End groups of some α-amylases have also been identified.

Human-[40], rabbit-[41], lobster-muscle[42] and potato[26] phosphorylases contain pyridoxal phosphate. Although the rabbit- and lobster-muscle enzymes are reversibly inactivated on removal and re-addition of pyridoxal phosphate, removal being effected by acid, reduction with sodium borohydride renders the pyridoxal acid stable yet does not destroy enzymic activity[43]. The reduced *a* and *b* enzymes are, moreover, still interconvertible. Rabbit and human *a* enzymes contain 4 molecular proportions of pyridoxal phosphate, the *b* forms and potato phosphorylase 2 proportions. Although lobster- and rabbit-muscle phosphorylases *a* are converted into the *b* forms by lobster- or rabbit-muscle phosphorylase phosphatases[42] (this interchange of enzymes does not apply in the $b \rightarrow a$ reaction), the lobster enzyme does not alter in molecular weight[42] but the muscle enzyme is halved in weight[5]. Likewise dog-liver phosphorylases *a* and *b* are equal in weight[44]. Human-[40], rabbit-[23] and lobster-muscle[42] *b* enzymes require adenylic acid for activity, but dog-liver phosphorylase *b* is enzymically inactive[45]. Human- and rabbit-muscle phosphorylases are immunologically identical[40], but dog-liver and -heart phosphorylases are immunologically different[46]. The phosphate removed by phosphatase from rabbit-muscle phosphorylase *a* is attached to serine[6]. Digestion of *a* enzyme with trypsin removes both serine and phosphate as a hexapeptide, Lys. Glu(NH_2).Ileu.Ser(PO_4).Val.Arg, leaving a phosphorylase *b'*, active in presence of adenylic acid, but no longer convertible into the *a* form. Therefore serine phosphate is not concerned with phosphorylase activity.

Outstanding Problems

Of the enzymes listed in Fig. 1, only the phosphorylases[47,48] and *Q*-enzyme[49] have been crystallized. Purification of enzymes has so often led to the discovery of new catalysts and such experiments are essential preliminaries to studies of specificity. Some unsolved problems of specificity have been touched on, for example, the requirements of animal phosphorylase and glycogen synthetase for branched primers of high molecular weight. The suggestion is prompted that they may need to form an alliance between pairs of chains before adding a glucose unit. Such an alliance is the present accepted idea of the action of the branching enzymes[50]. Searching inquiries into substrate specificities require a wider range of substrate than is provided by Nature and specific chemical modifications of amylose are being made, to provide such materials[51]. It is now known, for example, that removal of the oxygen atom of the primary hydroxyl groups in amylose renders the polymer resistant to β-amylolysis and phosphorolysis, but not α-amylolysis[52].

Phosphorylase has been cited as an example of an enzyme capable of performing a transformation *in vitro* which it is not called on to do *in vivo*. Another such example is provided by the enzyme capable of the direct biosynthesis of sucrose from uridine diphosphate glucose (Fig. 1). This enzyme has now been assigned the reverse role *in vivo*, synthesis of sucrose being believed to take place via sucrose phosphate[53]. Another case where reconsideration may be necessary is with the hydrolases. Many of these are now found to transfer to sugar hydroxyls as well as to water. Calculations show that the affinity of the enzyme for hydroxyls in the alternative receptors must be far greater than for water[54] and the possibility thus emerges that the *in vivo* function of such an enzyme may have nothing to do with hydrolysis.

There are some carbohydrases the function *in vivo* of which is still a matter for conjecture. Prominent among these are the amylases. β-Amylase occurs in plants in large amounts—animals seem not to require it—but no enzyme of comparable activity occurs along with the amylase to cope with its product of action, maltose. The enzyme occurs both where starch is abundant, in cereals, or absent, as in the soya bean. α-Amylases in the digestive tract are easily accounted for but what is the function of the small amounts of α-amylase that pervade most living tissue? Fig. 1 is a conventional representation of glycogen and starch metabolism and is incomplete, in that it offers no means of increasing or decreasing the total number of polysaccharide molecules. Possibly α-amylase functions in the glycogen-storage organs by multiplying the number of priming nuclei by hydrolysis. Given a supply of glucose, *D*-enzyme can do the same thing and can also assist in removing primer, as explained elsewhere[55]. It is worth noting also that Fig. 1 contains no means of creating primers where none existed. α-Amylase decreases the number of α-1,4-bonds, *D*-enzyme functions without changing their number and whereas phosphorylase and glycogen synthetase synthesize such bonds, they can only do so if preformed α-1,4-polymers are present. Is there perhaps in plants and animals a system to create the essential primer? Such systems are known in other organisms. Honey invertase acting on sucrose forms maltosylfructose and higher homologues[56], and *Neisseria meningitidis*[57] and beer lactobacilli[58] synthesize maltose from β-glucose 1-phosphate and glucose. Such a system, coupled with a transglycosylase like *D*-enzyme, could provide the necessary primer.

Beyond these studies of isolated enzymes must come attempts to integrate the enzymes into self-sufficient cycles operating *in vitro*. Though the advances in the glycogen and starch field have far outstripped those in any other field of polysaccharide biosynthesis, the goal of the multi-enzyme system may well become a practical objective only in the decade beyond this.

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OBITUARIES

Sir David Rivett, K.C.M.G., F.R.S.

With the death of Sir (Albert Cherbury) David Rivett in Sydney on April 1, there passed from the Australian scene a man who had contributed perhaps more than any other to the present healthy state of Australian science. Entrusted as Chief Executive Officer in 1927 with the management of the Council for Scientific and Industrial Research, Rivett and his colleagues contrived, in a country woefully weak in research, to create an atmosphere in which it could flourish.

The men they appointed and supported wholeheartedly did much to inspire the confidence of the people of Australia in the economic value of research. Rivett saw clearly from the outset that his scientists had to be free to tackle their problems on as fundamental a basis as was necessary for their solution. So the men who solved some of the outstanding problems of the farmer and the pastoralist also contributed to the development of science. Nowadays, basic research is a recognized prerogative of the Commonwealth Scientific and Industrial Research Organization, which continues to be known almost as well overseas through its contributions to science as it is within Australia for its long run of outstanding practical successes. To Rivett, first and foremost, should go the credit for this.

Rivett, while chairman of the Council for Scientific and Industrial Research during 1946–49, saw more clearly, and at an earlier date than most scientific leaders throughout the world, the troubles that come to a scientific research body from secrecy. He was persecuted by minor politicians for his belief that security and secrecy are not synonymous terms. But he never ceased to fight for the principle that scientific research needs freedom, and I believe that he thereby saved his beloved Council for Scientific and Industrial Research from lethal restrictive controls.

What sort of background produced this man for whom Australia had so great a need ? David Rivett was born in Tasmania in 1885, the son of a Congregationalist minister of large family and slender means. His secondary and tertiary education, mainly in the State of Victoria, had to be self-supporting. It culminated in a Rhodes scholarship which took him to Oxford for work under Sidgwick. In Stockholm, with Arrhenius, he became recognized as a physical chemist of great talent; his own notable contributions were mainly in phase rule studies and heterogeneous equilibria.

After munitions work in England he returned as a lecturer to the University of Melbourne. There he rejoined Sir David Orme Masson, who from their first meeting had had a boundless admiration for his abilities. It was inevitable that when Orme Masson resigned in 1924 Rivett should be invited to succeed his mentor. He filled the chair with distinction, but after only three years the call came to wider responsibilities with the Council of Scientific and Industrial Research; this he accepted.

Rivett was acknowledged a leader wherever he went. He was general president of the Royal Australian Chemical Institute in 1940 and 1949, president of the Australian and New Zealand Association for the Advancement of Science 1937–39, and president of the Society of Chemical Industry of Victoria in 1930 and 1931. A foundation member of the Australian Academy of Science, he was one of its first vice-presidents. The Australasian Institute of Mining and Metallurgy made him an honorary member in December 1943 for his services to the mining industry of Australia.

Well known abroad, he was president of the Society of Chemical Industry of London in 1949, and was a Fellow of the Royal Society from 1941. He was created K.C.M.G. in 1935, and held the honorary degrees of D.Sc.(Oxon.), D.Sc.(Manchester), and LL.D.(Australian National University).

Despite all his honours and distinctions, Rivett's greatest love was to visit one of his laboratories and to hear at first hand what the young research officers were doing. He was proud of the staff he had gathered, trusted its scientific ability implicitly, and fought for its freedom. No believer in the planning of science, he had the acumen to sense and oppose any impending restraint before it was openly formulated. Once one had gained his confidence he was a magnificent friend and backer; he believed in delegating responsibility and with it any credit that accrued, but in times of adversity he it was who wished to shoulder the blame.

Rivett married, in 1911, Stella Deakin, the daughter of a distinguished Australian statesman. She and their two sons survive him.

His friends throughout the world will deeply mourn his death. Their final thought of David Rivett will be of a man who gave magnificent service to the joint causes of science and humanity.

I. W. Wark

Prof. K. Koshtoyants

Prof. Kachatur Koshtoyants, whose death occurred on April 2, was a well-known figure among the physiologists of Moscow, the director of an active research institute and a stimulating and popular teacher in the University. Kachatur Sergeyevitsh Koshtoyants graduated in medicine in 1926, but his interests always lay in the field of comparative physiology, particularly that of the nervous system. During 1930–31 he had been to Holland to work in Prof. Jordan's laboratory, and five years later he was made head of the Department of Evolutionary Physiology in the Institute of Animal Morphology of the Academy of Sciences in Moscow. From 1943 onwards he was also professor of animal physiology in the University of Moscow. His more recent research dealt with neuro-muscular transmission from a comparative biochemical point of view, and he was the author of a comprehensive treatise on comparative physiology as well as of several monographs on the history of physiology in Russia. Physiologists who have visited his laboratory in Moscow will recall his kind hospitality as well as the range of his research interests, and will wish to express their sympathy to his colleagues in the University and the Academy.

Adrian

NEWS and VIEWS

Prof. Olaf Holtedahl, For.Mem.R.S.

Prof. Olaf Holtedahl, of the University of Oslo, recently elected a foreign member of the Royal Society, is best known for his geological researches on the Caledonian mountain chains of Norway, but he has also distinguished himself as an arctic and antarctic explorer, as a physiographer, and as a palæontologist. Born in 1885, he published his first paper while a student at Oslo in 1909, on Lower Palæozoic rocks of the classical Lake Mjøsa region nearby. Few cities in the world have the natural advantages of Oslo as a centre for studying geology, and no doubt inspired by the unusually fine sequence of Eocambrian–Palæozoic strata here exposed, he soon extended his researches to similar rocks in the northern district of Finnmark, much of which he mapped as a State geologist. A long list of publications during this period includes an outstanding palæontological monograph on the Strophomenidæ. In 1920 he was appointed professor of historical geology in Oslo, where he worked up the geological results of an earlier expedition to Novaya Zemlya, and a few years later he led the Lars Christensen expedition to Antarctica, afterwards editing two large volumes of reports. Attempts to correlate the geological structures of Norway and Britain gave rise to close contacts with British geologists, cemented during a Norwegian excursion which he conducted for the Geologists' Association in 1934 and resulting in his William Smith Lecture to the Geological Society of London, in 1951, on "The Structural History of Norway and its Relation to Great Britain". His two-volume survey of the geology of Norway, published in Norwegian in 1953, was written in simple form to encourage a local revival of geological studies, particularly in schools. A Russian translation appeared during 1957–58, and an abridged, re-written, version in English, with contributions from colleagues, was produced for the International Geological Congress last year.

Elmer Verner McCollum, For.Mem.R.S.

Prof. McCollum, also elected a foreign member of the Royal Society, is best known for his pioneer work on vitamins, which ranks in importance with that of Gowland Hopkins. Like Hopkins, he found that rats would not grow and thrive on 'purified' diets containing proteins, fats and carbohydrates in correct proportions. Unsuccessful attempts to make such diets life-sustaining by improving their palatability led him to the classical experiment (with M. Davis), published in 1913, showing that butter and the ether-soluble material in egg-yolk contained a growth-promoting substance lacking in olive oil and lard, which was designated "fat-soluble *A*". In 1915, McCollum discovered another growth factor, present in water-extracts of wheat germ and egg yolk, which was called "water-soluble *B*", and provisionally identified with Funk's "anti-beriberi vitamine". McCollum initiated the practice of assigning letters to the vitamins, which later proved somewhat unsatisfactory, but the combination of letters with Funk's new word gave the world a term with great popular appeal. Born in Kansas in 1879, McCollum has had a most productive scientific career. Originally trained in chemistry, he turned to biochemistry during an appointment in agricultural chemistry in Wisconsin. During 1917–46 he was professor of biochemistry in Johns Hopkins University in Baltimore, and afterwards professor emeritus. In addition to his investigations on vitamins, he has made remarkable observations on the effects of dietary deficiency of zinc and manganese. He was among the first to grasp the bearing of modern nutritional science on human welfare, and the earlier editions of his book, *The Newer Knowledge of Nutrition* (with Nina Simmonds), became the 'Bible' of a generation of nutrition workers. During recent years he has made scholarly studies of the history of the science of nutrition.

Academician A. N. Nesmeyanov, For.Mem.R.S.

Academician A. N. Nesmeyanov, another of the new foreign members of the Royal Society, has just retired from the presidency (see following paragraph) of the Academy of Sciences of the U.S.S.R. He has thus taken a leading part in the organization of science in his country. He is the director of the Organo-Element Research Institute of the Academy. He is eminent as an organic chemist, particularly for his development of the chemistry of organic molecules which contain a metal or some element outside the usual few which compose most organic chemical compounds. It is a striking fact that, in the first century of its rapid development, organic chemistry has used very few elements. With the whole Periodic Table to choose from, Academician Nesmeyanov has set its head towards a much more expansive programme in its second century. Outside his main field he has developed several fields of great importance, notably the telemerization reaction, with applications including the synthesis of ω-amino-acids, substitutions in the ferrocene nucleus, and organo-silicon chemistry, including telemerizations involving silicon.

New President of the U.S.S.R. Academy of Sciences: Academician M. Keldysh

Academician Mstislav Keldysh, a prominent mathematician, mechanical engineer and computer expert, has been elected as president of the U.S.S.R. Academy of Sciences. He succeeds Alexander Nesmeyanov, who has resigned at his own request, pointing out that he had held this administrative office for more than ten years. Nesmeyanov himself nominated Keldysh as his successor. Academician Keldysh graduated from the physics and mathematics faculty of the University of Moscow in 1931 at the age of twenty and won his doctorate in 1938. He worked for several years in the Central Institute of Aerodynamics. Theories he elaborated have led to effective methods of eliminating 'flutter' in wings and tail units of aircraft. His theories also resulted in new designs for hydro-foil ships. From 1932 he was first a reader, and later a professor, in the Moscow State University. In 1943 he became a corresponding member of the Academy of Sciences and in 1946 was elected a full member. In 1960 he became a vice-president of the Academy. In mathematics, Academician Keldysh has made major contributions to the development of the calculus, to computing techniques and to various aspects of automatic remote control.

Physics at Sheffield : Dr. R. A. Smith, C.B.E.

DR. R. A. SMITH, who has been appointed to the chair of physics at Sheffield in succession to Prof. W. Sucksmith, is at present head of the Physics Department, Royal Radar Establishment, Malvern. After graduating with first-class honours in mathematics and natural philosophy at Edinburgh in 1930 and with first-class honours in mathematics at Cambridge in 1932, he did research work in the Cavendish Laboratory for which he received the degree of doctor of philosophy in 1936. From then until he became lecturer in physics at the University of Reading in 1939 he was Carnegie Research Fellow in the University of St. Andrews. In 1939 he was invited to join the Telecommunications Research Establishment, becoming head of the Physics Department in 1945. The research programme of the Physics Department at Malvern covers a wide field including semi-conductor physics, low-temperature physics, infra-red spectroscopy, microwave physics, electronics and plasma physics. It includes a theoretical physics group and a mathematical group, the latter being specially interested in electronic digital computation. Dr. Smith has written a number of papers and books including *Radio Aids to Navigation* and *The Wave-mechanics of Crystalline Solids*. He was made C.B.E. in 1960. Dr. Smith's appointment as professor of physics will date from October 1, 1961, and he will succeed Prof. Sucksmith as head of the Physics Department when the latter retires in September 1962.

Director of the British Industrial Biological Research Association : Dr. Leon Golberg

DR. LEON GOLBERG has been appointed director of the recently formed British Industrial Biological Research Association (see *Nature*, 188, 1072; 1960). Dr. Golberg is at present medical research director of Benger Laboratories, Ltd., and it is expected that he will take up his appointment with the Association towards the end of the year. Born in Cyprus, Dr. Golberg was educated in South Africa, where he graduated in 1935 with honours in organic chemistry at the University of Witwatersrand. The following year he gained an M.Sc. in physical chemistry. He then came to the United Kingdom and for two years was a member of Sir Robert Robinson's research team at Oxford, carrying out investigations in organic chemistry. In May 1939 he returned to the University of Witwatersrand, and he remained in South Africa throughout the Second World War, working on a variety of nutritional problems at the biochemical laboratory (which he himself established) of the Union Defence Force at the South African Institute for Medical Research. After the War he returned to the United Kingdom and spent six years studying medicine at Emmanuel College, Cambridge, University College Hospital Medical School, London, and Addenbrooke's Hospital, Cambridge. After qualifying in 1951, he became senior lecturer in chemical pathology at the University of Manchester, and in 1955 medical research director of Benger Laboratories, Ltd.

Research Reactors for Universities and Technical Colleges

IN reply to a question in the House of Commons on May 9, Mr. D. Freeth, the Parliamentary Secretary for Science, said that the Government had approved in principle the provision of three nuclear reactors which would be available to universities and colleges of technology for teaching and research. One would be sited in Scotland and one in Lancashire, and it was proposed to site the third in the London area, where it would be available to the Imperial College of Science and Technology, and other institutions in London. Mr. Freeth was advised that the Atomic Energy Authority was not at present developing small and medium reactors, but was undertaking research in many fields which would be applicable both to small and large reactors. In a written answer on May 9, Mr. Freeth said that the Atomic Energy Authority planned to concentrate at Culham all its work on plasma physics and controlled thermonuclear reactions. The site was being developed in three stages, of which the first, including a large experimental hall, should be completed early in 1962, while the second stage, to be developed by mid-1963, included further provision of laboratories and a lecture theatre.

U.K. and U.S.S.R. Collaboration on Peaceful Uses of Atomic Energy

A FORMAL agreement providing collaboration on the peaceful uses of atomic energy between the U.S.S.R. State Committee of the Council of Ministers for the Utilization of Atomic Energy and the United Kingdom Atomic Energy Authority was drawn up, following a meeting of representatives of the two bodies, held at the headquarters of the Authority in London during May 15–16. Among the representatives, and signing the agreement on behalf of the two bodies, were Prof. V. S. Emelyanov, chairman of the State Committee, and Sir Roger Makins, chairman of the Authority. Under this agreement, the bodies concerned will organize conferences and visits to facilities in their respective countries by comparable groups of specialists from the other country; normally, these will last for periods of 1–2 weeks, and provide for visiting groups of 4–8 specialists. The Authority and State Committee will undertake reciprocal exchanges of unclassified reports concerned with the peaceful uses of atomic energy. Information of commercial value will be exchanged only at such times and on such commercial terms as may be agreed in each case. It is hoped that this agreement will enable the interchange of new scientific instruments and equipment, including radioisotopes and apparatus designed for their application in medicine, biological research and industry. The agreement will remain in force for a period of five years. Among the fields of collaboration envisaged in the agreement are : nuclear physics ; solid-state physics; basic radiation chemistry; inorganic chemistry of elements of importance in nuclear science and technology; research on isotopes and research on the application of isotopes; plasma physics; high-energy physics; reactor physics; experimental fast reactors.

Conservation of Wild Life

A RECENT issue of *The Bulletin of the International Union for Conservation of Nature and Natural Resources* (9, No. 7, 3; 1960) outlines the efforts being made in various countries, especially Thailand and the

Netherlands, to promote the objectives of nature conservation. In Thailand the game law is being revised according to the experience of game laws in other countries, and the forest law, too, is also being examined since it is ineffective in preventing destruction; setting aside of the primitive regions as natural reserves and national parks is also under consideration. In the national budget of the Netherlands for 1961 a sum of about 2·2 million dollars has been earmarked for nature protection (as compared with the sum of 1·2 million dollars for 1959). 1·3 million dollars is intended for the purchase of reserves and 300,000 dollars for their management, figures which compare with 630,000 dollars and 210,000 dollars respectively for 1959. In addition, the personnel of the Institute of Ecological Research for Nature Protection has been increased from 16, in 1959, to 21.

The City Museum, Sheffield

The combined annual reports of the City Museum, Sheffield, for the two years 1958–60 form an inspiring record of progress and initiative by a relatively small staff (Pp. 20+4 plates. Sheffield: City Museum, 1961). The theme of service to the community underlies the work of this Museum. Prominent among its activities is that of evening opening in the summer until 8.30 p.m. and the organization of more than a hundred demonstrators at the Unesco exhibition, "The Earth as a Planet". New displays include Saxon jewellery and metal-work, medieval pottery and sculpture, British bats, and one illustrating the variety and forms in extinct animals.

South African Prehistory

The January and February issues of the *South African Journal of Science* contain articles of considerable interest to archæologists. R. J. Mason discusses the problem of the earliest tool-makers in South Africa, P. V. Tobias brings forward new evidence and new views on the evolution of man, and Gordon Cramb continues his report on the excavations undertaken at the Holley shelter near Wartburg, Natal—the first report appeared in the same journal as long ago as 1952. Mason's conclusions are certainly very interesting in view of the human finds of very early date in the region. He says that up to now in South Africa there is no evidence for stone tool-making earlier than the complex earlier Acheulean stage, which is possibly equivalent to Olduvai Gorge Bed 2. There seems to be evidence at the limeworks site for the use of bone for tool-making purposes at an earlier date, but at present no stone implements have been found with them. Tobias does not agree with Dr. Wells, who has suggested that the basic human type in South Africa was possibly proto-sapiens. He claims that an early proto-sapiens such as that found at Kanam in Kenya is not early Pleistocene but Middle Pleistocene in date. He argues that the 'Rhodesioids' are derived from the African Middle Pleistocene hominids, and, in turn, these later gave rise to the Boskopoid and Bush types. Tobias also discusses the roles of cultural and natural selection in South African human evolution. A very extensive bibliography is appended. The Holley rock-shelter site has yielded artefacts belonging to the later Stone Ages from the upper layers. Below them a series of Middle Stone Age tools has been unearthed. From both these levels carbon samples were collected and these were analysed at the British Museum. The results would seem to be: 18-in.–24-in. layer 4,490 years ± 150 years, and 24-in.–30-in. layer 18,200 years ± 500 years.

Instrumentation in Industry

As a means of increasing the application of scientific method to industry, the Department of Scientific and Industrial Research has produced another of its popular booklets. This one, *Instruments in the Factory*, has been written by Dr. J. Thomson, director of the British Scientific Instrument Research Association, and shows that, despite the increasing use of instruments in modern manufacturing processes, there is still scope for improving instrumentation in most industries (Pp. 12. London: Department of Scientific and Industrial Research. 1961). Thermostatic control of water temperature is an example of how instrumentation can be extended. The smoke detector is another; its usefulness is easy to recognize, and the absence of smoke is a good indication of the efficient use of fuel. Another example occurs in flow measurement: often, particularly in smaller firms, accurate measurement of the flow of liquids in manufacturing processes can save a large amount of money. Simple robust methods are available. In the same way the 'thickness' of a liquid can also be measured with advantage. Manufacturers who would like to pass from 'instrumentation' to 'automation' will find from a study of this booklet that automatic control can often take very simple forms; instrumentation and automation can be used to improve products.

Hyperon Stars

The extension of V. A. Ambartzumian's hypothesis of stellar evolution led G. S. Saakyan (*Priroda*, 11, 14; 1960) to develop a new hypothesis of stellar evolution based on the findings of certain recent discoveries of new elementary particles. According to this hypothesis dense stars of the type of 'white dwarfs' on further contraction become neutron stars. During this process their density changes from approximately 10^4 gm./cm.^{3} to about 10^8 gm./cm.^{3}. With a further increase of density to 10^{15} gm./cm.^{3} these stars become hyperon stars representing a more stable state under such conditions. The mass of such hyperon stars in equilibrium is approximately the same as that of the Sun, while the size of their radius is of the order of a few kilometres. Only the central part of such stars contains hyperons. The outer zone is made of neutrons, while the outermost part is made of bare nuclei and electrons. The enormous pressure inside such stars is balanced by the force of gravity, but when such an equilibrium is disturbed hyperon stars are liable to explode with a terrific violence, not unlike the explosions leading to the formation of the 'nova' stars.

Scientific Information Notes

Scientific Information Notes for February–March 1961, issued by the National Science Foundation, notes that the Foundation is supporting a two-year investigation of the line-formula notation for representing chemical structural formulæ as linear arrays of symbols. This is part of an extensive study of chemical notation systems. The Foundation is continuing to support the Harvard University project for research on automatic translations and mathematical linguistics. It is also giving partial support to the abstracting office of the International Federation for Documentation, which is developing a world inventory of scientific abstracting services, and establishing an international clearing house for information on abstracting services and techniques. The Federation has also recommended the formation of a Group on Mechanical Storage and Retrieval; the Ford Foundation has announced a grant of 8 million dollars to the Council on Library Resources, Inc., for continued research to improve library methods, with emphasis on ways of storing and finding information in the library of the future.

Peasant Agriculture in the Tropics

A VALUABLE review of different systems of peasant agriculture in various parts of the tropics is made by E. W. Momber, School of Agriculture, University of Cambridge, in a recent issue of *Rural Life* (6, No. 1; 1961). Momber analyses existing practices in Northern Rhodesia, Tanganyika and Nigeria and shows that nearly all peasant systems were evolved to carry a subsistence economy where there was plenty of land and few people, and that the traditional systems of peasant agriculture are not basically unsound for the purpose for which they were designed. To-day they are being asked to perform tasks which they were not designed to carry out.

Sheep Husbandry in Northern Ireland

A FEATURE of farming in Northern Ireland during recent years has been, says the Ministry of Agriculture of Northern Ireland (Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 47 : Sheep Husbandry. Pp. 26. Belfast: Ministry of Agriculture, 1961), an increase of the number of sheep reared from 654,000 in 1945 to 1,097,000 in 1960. Important factors in this development have been the improvement in pasture manuring and management and the relatively high cost of store cattle. The leaflet discusses breeds of sheep, the types and sizes of sheep flocks, the mating, management and diseases of sheep and the handling and care of wool. It is written in clear, non-technical language and is certainly a very good exchange for a postcard sent to Belfast.

Damage to Maize in Nigeria

EXAMINATION of samples of maize obtained from market traders at Ibadan, Nigeria, over a period of fourteen months by G. H. Caswell of the Department of Agriculture, University College, Ibadan, Nigeria, showed that damage to maize by *Calandra oryzae*, L., the only important pest of stored maize in this region, is slight compared with damage by the same insect in other countries (*J. West Africa Sci. Assoc.*, 6, No. 1, February 1961). This is probably due to the fact that farmers use a system of shifting agriculture, and store maize on their farms where *Calandra* is not endemic. Should the practice of central storage become more common, damage is likely to increase.

The Institution of Metallurgists

AT the seventeenth annual general meeting of the Institution, held in London on May 17, Dr. N. P. Allen, superintendent of the Metallurgy Division, National Physical Laboratory, was elected president for the year 1961–62, in succession to Mr. W. E. Bardgett. The following elections were also made: *Senior Vice-President*, Dr. E. G. West, technical director, Aluminium Development Association; *Vice-Presidents*, F. Dickinson, director and general manager of the Development and Research Department, International Nickel Co. (Mond), Ltd., and Dr. L. Northcott, principal superintendent, Materials Division, Armament Research and Development Establishment, Fort Halstead (War Office); *Honorary Treasurer*, L. W. Derry, head of the Metallurgy Department, Battersea College of Technology; *Members of Council*, H. Morrogh, director, British Cast Iron Research Association, Alvechurch, Birmingham, S. S. Smith, research manager, Imperial Chemical Industries, Ltd., Metals Division, Kynoch Works, Witton, Birmingham, and Dr. J. C. Wright, reader in Industrial Metallurgy, Birmingham College of Advanced Technology.

The Linnean Society of London

AT the anniversary meeting of the Linnean Society of London, held on May 24, the following were elected officers for the session 1961–62: *President*, Prof. Thomas Maxwell Harris; *Treasurer*, the Earl of Cranbrook; *Secretaries*, Dr. C. R. Metcalfe (Botany), Dr. H. G. Vevers (Zoology), Dr. John Smart (Editorial); *Vice-Presidents* appointed were: Prof. A. R. Clapham, Prof. C. F. A. Pantin, the Earl of Cranbrook and Dr. W. T. Stearn. *New Members of Council* elected : Mr. P. R. Bell, Mr. J. B. Gillett, Prof. Tom Harris, Dr. D. M. Kermack and Dr. Sydney Smith. *Linnean Gold Medals* were presented to Mr. Edmund William Mason and Dr. Frederick Stratten Russell. Dr. Mary Agnes Chase, of the United States, and Dr. Franz Petrak, of Austria, were elected foreign members.

The H. G. Wells Society

THE H. G. Wells Society has recently been founded to promote and encourage an active interest in, and an appreciation of, the life and work of one who made such great contributions in many directions during the first half of this century. Educated as a scientist, he inspired persons of all persuasions to take nothing for granted and to examine fundamental principles in all activities, particularly Government, history, planning, possibilities of science and its potential assistance to mankind. The new Society is non-political and non-sectarian, and seeks only to find that which is good in the thought, word and deed of a great man and which can be used as a memorial to one who filled a great gap in human thought. Further information can be obtained from the hon. secretary, Mr. J. R. Hammond, 39 Rugby Road, West Bridgford, Nottingham.

The Royal College of Surgeons of England

A GRANT of £250,000 has been made to the Royal College of Surgeons of England by the Wolfson Foundation. The gift will be applied primarily to the provision, furnishing and equipment of the Hunterian Museum. The Museum—now being rebuilt following its destruction by bombing—is expected to be finished by the spring of 1962, and is to house the collection of anatomical and pathological specimens collected by John Hunter and purchased by the Government of the day, after his death in 1793.

College of Advanced Technology, Birmingham

IN support of the College policy to promote and develop research activities, Messrs. Joseph Lucas, Ltd., Birmingham, have established a fellowship, to be known as the Joseph Lucas research fellowship, in production engineering which will be tenable normally for a period of two years with provision for extension to three years, and valued at £1,300–£1,500 per annum.

University News : Cambridge

The following appointments of overseas fellowships at Churchill College have been made: Prof. A. Schlüter of the Max Planck Institute, Munich, during March–September, 1961; Prof. G. Fodor of the Hungarian Academy of Sciences during April–July, 1961.

Leeds

The following appointments have been announced: Dr. A. E. Guile as senior lecturer, and J. M. Stephenson as lecturer, in the Department of Electrical Engineering; Dr. D. H. Niblett as lecturer in the Department of Physics; Dr. T. A. Scott as lecturer in the Department of Biochemistry; D. J. Harberd as lecturer in the Agricultural Section of the Department of Agriculture.

Liverpool

The following appointments have been announced. *Readerships:* Dr. R. S. Benson (mechanical engineering); J. O. L. King (animal husbandry); Dr. J. H. Leck (electronic engineering); Dr. R. B. Whittington (civil engineering); Dr. A. Young (numerical analysis). *Senior Lecturers:* J. W. Dransfield (veterinary anatomy); Dr. W. H. Evans (physics); Dr. D. Flinn (geology); J. Halling (mechanical engineering); Dr. D. Hull (metallurgy); Dr. J. W. Lynn (electric power engineering); Dr. W. R. H. F. Muirhead (physics); Dr. N. Rast (geology); Dr. J. P. Riley (oceanography); Dr. C. F. H. Tipper (inorganic and physical chemistry); Dr. H. Wilson (pharmacology).

London

Dr. M. G. Fleming, reader in mineral technology in the Imperial College of Science and Technology, has been appointed to the University chair of mineral technology tenable at that College. Dr. O. J. Lewis, senior lecturer at St. Bartholomew's Hospital Medical College, has been appointed to the University readership in anatomy tenable at that College Dr. R. F. Strickland-Constable, senior lecturer at the Imperial College of Science and Technology, has been appointed to the University readership in chemical engineering tenable at that College.

Titles have been conferred on the following: Dr. Alexander Lawson (professor of organic chemistry), in respect of his post at the Royal Free Hospital School of Medicine; Mr. Ralph Shackman (professor of urology), in respect of his post at the Postgraduate Medical School of London; Dr. E. G. C. Clarke (reader in chemistry), in respect of his post at the Royal Veterinary College.

Announcements

Mr. L. A. Thomas has been appointed chief physicist at the Hirst Research Centre of The General Electric Co., Ltd., Wembley, Middlesex. He will be a member of the staff of the Central Research Laboratories with special interest in the programmes of long-term and fundamental research. He was appointed head of the Materials and Components Division in 1960 and retains his responsibilities in this field of the work of the Centre.

The annual general meeting of the Royal Society of Tropical Medicine and Hygiene will be held in Manson House, 26 Portland Place, London, W.1, on June 15 at 7.30 p.m. The new president, Sir George McRobert, will be inducted in succession to Sir William MacArthur. The Chalmers Medal will be presented to Dr. C. E. Gordon Smith.

The third International Congress on Catalysis is to be held in Amsterdam during July 20–25, 1964. The theme of the Congress will be "The Mechanism of Heterogeneous Catalysis". Further information can be obtained from Dr. D. M. Brouwer, c/o Badhuisweg 3, P.O. Box 3003, Amsterdam-N, Netherlands.

The sixth International Hæmatology Symposium will be held at the State Agricultural College, University of Ghent, during July 24–28. Further details can be obtained from Prof. J. Van den Brande, Rijkslandbouwhogeschool, Coupure Links, 235, Ghent (Belgium).

The annual general meeting of the Freshwater Biological Association will be held in the meeting room of the Zoological Society of London, Regent's Park, London, N.W.1, on June 20. Further information can be obtained from the Director, Freshwater Biological Association, Windermere Laboratory, The Ferry House, Far Sawrey, Ambleside, Westmorland.

The British National Committee for Physics is organizing a conference on "Optical Instruments and Techniques", under the auspices of the International Commission for Optics, in London during July 10–14. Further information can be obtained from Mr. K. J. Habell, National Physical Laboratory, Teddington, Middlesex.

The Building Research Station of the Department of Scientific and Industrial Research is holding open days for the new Materials Laboratory during July 10–13. Further information can be obtained from the Director, Building Research Station, Bucknalls Lane, Garston, Watford, Herts.

The Institute of Physics and the Physical Society is organizing a conference on "Thermoelectricity" at Durham Colleges, University of Durham, during July 10–12. Among the topics to be considered are: basic properties of thermoelectric materials, solid and liquid; methods of determining electrical conductivity, thermal conductivity, and thermoelectric power; properties of materials, problems and techniques associated with device developments; thermoelectric devices and their applications. Further information can be obtained from Prof. D. A. Wright, Department of Applied Physics, Durham Colleges, Durham.

The B.B.C. and the Council for Nature announce that they are organizing a competition for amateur cine-photographers for natural history films suitable for showing on television (35 mm. and 16 mm. at 24 frames per sec.). The closing date is February 28, 1963. Further information can be obtained from the Council for Nature, 41 Queen's Gate, London, S.W.7.

The Trustees of the Plastics Industry Education Fund invite, from young people working in the plastics industry, applications for a limited number of training grants towards the cost of full-time study on an approved course leading to a degree or diploma in science, technology or industrial design. Applications must be submitted to the Secretary, Plastics Industry Education Fund, 6 Mandeville Place, London, W.1, by July 15.

The article entitled "Measuring Indigestion", which appeared in the May 13 issue of *Nature* (p. 596), refers to an original paper appearing in *Research in Industry*, published by Stanford University.

UNIVERSITY COLLEGE OF SWANSEA

NEW CHEMISTRY LABORATORIES

NEW chemistry laboratories were opened at University College, Swansea, on May 1 by Sir Alexander Todd. When, in 1920, a department of chemistry was established by J. E. Coates in Swansea, accommodation was provided in temporary buildings the useful life of which was expected to be ten years. In fact, the Chemistry Department has occupied this accommodation until this year, when the new laboratories became available.

The building consists of three major units: a north wing with teaching laboratories; a south wing with research laboratories; and a central spine which contains the lecture theatres, main stores and central services. Accommodation is on three floors, with a total area of 66,000 sq. ft.

The three floors of the teaching wing are essentially the same. Each consists of one general and one honours laboratory accommodating 60 and 48 students respectively, and built on either side of common preparation and balance rooms. The ground-floor honours laboratory has special facilities for organic chemistry; the first and second floor honours laboratories are specially designed for physical and inorganic chemistry respectively. In the general laboratories, each working space is provided with two sets of lockers so that a total of 360 elementary students can be accommodated during one session.

The research wing contains principally staff rooms and research laboratories. The ground-floor is devoted to organic chemistry and has six staff rooms facing south and two six-man research laboratories with an interconnecting service laboratory facing north. The arrangements on the first floor (physical chemistry) and the second floor (inorganic chemistry) are similar. This wing also contains professors' suites, a departmental library with book-stack, microfilm room and reading room, and a seminar room.

There are a number of general facilities for both teaching and research in the central spine. These include two lecture theatres, both stepped, windowless and mechanically ventilated, with seating for 320 students in the larger theatre and 105 students in the smaller one; at second-floor level above the large lecture theatre is a combined lecture theatre and examination room seating 120 students; there are three main stores directly above each other in the central spine and connected by a goods lift. In addition, in the central spine, there is an engineering workshop, an instrument room and separate workshops for glass-blowing and for electronics. A semi-technical area is situated under the small lecture theatre and is equipped for operations such as fractionation and solvent extraction on a pilot plant scale. A number of rooms with special facilities open off the semi-technical area: a cold room; a walk-in fume cupboard; a fire and flood laboratory; a centrifuge room and a constant-temperature room. The first floor of the central spine included a microchemistry laboratory, dark rooms and special laboratories for infra-red and Raman spectroscopy. On the second floor are a radio chemistry laboratory, special instrumentation rooms and a liquid-air plant.

Benches in the laboratories have been designed to give flexibility and ease of access to services for

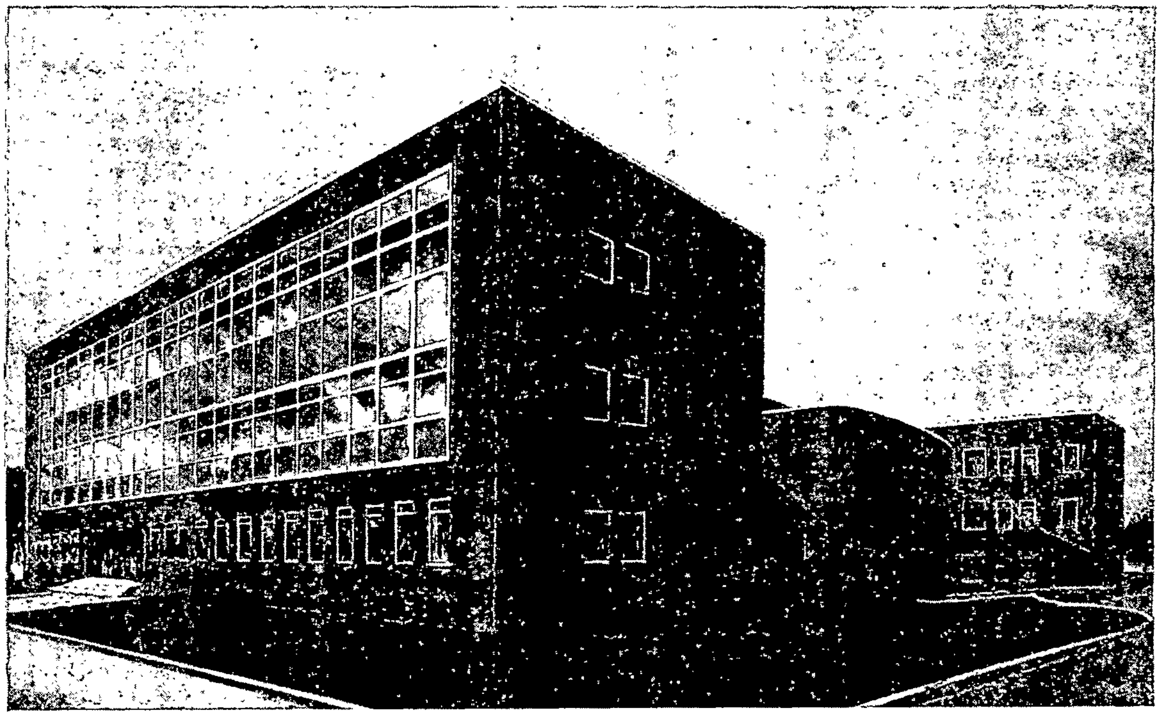

Fig. 1. New Chemistry Laboratories, University College of Swansea

maintenance. Most of the benches, both of the island and wall type, have tops supported by cantilever brackets which fit into specially designed service stands fixed to the walls and floor. Underbench cupboard and drawer units then slide into a position below the bench tops and conceal the service racks. In the research laboratories the tops can be fixed at either standing or sitting height, or can be removed entirely leaving only the stands and associated services. The service racks are formed by a number of metal supports spaced at approximately 3-ft. centres and connected at bench-level by a 9-in. wide horizontal teak strip and a 6-in. deep teak facia. Curved brackets are fixed to the metal strands which support the service pipes, from which outlets are taken at suitable positions. Where a concentration of service is required, plumbing has been simplified by the use of service towers, which results in a clear bench top and a generally neater appearance. In the physical chemistry research laboratories the bench tops and cupboards form movable integral units. All services are available along the walls, with electrical services also available from ceiling outlets. This gives great flexibility in the arrangement of the laboratories.

Bench tops are of Burma teak (*Tectona grandis*); all other exposed timber and veneers are of West African mahogany. Teak has also been used throughout as flooring in laboratories. Reagent shelves have been covered with white 'Vitrolite' and the working tops of fume cupboards formed from a sheet of armour-plated glass.

The number of students taking courses in chemistry now exceeds 250, including 37 honours students and 26 research students and fellows. These numbers are expected to increase considerably in the next five years, during which period the College is planning to increase the number of students from 1,500 to 3,000.

The new facilities make possible very substantial expansion of the research programme in Swansea. As it is, the work of the Department covers a wide range of interest. There is a group working with D. A. Long in the field of molecular spectroscopy with special emphasis on Raman studies. Other groups are concerned with the infra-red spectroscopy of amides (H. E. Hallam *et al.*), the kinetics of nitrosation reactions (G. Stedman *et al.*) and solid-state chemistry (K. J. Gallagher *et al.*). The organic chemists are largely concerned with natural products. This work, which is under the general direction of Prof. C. H. Hassall and Dr. R. F. Curtis, involves work on peptides, cardenolides and alkaloids. A major investigation, dealing with the pathways of biosynthesis of complex phenolic compounds, combines techniques of organic chemistry with others drawn from the relatively young field of chemical genetics. There are also investigations in the field of steroid chemistry (G. H. R. Summers *et al.*) with particular emphasis on reaction mechanisms.

SCIENCE AND INDUSTRY—THE PROBLEM OF COMMUNICATION

A CONFERENCE, organized by the Department of Scientific and Industrial Research and held in Swansea during April 18–19, had as its primary object the critical examination of present-day methods of disseminating scientific and technical knowledge. Both the papers and the lively discussions which followed indicated the growing concern in scientific and industrial circles with the many-sided nature of this problem.

Opening the conference, Lord Brecon, Minister of State for Welsh Affairs, emphasized the relation between the growth of scientific knowledge, the resultant industrial innovation and the growth of industry. His reference to the time-lag between technical advance and industrial use of new techniques and new materials was echoed by several speakers.

Nine papers were briefly presented to enable delegates themselves to participate in the frank and critical appraisal of the problems and solutions as presented. The first paper, by W. S. Robertson, chief executive of the Scottish Council for Development and Industry, reminded delegates that as the volume of available information multiplied there was a proportionate increase in the difficulty of condensing and making that information effectively available to those who could use it. The answer to the increasing volume of literature, it was suggested, might lie in the development of mechanized recording and retrieval systems, but Mr. Robertson maintained that there was still nothing quite so effective as personal contact for passing on new ideas and know-how. He thought that scientific and technical, as well as commercial, people should be encouraged to travel more widely and more frequently, and he condemned the inhibiting parochial attitude of many firms which restricted the development of this most useful means of exchanging ideas.

Dr. D. J. Urquhart, director of the National Lending Library for Science and Technology, also emphasized personal contact, and likened the seeker after scientific information to the stranger in a vast, growing, changing city, the maps of which became out of date almost as soon as they were published. The best and almost only way of reaching an objective quickly was to ask other people working in the specified field. Dr. Urquhart stressed the assistance that libraries and information scientists could provide in making these contacts and warned the conference that existing facilities were inadequate. Quoting Wales as an example, he pointed out that there were only two public libraries with a reasonable holding of scientific periodical literature. The educational aspects of the problem were discussed in the paper by Dr. M. R. Hopkins, senior lecturer in physics at Swansea. He criticized the attitude of narrow utilitarianism which leads to a programme of teaching preoccupied with its immediate purpose. He suggested that the training of every educated man and woman should include some teaching of the fundamental principles of scientific thought, and that both scientists and non-scientists should be educated in the basic skills of communication. Speakers from both platform and floor supported this view; it was pointed out that not one university department specializing in documentation studies existed in Britain. A large increase in the British research effort in this field and the allied one of information retrieval was called for. Following Dr. Hopkins several speakers demanded better facilities for documentation research and indicated how poorly this field was supported in Britain. Dr. R. M. Lodge, research manager of British Nylon Spinners, Ltd., spoke on the communication network in an industrial organization and indicated the difficulties of presenting technical information at many different levels within the firm.

The mass media of communication—press, radio, television, films, exhibitions and conferences—were each dealt with in turn by acknowledged experts. It was generally accepted that the primary value of radio, television and films was in creating a healthy climate of opinion to encourage scientific and technical development. Dr. T. Margerison, science editor of the *Sunday Times*, felt that more use could be made of television by the inclusion of news reports of scientific and industrial developments in magazine-type programmes, and the possibility of implanting the germ of an idea in this way should not be overlooked. Mr. P. Cudlipp, editor of the *New Scientist*, reviewing the function of the general science journal, stressed the value of scientists and technologists keeping themselves broadly informed on a wide range of scientific disciplines. He referred also to the need for scientists themselves to take a hand in the preparation of material that the layman could understand, and noted the growing number of science correspondents in the popular press—which was a recognition of the public interest in science. A better balance and more careful reporting of science news had been achieved, but there was still room for improvement. Exhibitions and conferences were dealt with by Mr. E. H. Underwood, the director of public relations for the U.K. Atomic Energy Authority, who stressed the importance of defining objectives and the audience aimed at, but once again, however, personal contact was cited as being very often the most valuable result of attending such functions. Mr. E. Anstey, of the British Transport Commission, and president of the Scientific Film Association, spoke on "Films to Illuminate and Persuade", directing attention to the use of film as a tool not only for spreading scientific knowledge but also for the popular understanding of science.

In a paper by Mr. L. W. Stevens-Wilson, head of the Steel Users Section, British Iron and Steel Research Association, attention was directed to the need for closer collaboration between specialist or vertically oriented technical information services and horizontal or regionally based services. The Department of Scientific and Industrial Research was strongly recommended to set up more branch offices, similar to those existing in Cardiff and Edinburgh.

The widely differing views of delegates and the relative value of the different methods of communicating information made it clear that there is no one ideal solution to the problem. It would appear that there is still need to strengthen all existing techniques. There was general agreement that in many sectors of industry the prevailing level of interest in research and development was not very high, and that the outstanding difficulty was not simply to improve the means of transmitting scientific and technical information but to stimulate interest at the receiving end in industry. How this was to be achieved remained largely an open question, but it must involve a strong element of personal contact to make an effective change in the habits of thought and climate of opinion in both industry and science.

E. E. WILLIAMS

AN INTERNATIONAL ANDEAN YEAR

ON the initiative of Unesco and by invitation of Dr. Angel Establier, head of the Scientific Office in Montevideo of that Organization in Latin America, who has been interested in such a project from its beginning, a meeting was held in Buenos Aires, during April 3–4, to discuss the planning of an International Andean Year. During such a programme, an intensive study would be carried out on the range of mountains known as the Cordillera de los Andes, from Panama to the southern part of the continent. A small number of scientists from different countries interested were present: Dr. H. O'Reilly Sternberg (Brazil); Dr. Guillermo Mann (Chile); Dr. Tobías Lasser (Venezuela); Dr. Ovidio Suarez (Bolivia); Eng. Eduardo Baglietto, General M. Olascoaga and Drs. F. Bonorino Udaondo, J. Roederer, Angel Cabrera, M. Sadosky, B. A. Houssay and V. Deulofeu, all from Argentina. Dr. A. Hurtado, from Peru, unfortunately could not attend. Dr. A. Establier represented Unesco and his office will be the centre for the various groups in preparing the project.

After an introductory welcome by the vice-dean of the Faculty of Exact and Natural Sciences at Buenos Aires, Dr. M. Sadosky, Dr. Venancio Deulofeu was elected chairman and Dr. Establier acted as advisory secretary. Four sessions were held.

All present agreed in considering that the project to institute an International Andean Year will be of great importance not only because of its width of interest and of the scientific results which are likely to accrue, but also because of the number of scientists involved and the expected international participation from countries outside South America. It was also considered that a project of this magnitude will have a favourable impact on the progress of science and the future scientific development of most of the South American countries.

Discussions dealt with the fields which it would be desirable to cover during the International Andean Year. As a preliminary list it was considered possible and convenient to work on the following subjects: 1, geology; 2, botany; 3, geophysics; 4, human biology; 5, zoology; 6, human ecology; 7, geomorphology including glaciology; 8, physics of high altitudes; 9, soil sciences; 10, geography; 11, agronomic sciences (in collaboration with the Food and Agriculture Organization); 12, sociology; 13, ethnography (both 12 and 13 in collaboration with institutes of social sciences); 14, archæology; 15, anthropology; 16, economics (in collaboration with the Comisión Económica para América Latina).

In each of these fields some subdivisions have already been made and, as an example of the type of work which would be involved, some specific projects were mentioned. For example, it was suggested that in studying the geology of the Andes, it would be desirable to concentrate the efforts in examining the evolution of the Andean range, especially the tectonic and magmatic cycles of the Andean syncline, tertiary and quaternary vulcanism and the magmatic provinces in the Andes.

A further meeting is being planned for December 1961, and will be held in Santiago de Chile, to consider reports to be presented by the scientists participating in the meeting in Buenos Aires and to elaborate further and develop the specific projects in each scientific field.

In the meantime, the Unesco Scientific Office in Montevideo will start compiling a bibliography of research done on the Andes, and collecting the names of scientists who have been active in that field, or who, by their training, are likely to be interested in participating in any of the activities planned for the International Andean Year.

Further information on this project can be obtained from Dr. A. Establier, Centro de Cooperaciôn Científica de la Unesco para América Latina, Boulevard Artigas 1320, Montevideo, Uruguay.

V. DEULOFEU

SOLAR VARIATIONS, CLIMATIC CHANGE AND RELATED GEOPHYSICAL PROBLEMS

UNDER the above title, a symposium was arranged by the New York Academy of Sciences, through the Academy's Section of Geological Sciences and its Division of Oceanography and Meteorology, in co-operation with the American Meteorological Society. The meetings were held in New York during January 24–28. The papers given at the symposium are to be published by the Academy in a forthcoming volume of its *Annals*.

Understandably, the United States provided about four-fifths of the sixty or so participants who offered papers or contributed to the discussions, but a number of workers active in the fields covered by the symposium were present from Canada, the United Kingdom, Holland, Germany and Hungary. Swedish, Russian and Australian contributions had been invited, but their authors were unable to be present. Abstracts of the papers were provided and the meeting as a whole was admirably organized. The generous hospitality of the New York Academy of Sciences was indeed a feature.

The organization of the conference and the arrangements for overseas visitors were largely in the hands of Dr. Rhodes W. Fairbridge, professor of geology at Columbia University, to whom indeed a great part of the success of the meeting was due. Dr. Fairbridge's arguments for the relationship of changes of sea-level, great and small, to the fluctuations of climate which have affected and continue to affect the volume of the Earth's ice-sheets and glaciers, have become widely known (cf. his recent article in *Scientific American*, 202, No. 5, 70; 1960). A fuller statement has now been published in *Physics and Chemistry of the Earth*, 4 (Pergamon Press, 1961). Developing as they do from his earlier work on the eustatic changes shown on the coast of West Australia, they integrate a vast amount of carefully assembled field evidence on a world-wide scale and, cautiously yet persuasively presented, they will be found acceptable by many workers. Nevertheless, it can be agreed that we do not yet know enough about the other agencies that may have affected the volume of the hollows in which the oceans lie.

Climatic change is demonstrably a subject with exceptional ramifications, and the contributions of a great many scientists from differing disciplines are repeatedly called for. Accordingly, the scope of the symposium was enormously wide, beginning with six papers on solar physics under the chairmanship of Dr. Donald H. Menzel (Harvard University). Climatic effects potentially attributable to solar variation were discussed under the chairmanship of Dr. Sigmund Fritz, replacing Dr. H. Wexler, who could not be present. It is evident that the only effects of solar variation that are so far generally accepted are those observable in the extreme ultra-violet end of the solar spectrum, affecting the high atmosphere and the Earth's magnetic field, although some of the apparent relationships with atmospheric circulation patterns are sufficiently suggestive to justify further attention. Among a variety of papers on various aspects of the radiation balance and the general circulation, H. H. Lamb (Meteorological Office, Harrow) presented some of his recent work on displacements of the circulation pattern (cf. *Geografiska Annaler*, 1960). A further session was devoted to meteorological satellites. Dr. Helmut E. Landsberg (United States Weather Bureau) took the chair at a session entitled "Glaciology, Oceanography and Climatology" ; here Dr. William L. Donn presented a summary of the Ewing - Donn theory of the ocean control of Quaternary climate (*Science*, 1958), unfortunately with insufficient time for discussion of a controversial topic in which many would gladly have joined. A preceding paper by Dr. T. N. V. Karlstrom (Coast and Geodetic Survey, Washington) on the glacial history of Alaska provided evidence manifestly difficult to reconcile.

With Dr. R. F. Flint (Yale University) in the chair, followed by Dr. E. S. Deevey (Yale University), palæoclimatology came under review, including several papers around the theme of palynology. Dr. E. H. Willis (University of Cambridge) commented on some of the Fenland evidence. Among the most interesting contributions was that of Dr. Calvin Heusser (American Geographical Society), who gave evidence for the approximate contemporaneity of Late- and Post-Glacial events in the Rockies and the Southern Andes. With Prof. F. E. Zeuner (University of London) in the chair, a final session was devoted to papers around the theme of "Climatic Change and Man". Archæological studies in the United States and in the Near East were noteworthy; Dr. Ralph Solecki's discussion of some of the preliminary findings at the Shanidar cave in Northern Iraq was of great interest, and attracted attention.

It will be evident from this brief summary that a remarkable diversity of papers came up for discussion. Throughout the symposium the impression prevailed that there are many who are still hopeful that some kind of periodicity will appear, based on such solar manifestations as we can observe. From others, dispiriting criticisms equally abound. Dr. Reid Bryson (University of Wisconsin), for example, has applied modern statistical methods to the analysis of tree-ring spectra and found that the usual sunspot cycles fail to appear. On the other hand, several contributors provided support for relationships between solar variations and the pattern taken by the general circulation.

It became abundantly clear how large a number of investigators are patiently accumulating evidence of the amplitude, character, effects and especially the dating of climatic fluctuations all over the world. Speculations regarding the causes abound; supporters of each of the popular theories—solar variation, atmospheric turbidity, carbon dioxide, ozone, variations in the Earth's orbital elements—find their several gods alternately set up and cast down. Workers in one field find themselves unable to judge the validity of the evidence from other disciplines; in this regard, the sessions and the informal discussion resulting from personal encounters between different kinds of specialists are for many a real help. A great deal of enjoyment is had by all, and those who are tempted to advocate simple explanations are repeatedly reminded of the complexity of the problems they so hopefully seek to solve. It now seems almost certain that no one simple panacea is in any way adequate to explain the intriguing patterns of climatic fluctuation to which the evidence points. Almost all the many processes involved seem likely to play some essential part, small or large. The forthcoming volume of papers will provide welcome reading.

GORDON MANLEY

EXPERIMENTAL TAXONOMY OF FLOWERING PLANTS

A SYMPOSIUM on the experimental taxonomy of flowering plants was held at the Linnean Society on March 3. The chair at the morning session was taken by the president, Prof. C. F. A. Pantin, and at the afternoon session by Dr. W. G. Templeman.

In a brief introduction, Prof. D. H. Valentine said that the symposium should provide a good illustration of both the coherence and the wide range of experimental taxonomy. In the papers to be heard, groups at all levels, from the local population to the genus, would be treated, and the problems would be approached from a variety of points of view—genetical, ecological and taxonomic.

Dr. A. D. Bradshaw, of the University College of North Wales, Bangor, then gave his paper on "Evolution within Plant Species in Relation to Soil Mineral Factors". He pointed out that while a considerable amount is known about evolution in relation to climatic and biotic factors, soil factors have, comparatively, been neglected. This is surprising, as an examination of the differences between closely related species shows that they often possess markedly different soil preferences, which indicates that soil factors must play an important part in evolution. Experiments have been done in sand cultures on a number of common species, especially *Festuca ovina* and *Trifolium repens*. The fescues used were all of the 28-chromosome type, and selected tillers were grown in a range of solutions with varying concentrations of calcium. Populations from chalk and peat soils showed a markedly different response to growth; and an experiment on samples from base-rich and base-poor soils from the Breckland showed a similar difference, although the populations were morphologically identical. *Trifolium repens* gave the same kind of response in relation to variation in both calcium and phosphate concentrations, but the phosphate and calcium responses were not parallel. It would appear that populations of both species are physiologically differentiated in relation to their mineral nutrition; and such differentiation cannot be neglected in considering the evolutionary processes in these and other common species.

In the discussion on this paper, it was pointed out that much information of a similar kind was available for cultivated plants, notably rice, but that a great deal of it was inaccessible or not widely known.

The second paper, on "Interspecific Hybridization as an Evolutionary Stimulus in *Œnothera*", was given by Dr. P. H. Raven, of the Rancho Santa Ana Botanic Garden, California. Dr. Raven first described *Œ. brevipes* and *Œ. clavaeformis*, a pair of annual species which occur in arid areas in western North America. The species differ in a number of morphological characters (habit, flower colour, etc.), in habitat, and also in their floral biology. In one, the flowers open at about sunrise, in the other, in the late afternoon, and they are pollinated by different but closely related species of bees, which are highly specific to these flowers. Geographically, the species have a rather broad area of overlap, and natural hybrids are sometimes formed (the species are occasionally visited by insects other than the bees mentioned above). Both species have 14 chromosomes; 7 bivalents are formed in most cells, though occasionally rings of 4 or 6 chromosomes are observed; fertility is high. In the hybrids the multivalents are more frequent, larger rings or chains of up to 12 chromosomes being recorded; fertility is low, and only 5–15 per cent of good pollen is formed. Although most of the hybrids between the particular pair of populations studied appeared to be F_1's, other populations can be found (taxonomically of subspecific status) which are probably of hybrid origin. It would appear that occasional hybridization of the kind described may well play an important part in increasing the number of evolutionary pathways available, especially in areas such as this, in which considerable climatic changes have been occurring. In general, it may be said that the retention of the ability to hybridize and to form stable recombinant phenotypes may be regarded as a significant part of the adaptive system in higher plants. There was a short but active discussion of this paper, in which the evolution of pollinating mechanisms and the part played by chromosome interchanges in the genus were discussed.

The final paper in the morning session was given by Dr. N. W. Simmonds, of the John Innes Institute, on "Classification and Nomenclature in Bananas and Potatoes". Dr. Simmonds dealt with bananas first. He pointed out that we now had a good knowledge of the probable course of evolution in the group. The cultivated bananas had arisen from the diploid species *Musa acuminata* and *M. balbisiana* as a result of polyploidy and hybridization, and consisted of a group of sterile, parthenocarpic clones, mainly triploid in constitution. In the potatoes, the cultivated plants probably originated from wild diploid species, as yet not certainly identified, which had developed into the cultivated tetraploid species *Solanum andigenum;* and this in turn, introduced into Europe as a small sample some four hundred years ago, had given rise to *S. tuberosum*. This resembled the banana in being clonally propagated, but was unlike it in being sexually fertile. In both groups, it is practically impossible to apply the normal nomenclatural rules in any useful way. The banana now comprises six groups of sterile clonal cultivars which are not species in any reasonable meaning of the word; and to attempt to apply the Linnean names *M. paradisiaca* and *M. sapientum*, which represent two of these cultivars, would merely lead to confusion. In potatoes, many of the described species with Latin names refer to single clones, others to an arbitrary selection of plants from continuously varying populations; further difficulties arise in the identification of the Linnean type. In both groups, the best procedure is to discard the Latin nomenclature below the generic level, and apply the *Code for Cultivated Plants*. Rates of evolution in crop plants are high, and crops change; and this must be recognized by an appropriate flexibility of nomenclature. In the discussion, sympathy was expressed with Dr. Simmonds's view of the problem; nomenclatural divisions should resemble a neat fence rather than a barbed-wire entanglement.

In the first paper of the afternoon session, Dr. J. R. Ellis, of the Galton Laboratory, University College, London, spoke on "*Fragaria–Potentilla* Intergeneric Hybridization and Evolution in *Fragaria*". He first reviewed briefly the cytology and distribution of the genus *Fragaria*, and the preliminary experiments on hybridization in this group by Mangelsdorf and by J. K. Jones. Dr. Ellis then described in detail his own experiments, in which he used as seed parent both the garden strawberry, *Fragaria grandiflora* ($2n = 56$), and the artificial allopolyploid *F. vesca-grandiflora* ($2n = 70$). When *Potentilla erecta* ($2n = 28$) was used as pollen parent, hybrid seedlings were produced, but none survived to maturity; but with *P. fruticosa* ($2n = 14$) and *P. palustris* ($2n = 42$) as pollen parents not only did many hybrid seeds germinate but also a number of seedlings grew to maturity. There was great variation in the vigour of the hybrids, which were sterile, and all resembled their *Fragaria* more than their *Potentilla* parent; fertile amphiploids, which may be of value in strawberry breeding, have not yet been obtained. Studies of meiosis in the hybrids have been useful, as they have thrown light on the homologies of the genomes comprising the garden strawberry. Dr. Ellis also suggested that the wild species *Duchesnea indica* might be a *Fragaria–Potentilla* amphiploid. In the discussion on this paper, the bearing of Dr. Ellis's results on the taxonomy of *Potentilla* and its allied genera was referred to, as well as the cytological phenomenon of double non-reduction, observed in both *Fragaria* and *Solanum*.

The final paper, on "The Experimental Taxonomy of *Anthoxanthum* L.", was given in two parts. Dr. M. Borrill, of the Welsh Plant Breeding Station, Aberystwyth, spoke first, on the genetic aspects. In Europe and the Mediterranean, three main groups can be recognized: the arctic-alpine perennial diploid *A. alpinum*, the Mediterranean annual diploids *A. puelii* and *A. aristatum*, and the widely distributed tetraploid *A. odoratum*. Although the latter resembles *A. alpinum* very closely, it also possesses certain features, such as leaf pubescence, which occur in the Mediterranean diploids. *A. odoratum* hybridizes readily with the Mediterranean diploids, *A. alpinum* only with difficulty. However, a sterile diploid hybrid between *A. puelii* and *A. alpinum* was obtained, and from this a tetraploid plant was made by colchicine treatment. This plant is a vigorous perennial, is reasonably like *A. odoratum*, and is fairly fertile. It is also moderately self-fertile and can be inbred, unlike the very self-incompatible natural tetraploid. It seems reasonable to suppose that *A. odoratum* arose from a cross similar to that made artificially, though not necessarily from exactly the same parents.

Dr. K. Jones, of the Royal Botanic Gardens, Kew, then spoke about the cytological aspects of the problem. Natural populations of *A. odoratum* have invariably been found to be composed entirely of interchange heterozygotes. Some bivalents are always formed, but quadrivalents are common, and up to 10 chromosomes may be associated in a ring. These facts can be related to the probable mode of origin of the species. The artificial F_1 hybrid *A. alpinum* × *A. puelii* (described by Dr. Borrill) is a complex interchange heterozygote in which pairing varies from 5 bivalents to 1 bivalent and a ring of eight. The artificial amphidiploids derived from this hybrid are thus polyploid interchange heterozygotes, and, in the plants which have been studied, disjunction is not always regular. As a result of selection, the fully fertile natural tetraploid has developed in which there is regular disjunction and self-incompatibility. *A. odoratum* has thus become a stable and permanent interchange heterozygote.

A general discussion on all the papers was opened by Prof. Irene Manton, of the University of Leeds. After referring to a number of specific points, she raised the general question of the time scale of evolution, pointing out that in many of the groups discussed some fairly precise statements had been made which, if followed up, could give more exact information about the speed at which evolutionary adjustments happened than had hitherto been available. Thus it seemed certain that the physiological adjustments to soil described by Dr. Bradshaw must in some cases have occurred rapidly, since one example involved a disused quarry the age of which could be precisely determined. Evolution in *Œnothera*, described by Dr. Raven, seemed to be related to recent changes in the climate of western North America. In bananas, where parthenocarpy was a significant character, artificial selection by man must have been involved in the original Malayan habitat which could perhaps be roughly assessed in years; a significant part of the evolution of the cultivated potato as we know it to-day must have taken place in historical time. Recent advances in the experimental approach to evolution would now justify closer attention to the question of timing than would have been possible a few years ago, and it was strongly recommended that this attention should be given. In the discussion several speakers endorsed the view that certain sorts of evolutionary adjustment can occur surprisingly rapidly. Prof. G. L. Stebbins pointed out that the first stages in the evolution of the potato had not been quite so rapid as Prof. Manton had suggested, as the crop had a very long history in South America. He agreed with Prof. Manton as to the difficulty and complexity of the problem. The rate at which chromosome changes occurred often seemed to show little correlation with rate of change in the gene complexes which controlled morphological and physiological characters, and the causes of both kinds of change were still obscure. More accurate and detailed knowledge of the history of Tertiary floras might eventually provide a basis for firm conclusions.

D. H. VALENTINE

THE INDIAN INSTITUTE OF SCIENCE

THE Indian Institute of Science owes its existence to the vision of the late Mr. Jamsetti Nusserwanji Tata. In 1889, Mr. Tata, realizing the vital part that science could play in the advancement of human welfare, conceived the idea of founding a research institute which would have as its object the industrial welfare of India. Unfortunately, the founder did not live to see the fruition of his idea and it was left to his two sons, the late Sir Dorab Tata and the late Sir Ratan Tata, to fulfil their father's wishes, with the active support of the late Lord Curzon, then Governor General of India, and Sir K. Seshadri Iyer, who was at that time the Dewan of Mysore. The history of the Institute is presented in the February issue of *Science and Culture**; a short account of the Institute on the occasion of the jubilee also appeared in *Nature*, 183, 644 (1959).

Mr. Tata offered property then worth two hundred thousand pounds (twenty-seven lacs of rupees) as an endowment for launching the Institute. In addition, the Government of Mysore offered 372 acres of land at the north end of Bangalore as a gift so that the Institute would be situated in one of the most favourable locations in India.

The Indian Institute of Science came into formal existence on May 27, 1909, as a postgraduate institution for the promotion of advanced studies and original research. The first batch of students was admitted in July 1911 to the Departments of General and Applied Chemistry and Electrical Technology. The Department of Organic Chemistry was added soon after. The Department of Biochemistry was opened in 1921, and the Department of Physics in 1933, when Sir C. V. Raman took charge as the first Indian director of the Institute.

From such modest beginnings it has progressed in a number of directions, and is to-day considered to be the leading centre of research and training in engineering and science in India.

The Institute functions as an autonomous body in accordance with a scheme formulated by the Government of India, under the Charitable Endowments Act. The Quinquennial Reviewing Committee (1955) recommended that, in place of the diplomas which it had so far been conferring, the Institute should confer degrees. The Institute is now deemed a university under the University Grants Commission Act of 1956.

During the Second World War the Institute took up for investigation a large number of schemes of research sponsored by the Council of Scientific and Industrial Research, the Council of Agricultural Research, the Indian Medical Research Council, and the Governments of Madras and Mysore. During that period, the Institute planned and established the Department of Aeronautical Engineering. At the close of the Second World War, a further four-year expansion programme was launched with grants from the central Government. Under the programme, the Departments of Metallurgy (1945) and Chemical Technology and Chemical Engineering (1951) were established and laboratories to carry out work on fermentation technology and pharmacology were set up.

For the training of power engineers, a Power Engineering Department was established in 1947, with separate laboratories for electrical, mechanical, civil and hydraulic engineering. A section on Economics and Industrial Psychology, including industrial relations, and affording facilities for research in these subjects, was also started in the same year.

In 1955, under a scheme for the development of facilities for technical education, additional postgraduate courses in automobile engineering (1956), foundry engineering (1956), soil mechanics and foundation engineering (1957), electronic engineering (1956), ultra-short and microwave engineering (1956), line communication engineering, and acoustical engineering were provided. Further facilities for research in technical gas reactions, physical metallurgy, hydraulic machines, internal combustion engineering, electrical measurements and measuring instruments, transmission, distribution and net-work practices have been added.

Recently a Department of Applied Mathematics and a section of Industrial Engineering Administration have been established.

The Library of the Institute, with about 63,000 volumes of current scientific and technical journals and books, occupies a high place among scientific libraries in India. The Institute celebrated its golden jubilee, commencing February 2, 1959, to mark the completion of fifty years of notable service to the cause of science and engineering in India.

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* *Science and Culture*, 27, No. 2 (February 1961).

THE INSTITUTE OF SEAWEED RESEARCH

THE annual report of the Institute of Seaweed Research, Inveresk, Midlothian, Scotland, outlines the expansion in the seaweed industry both in Britain and overseas*. This is due partly to an increase in the use of the seaweeds themselves and partly to the greatly increased use of seaweed extracts.

Seaweed meal factories at Gairloch, Lochmaddy, Kirkwall and Sanday all increased their production during 1960, and the products have found a ready market especially to compounders of animal feeding stuffs.

A retail market for seaweed manure has been developed by eight firms, and a new company has produced a soil conditioner made from seaweed, a liquid seaweed extract and a compost accelerator with a seaweed base. Liquid extracts are being used increasingly in agricultural practice.

Alginates continue to be of primary importance, and the Scottish market is holding its own against overseas competition, particularly from France and

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* Institute of Seaweed Research, Inveresk. Annual Report for 1960. Pp. 23. (Inveresk, Midlothian: Institute of Seaweed Research, 1961.)

Nova Scotia. America, Iceland and Norway are all producing alginates commercially and there are new ventures in Holland, Australia and South Africa. Increased attention is being paid to the technology of seaweed products, and their use increases, especially in the food, textile-printing and paper-making trades.

The commercial use of mannitol is extending and it may be that its extraction from brown algae will become necessary, since its availability as a by-product in the manufacture of sorbitol does not meet the demand.

The Institute has continued to support fundamental research in a number of universities, and a summary is given of work on algal chemistry carried out in the University of Edinburgh under Dr. E. Percival and Dr. D. J. Manners, and on microbiology under the direction of Prof. G. E. Fogg at University College, London.

The report concludes with lists of publications during 1960, some of which are linked with the Institute while others concern relevant work of scientific importance or commercial development elsewhere.

PUBLIC HEALTH ENGINEERING

POSTGRADUATE courses in public health engineering were first given as a joint venture between the Imperial College of Science and Technology and the London School of Hygiene and Tropical Medicine during 1950. They were inspired by the International Health Division of the Rockefeller Foundation, which, besides giving generous financial aid during the first three years, also awarded many bursaries to enable students to attend the courses.

The courses have now become well established and, during the past eleven years, have been followed by 130 full-time and numerous part-time students from many parts of the world. From the start the aim of the courses has been to give a broad education in public health engineering, and emphasis has been placed on the importance of the public health aspects of the engineer's work.

The engineering studies have been supported by specially arranged courses in basic sciences, especially biology and chemistry, which are essential to the public health engineer but not normally taken by engineers, if at all, beyond the level achieved at school or first year at university. This broad treatment has made it possible to satisfy to a considerable degree the requirements of students from many different countries and of varying interests and experience.

It is now felt that some provision for alternative courses would be a great advantage, and it is proposed to provide two types of course from October 1961*. The first is similar to the course which has been given since 1950. It is intended for engineers who will be employed mainly on the design or control of public health engineering works in Government or local authority departments, public works departments, water or sewerage undertakings or consulting engineers' offices. The second will be a new, broader type of course intended for persons with education and experience in engineering who are concerned with the general application of public health engineering measures rather than with detailed design. Such students will usually find employment in newly developing countries where many of the works with which they deal may be comparatively simple from the engineering point of view. These students require a complete knowledge of public health principles and practice, since their work is closely integrated with the public health services and they may have to initiate new methods of environmental control to meet a variety of health problems.

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* Imperial College of Science and Technology; and London School of Hygiene and Tropical Medicine (University of London). *Postgraduate Courses in Public Health Engineering.* Pp. 15. (London: Imperial College of Science and Technology; and London School of Hygiene and Tropical Medicine, 1961.)

CHEMOTHERAPY OF MALARIA

IN a recent issue of the *W.H.O. Chronicle**, Dr. L. J. Bruce-Chwatt, head of the Research and Intelligence Section of the World Health Organization's Division of Malaria Eradication, has outlined changes being made in the drugs used in the campaign to eradicate malaria. Dr. Bruce-Chwatt suggests that research on the biochemistry of anti-malarial drugs is badly needed and directs attention to the renewed interest in drug combinations and associations.

The rationale of the present use of drugs in malaria eradication programmes carried out by the World Health Organization is as follows:

(1) Schizontocidal drugs (chloroquine or amodiaquine) are used occasionally in the early stage of the attack phase, more generally in the late stage of the attack phase, and always in the consolidation phase for the 'presumptive treatment' given to all suspected cases of malaria for relief of clinical symptoms. The administration of a single dose of the drug to all detected fever cases is one of the characteristics of the surveillance activity that starts towards the latter part of an attack phase of malaria eradication, covers the whole of the consolidation phase, and extends over at least part of the maintenance phase.

(2) A sporontocidal drug (generally pyrimethamine) is often associated with the schizontocidal drug in the 'presumptive treatment' of the attack and consolidation phases with the purpose of making the suspected case of malaria non-infective to *Anopheles* vectors.

(3) Mass administration of schizontocidal drugs, alone or in association with such sporontocidal drugs as pyrimethamine or primaquine, has also been adopted during the attack phase together with residual spraying when the conditions are such that anti-anopheline measures are unlikely to have a rapid or complete effect. This use of drugs is still tentative; they have been given either as a single dose at long intervals at the time of residual spraying or as a repeated (weekly or monthly) dose for some definite period.

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* *W.H.O. Chronicle*, 15, No. 4 (April 1961).

The distribution of medicated salt is actually a variant of mass drug administration, applicable in conditions when residual spraying is not, for various reasons, feasible. Chloroquine or pyrimethamine has been used for admixture with salt, but the latter drug is no longer advocated for this purpose.

(4) Radical treatment of relapsing malaria means generally the use of 8-aminoquinolines for infections due to *Plasmodium vivax* and *P. malariae*, since that due to *P. falciparum* is cured easily by a standard three-day treatment with chloroquine or amodiaquine. The proper administration of daily primaquine or quinocide over a fourteen-day period is often difficult without a wide network of medical services, and in such cases large single doses or smaller repeated doses of a sporontocidal drug (pyrimethamine) have been tentatively given.

The following aspects of chemotherapy are among those of immediate interest for malaria eradication. There is a great difference between the approach of the clinician interested in treatment of a case of malaria and that of the malariologist whose aim is the eradication of this infection from a large community. From the point of view of the clinician no new anti-malarials are really necessary; he has quinine or, preferably, one or other of the 4-aminoquinolines for treatment of severe infections; if he wants to prevent relapses one of the modern 8-aminoquinolines—safe when given with some elementary precautions and almost 100 per cent reliable—is available; for protection from infection the clinician has the choice of proguanil, pyrimethamine, and (in the second place) chloroquine or amodiaquine; any of these drugs will serve his purpose when given at proper and regular intervals.

For the malariologist, each of these drugs has one flaw: it must be given in regular, repeated doses to achieve its effect on the transmission of malaria. In this respect they are often a failure when used on a large scale in countries without extensive and well-organized medical and public health systems. In reassessing the problems of chemotherapy in malaria eradication two questions may be asked: How can the best use be made of the anti-malarials now available?, and, What new drugs are needed, if any ?.

Improvements in operational conditions, better co-operation from an enlightened community, or the introduction of some relatively simple technical innovations may remove a number of obstacles to a wider use of drugs in malaria eradication, but are not always feasible in under-developed areas with inadequately organized public health services.

What new drugs are needed ? The answer is that at least two types of anti-malarials must be developed : (1) A potent and safe schizontocidal drug which will maintain its effect for 3–6 months after a single dose. It would be advantageous if this drug had a rapid and prolonged sporontocidal action. (2) A good and safe anti-relapse drug capable of effecting a radical cure of *vivax* and *malariae* infections when given in a single dose or, at the most, in a three-day course of treatment. Over the past five years the development of new anti-malarial drugs has not been so fast or so successful as expected.

There are two reasons for the apparent lack of drive in research on new anti-malarials: one is the empirical approach to the investigation of new compounds and the other is the growing difficulty of gauging their value in human malaria; little is known about the metabolism of the infected cell.

PHYSICAL ANTHROPOLOGY OF AUSTRALIAN ABORIGINES

SINCE 1951 the Department of Anatomy of the University of Adelaide has sent out seven field expeditions to study the physical anthropology of the aborigines in different parts of Australia, and the first complete trans-continental survey—from Fowler's Bay (Yalata) on the south coast to the Liverpool River in northern Arnhem Land has now been compiled.

The survey covered most metrical and non-metrical characters. Much of the information collected has yet to be analysed statistically, but some features are emerging to warrant preliminary consideration. These have been reviewed by Prof. A. A. Abbie of the Department of Anatomy, University of Adelaide, South Australia*.

Divergent opinions on whether the aborigines are homogeneous or heterogeneous lend interest to a comparison of some physical measurements of three aboriginal groups, separated from each other by some 800 miles of territory and all living in different environments.

The groups are, respectively, (*a*) a composite southern group assembled near Yalata on the south coast ; (*b*) two neighbouring tribes of central Australia —the Pintubi and the Wailbrai : and (*c*) a tribe near the mouth of the Liverpool River in northern Arnhem Land, the Burera. In the present paper only adult males are considered as being most representative of their groups. The numbers examined—Yalata, 33 ; Pintubi, 38 ; Wailbrai, 30 ; Burera, 26—are about the maximum obtainable in any tribe in a reasonable time. All except the Yalata group had come under European influence only shortly before the time of examination.

The figures indicate that, right across the continent, through a wide diversity of environments, the aborigines exhibit an extraordinary degree of physical homogeneity.

Up to the age of about 5 years, the physical proportions of aboriginal children are about the same as those of comparable Europeans. Between 5 and 6 years, however, aboriginal children suddenly show a marked extension of the inferior extremities that reduces the relative sitting height to about 50 per cent —close to the proportions of European children of around 12 years. Thereafter, until the cessation of growth, the aborigines preserve their six years' advantage and finish with a relative sitting height close to 46 per cent.

So far this has been worked out fully only for the central Australian males, but a preliminary survey of the data indicates that it holds for both sexes and for most northern and most southern groups as well.

New-born aborigines are pinkish-yellow with some darker colouring around the nipples, navel, genitalia and buttocks. The rate of darkening of the skin seems to depend on the degree of exposure to the

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* *Australian Journal of Science*, 23, No. 7 (January 1961).

Sun and full pigmentation may be acquired in one week or not for two or three weeks. The oral mucosa may be patchily pigmented in infancy, but usually not until eruption of the first permanent molars. The darker colouring starts around the anterior gum margins and spreads to the general mucosa and the palate with advancing maturity. There is a tendency towards fading in old age. In tribes that practise circumcision, at the time of operation (about puberty) the glans penis is pink or only slightly pigmented; in adult circumcized males the glans is as dark as the surrounding skin.

In most aborigines the skin is reddish-brown or chocolate, but more intense exposure—as in the desert or far north—produces a much darker colour. Those who wear clothes regularly tend to lose some of their melanin and fade, sometimes to near the original pinkish-yellow in parts. Such people are liable to sunburn on unaccustomed exposure.

Head hair is usually dark brown to black, but blondness—ranging from quite flaxen to reddish-brown—is common in the children of central and south Australia. The fine hair of the body is also fair, but all other aspects of pigmentation—skin, eyes, eyebrows and lashes—are as in all other aborigines. In boys the scalp hair begins to darken at 8–10 years and becomes black or dark brown, like the hair of axillæ, pubes and beard, before the age of twenty. Girls start to darken later—in adolescence—and even when adult may still only be mid-brown on the scalp although axillary hair and pubic hair is dark brown or black. In the far north light scalp hair is less common, but it occurs, and usually with a redder tinge. Light body hair is quite common there in women and children, however. This blondness probably has a genetic basis; it is certainly not due to kwashiorkor, or other nutritional disturbance.

Aborigines living under native conditions have relatively low blood pressures. In the western desert men at 40 years averaged 75–109 mm. mercury and women slightly less. At Government settlements, where living is easier and some European food is available, male pressures were 79–119, female 76–110. In a more or less urban environment male pressures averaged 89–131 (very similar to European males under similar conditions), female 75–115. In northern Arnhem Land pressures expressed on the same basis were: males 84–116, female 82–113.

All aborigines so far examined have high serum protein-levels, especially in the γ-globulin range. Serum cholesterol-levels are comparatively low in desert-living aborigines and there does not appear to be any rise with age as occurs in Europeans. Aboriginal cholesterol-levels rise with increasing Europeanization (roughly paralleling the rise in blood pressures), but not, so far, to European-levels. Serum phospholipid and mucoprotein-levels also rise under the same conditions.

WEATHER FORECASTING BY NUMERICAL METHODS

THE advent of the electronic computer has made it possible to attempt weather prediction by numerical processes. The idea is to observe an initial state of the atmosphere, apply Newton's laws and the laws of conservation of energy and mass, together with the appropriate gas laws, and hence predict the condition of the atmosphere at some time in the future, rather as the motions of heavenly bodies are predicted in astronomy. The earliest attempt was made by L. F. Richardson between the World Wars and is described in his classic book. Richardson visualized an army of computers, just able to keep up with the weather. He did not visualize computing machines, and their widespread use has changed the position, for an electronic computer can get ahead of the weather quite easily.

Active work on this subject is now proceeding in the United Kindom, Belgium, Holland, Norway, Sweden, Japan, and several centres in the United States. A recent publication from the Meteorological Office* describes some of the work being done in the forecasting research division. This paper, considered together with recent work in the United States deserves special attention because, for upper-wind forecasting, numerical methods now surpass conventional methods of forecasting. This does not mean that the conventional forecaster with his weather-maps will disappear, because, as yet, it is only the winds which are forecast numerically, not the equally important weather. A housewife may like to know that Monday will be windy because this will facilitate drying clothes, but she is more interested in whether or not it will rain. Numerical methods at present either do not attempt to predict this or they do so very badly.

It is obvious that the atmosphere is a very complicated affair, yet the primitive (partial differential) equations expressing the laws mentioned above look beguilingly simple. They do, however, include all the motions of the atmosphere, the steady trade winds on the surface, the great jet-streams in the upper air, depressions and anticyclones, tornadoes and showers, to mention only a few of the varied phenomena. Almost always the first thing, therefore, is to simplify the equations so that only events significant to the scale of weather concerned remain. The resulting simplified system of equations and the somewhat artificial atmosphere it describes then become a 'model'.

In the United States experience is that the simplest and earliest model—the barotropic model—remains the best for forecasting large-scale phenomena. After pioneer work by J. Charney, the great labour of extensive trials has been borne by a joint numerical weather prediction group at Suitland, Maryland. The model regards the atmosphere as describable in every way by events at one level only, and the resulting simplified atmosphere is called the equivalent barotropic atmosphere. By extending the calculation to include almost the whole northern hemisphere, and by correcting artificially for the unexplained incorrect backward motion predicted for the phenomena of the largest scale, a useful extra skill above the conventional forecaster is obtained and, for long-range air navigation, winds forecast in this way are now used in the United States.

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* Air Ministry: Meteorological Office. Scientific Paper No. 5: *An Experiment in Numerical Forecasting*. By E. Knighting, G. A. Corby, F. H. Bushby and C. E. Wallington. (M.O. 682.) Pp. iii+67. (London: H.M. Stationery Office, 1961.) 5s. net.

In a barotropic atmosphere, by its very nature, kinetic energy cannot be generated, and this is clearly unnatural since without the continuous generation of energy the atmosphere would slow down and soon be stopped by the drag of the air on the ground. This fundamental unreality would appear to be very unsatisfactory. However, it is now generally accepted that the large-scale motions of the general circulation, for which the barotropic model succeeds so well, do not have their own source of energy, but derive it from the more ordinary phenomena, such as the depressions of temperate latitudes. In most fluid-flow problems the small vortices obtain their energy from the general flow, and it is a remarkable property of the atmosphere that here the reverse occurs. It is therefore reasonable that phenomena which have no internal energy source can be described by a model which provides none, but this model would be expected to fail when kinetic energy was obviously being generated. This is found to be the case and most of the active groups working on the problem are experimenting with more complicated models which can provide the necessary source.

In the Meteorological Office the effort available is relatively small, and it has been mainly concentrated on the two-layer models which arose naturally out of Sutcliffe's early work. In the work of all groups, much of the success which has been claimed for the complicated models appeared to rest suspiciously on the isolated events which had been used to test them, the model being adapted until it succeeded, but presumably its success was isolated because these models have not appeared in service use. The Meteorological Office, in its Scientific Paper No. 5, has now published the results of a substantial test of a two-layer model, and some significant success is achieved. The forecasts appear to have a just significant superiority over conventional methods. The workers have been handicapped by the need to work over a limited area so that the influence of events outside the region of computation is not negligible, and it is to be hoped that they will be able to increase their scale of operations in the future.

It seems that the slow improvement in the accuracy of weather forecasting must be accepted. Conventional methods using weather charts, the dynamic methods discussed above and elaborate statistical methods all seem to achieve a success only a little better than persistence. They then progress either no more, or very slowly. It may be that the block is because the weather is in fact indeterminate and it will never be possible to do much better, but surely that is a gospel of despair.

A. W. BREWER

PRECIPITATION OF SUB-MICRON DUST IN STILL AIR BY CLOUD-SIZE WATER DROPLETS

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A SEARCH of available literature indicates doubt as to the possibility of precipitating sub-micron dust dispersions in air by means of water droplets falling under gravity. The results of a preliminary investigation of this problem by Bhadani[1] indicate that such precipitation occurs readily under conditions described in this article.

Some fields, in which further extensions of research into the behaviour of fine aerosols in general may have special significance, are discussed briefly.

That such fine dust is produced in certain industrial processes is discussed by Sichel[2], who records large percentages of dust of less than $0{\cdot}1\mu$ from mine-drilling operations. In certain foundry operations, too, high percentages of $0{\cdot}05$–$0{\cdot}10\mu$ grains have been reported[3].

The potential significance of sub-micron particles in lung pathology has been established by Pollicard[4], Dautrebande *et al.*[5], and others, who have shown that such fine particles can penetrate into the lung alveoli.

A method of coagulation of fine dust and sodium chloride aerosols, both in Brownian motion, is described in refs. 6–8. When breathed into the lungs, the hygroscopic sodium chloride-dust agglomerations grow larger by absorption of moisture and are deposited in the larger air tracts before reaching the alveoli.

The finer particles present in atmospheric pollution comprise both man-made and naturally occurring substances. Cartwright and Skidmore[9], in surveys of airborne dusts in South Wales coal mines, concluded that atmospheric pollution entering the mines with the ventilating air current made it difficult to assess the sub-micron dust actually produced in the mining operations.

In meteorology great importance is attached to the function of fine particles present in the atmosphere in acting as condensation or freezing nuclei in the process of cloud and rain formation. Simpson[10] notes the presence in air of naturally occurring hygroscopic nuclei such as salt from sea spray and nitrous acid from atmospheric nitrogen, in addition to non-hygroscopic materials. Nolan and Doherty[11] have recorded the presence in Dublin air of condensation nuclei of $0{\cdot}01$–$0{\cdot}10\mu$ size and with varying concentrations up to about 9×10^4 particles/c.c. of air and more. Bowen[12] has produced evidence showing a possible relationship between regularly occurring meteoric dust streams and peak rainfall periods in various parts of the world. Cwilong[13] has found nuclei concentrations of about 10^5 particles/c.c. of air coinciding with abnormal rainfall and meteoric dust showers. Ludlam[14] has estimated that cosmic dust may settle into the atmosphere at a sufficient rate to provide concentrations of Aitken nuclei near ground-level of 100 particles/c.c.

When bulk coal from the top seam of the Khost-Sharig colliery, Baluchistan, Pakistan, or silica dust (that is, dust produced by grinding) is dispersed in air in a confined space, the larger particles soon settle out by gravitational effect, leaving behind a fine residual dust suspended in the air medium. Residual dust, prepared in this way in a metal chamber (17 in. × 17 in. × 30 in.), has been found to consist of particles largely in Brownian motion.

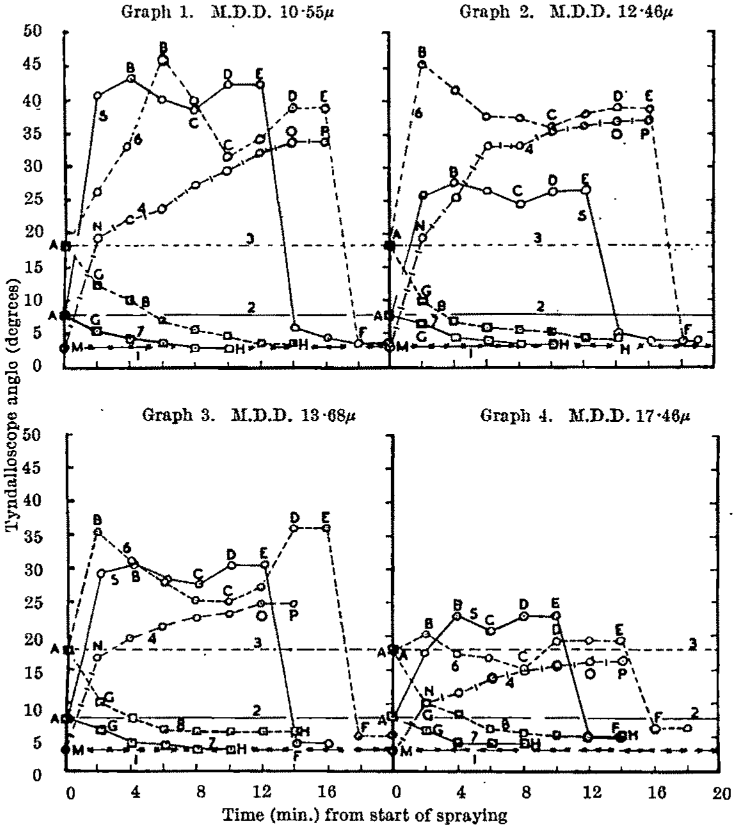

Fig. 1. Water spray precipitation for residual coal and silica dusts, with related curves. Average mean diameter of dusts used, 0·14μ. (1) Instrumental zero reading of +3°. (2) Constant scatter angle for residual Pakistani coal dust dispersion in the absence of spray. (3) Constant scatter angle for residual silica dust dispersion in the absence of spray. (4) Curve for spray in the absence of dust. (5) Precipitation cycle for residual Pakistani coal dust with continuous water spray. (6) Precipitation cycle for residual silica dust with continuous water spray. (7) (AGH). Precipitation curve for residual Pakistani coal dust with intermittent spray. (8) (AGH). Precipitation curve for residual silica dust with intermittent spray. M.D.D. = mean droplet diameter

The average mean diameter of the particles was 0·14μ and the concentration 9×10^4 particles/c.c. of air. The concentration in the chamber was measured by means of the light-scatter angle of a Tyndalloscope. The Tyndalloscope readings of the residual dust suspensions before commencement of spraying were found to remain constant for several hours, the values being 7·8° and 18° for the coal and silica dusts respectively. This indicates a steadily maintained dust dispersion in which coagulation, diffusion or settling were not important factors during the period of the experiments.

When the steady state of the residual dust suspension in the chamber was indicated by the constant Tyndalloscope reading, water droplets were introduced into the upper portion of the chamber by means of a May[15] type spinning top driven by compressed air. The Tyndalloscope angle was read at 2-min. intervals during the precipitation cycles.

Four sets of typical precipitation cycles and related curves for residual coal and silica dusts are shown in Fig. 1, graphs 1–4, using water spray with mean droplet diameters of 10·55, 12·46, 13·68 and 17·46μ in separate experiments. The same rate of water-flow (2·4 c.c./min.) was used in each case.

Particular consideration is given here to the continuous spray precipitation curves 5 and 6 for coal and silica dust, respectively.

Entering these curves at *A*, on the line of steady scatter angle of the coal or silica dispersed in the chamber, the sprayer is started. The Tyndalloscope scatter angle increases rapidly from *A* to *B* as the combined effect of mist in the presence of dust is registered. From *B* the angle decreases sharply to *C* and then soon again increases to *D*, after which it remains constant, indicating equilibrium conditions. At *E* the spray is stopped and the portion of the curve *E* to *F* is clearly related to the diminution of concentration, as the droplets plus remaining precipitable dust fall to the floor of the chamber.

An interesting feature of these continuous spray precipitation curves is the fall in scatter angle *B*–*C* followed by the rise *C*–*D*. It is thought that this may be in part due to a temporary shower of dust-laden droplets. Evidence of substantial dust precipitation by droplet assimilation is seen in Fig. 2. This shows an electron microscope photograph of a droplet which has fallen directly on to a 'Formvar'-covered specimen grid, placed in the floor of the chamber.

Comparing all the precipitation curves for the coal and silica dusts, it is noticeable that the smaller droplets are somewhat more effective in eliminating the dust from suspension than the larger droplets. Also the coal dust is more readily precipitated than the silica dust.

The reasons for the diverse precipitation characteristics of coal and silica are not fully understood. The contrasting physical properties of coal and silica in the presence of water vapour may be important in this connexion. It is well known that coal absorbs water vapour[16–19]. To illustrate this point in the present work, equal weights of the bulk silica and coal dusts were placed in separate vessels on the floor of the experimental chamber, sheltered from falling droplets, but with open

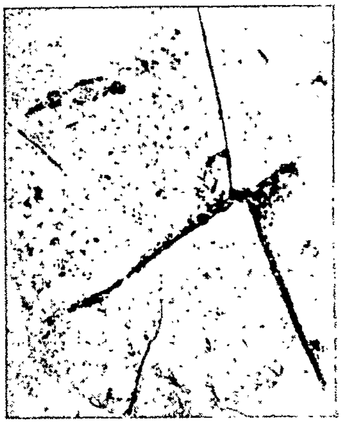

Fig. 2. Electron microscope photograph of dust-laden water droplet (× 1020)

access to the atmosphere of the chamber. It was found that the increase in weight of the coal dust attributable to water vapour adsorption was more than 100 per cent, whereas that of the silica dust was negligible.

Our thanks are due to Prof. Stacey G. Ward, head of the Department of Mining and Minerals Engineering, for help and advice in the research and in the preparation of this article.

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VISCOSITY OF DUSTY GASES

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IF dust is added to gas flowing turbulently through a pipe or other system, the difference, H, in pressure required to maintain the flow at its original (clean) rate, V, decreases immediately. For air, a dust concentration of ¼ kgm./m.^{3} decreases H about 13 per cent. Or, if H is held constant, the addition of the dust increases the flow-rate about 6½ per cent, the flow being proportional to the square root of H. These quantitative results were determined by Dr. Leo W. Briggs in our laboratory in 1945, but never published. The qualitative observation that the gas-handling capacity of a cyclonic dust collector increases considerably with the dust concentration was familiar here about 1935 or earlier.

Curiosity about this effect led to the present investigation. Obviously, addition of 0·25 kgm. of dust to a cubic metre of clean air, which has a mass of about 1·2 kgm., will increase its density to about 1·45 kgm./m.^{3}. Such a 21 per cent increase in the density could be achieved by, for example, cooling the air sufficiently, and both theory and experiment agree that such an increase in density due to temperature reduction will *increase* the value of H required to keep V constant by 21 per cent. Yet Briggs found that H *decreases* about 13 per cent when this same 21 per cent density increase is achieved by suspending dust in the air (or other gas).

The probable explanation seemed to be that addition of the dust must decrease the viscosity of the gas in order to account for its decreased resistance to flow through pipes, etc. Moreover, the decrease in the viscosity must be quite a large one, because empirical equations such as the so-called 'Fanning equation' show that, for a constant flow-rate V, H varies as the 0·25 power of the viscosity. Thus, the observed 13 per cent reduction in H for a dust concentration of ¼ kgm./m.^{3} would imply a 43 per cent reduction in the viscosity. This seemed difficult to believe; yet no other explanation could be found.

In 1958, I conducted some preliminary tests with a viscosimeter and found a decrease of about 40 per cent in the viscosity of air, from about 180 to about 110 μpoise, when limestone dust was suspended in it at a concentration of about ¼ kgm./m.^{3}. These results were published only in abstract form[1]. The viscosimeter used in 1958 was constructed with a horizontal disk, suspended by a torsion wire, 5 mm. above a second horizontal disk driven by a motor at 78 rev. per min. Dust settled rapidly on the disks at the heavy concentration required, and this left some room for doubt about the reliability of the results.

Therefore the experiments were repeated in 1960, using a viscosimeter having a vertical sensing cylinder suspended coaxially within a rotating cylinder, as shown in Fig. 1. It was not possible to work under steady-state conditions in either the 1958 or 1960 experiments. If dust-laden air from the dust-dispersing device had been blown steadily through the viscosimeter cabinet with a dust concentration of ¼ kgm./m.^{3} over a period of, say, 20 min., the dust settling within the cabinet would have been so great as to fill it about half full and completely spoil the operation of the viscosimeter. Even in 1 min., the dust settling on the floor of the cabinet amounted to about 1 in. at these heavy concentrations. Thus each test had to be conducted rapidly and a result obtained within 1 min. The procedure was to blow clean air through the viscosimeter cabinet at the maximum rate attainable without undue disturbance of the inner sensing cylinder. After noting the 'zero' deflexion, the outer cylinder was started rotating; the change in the deflexion was taken as a measure of the viscosity of the clean air (about 180 μpoise). Next, dust was fed into the disperser at a rate sufficient to yield the desired concentration, for about 1 min. During this time, the deflexion of the inner cylinder decreases due to the reduced viscosity, and the reduced value must be determined.

The annulus between the two cylinders was 5 mm. wide in some tests and 7½ in others. More than one value was also used for the speed of rotation, 25 and 50 rev. per min.; for the dust concentration, 60 and 240 gm./m.^{3} approximately; and two types of dust were used. One was limestone dust consisting 40 per cent by weight of particles smaller than 10μ diameter; the other was talc dust 99 per cent by weight smaller than 10μ. Although the density of the individual particles was nearly the same for these two dusts (2·5 gm./c.c. for talc and 2·6 gm./c.c. for limestone), the talc dust had a bulk density of only about 0·3 gm./c.c., as contrasted with about 1·3 gm./c.c. for the limestone dust. The results follow:

Test series	Dust	Approximate concentration (gm./m.^{3})	Cylinder speed (r.p.m.)	Annulus width (mm.)	Observed viscosity reduction (per cent)
1	Limestone	240	50	7½	40
2	Talc	240	50	7½	38
3	Talc	60	50	7½	10
4	Talc	60	50	5	10
5	Talc	240	50	5	35
6	Limestone	240	50	5	24*
7	Limestone	240	25	5	24*

Each of the seven values in the last column of the above table is an average of several tests. The last two, marked with asterisks, are to be discounted on the grounds that with the narrow 5-mm. annulus, the comparatively coarse limestone dust could not be forced through the apparatus fast enough to prevent excessive settling, without disturbing the sensing cylinder unacceptably. Consequently, settling reduced the dust concentration in series 6 and 7 to a value considerably less than the ¼ kgm./m.^{3} at which it entered the viscosimeter cabinet. These two series did indicate, however, that the result is insensitive to cylinder speed.

The tests of two such different dusts as limestone and talc, with nearly identical results, indicate that the concentration of the dust (mass per unit volume of the gas) is the main factor in the effect, and that the particle size and bulk density of the dust are relatively unimportant. The present results confirm the 1958 results and indicate that dusty gas flows through pipes with a smaller resistance than clean gas, because the dust reduces its viscosity. This work has also been published in abstract form[2] only.

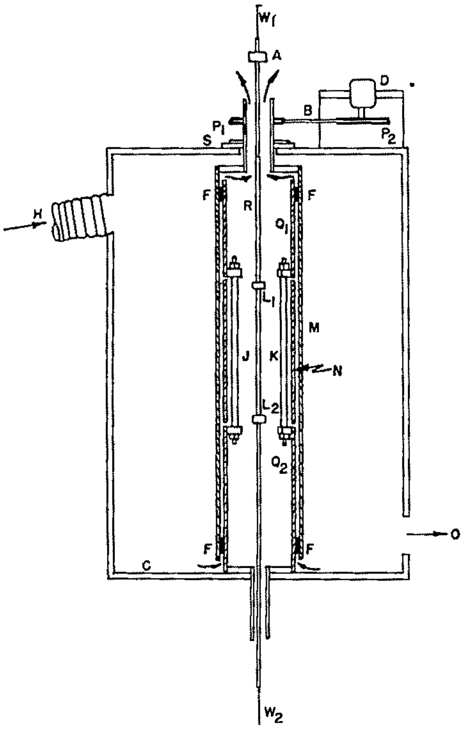

Fig. 1. Vertical section of the viscosimeter and enclosing cabinet. Dust-laden air is blown in at *H* and out at *O*. Some of it also escapes via the route shown by the arrows near *F* and *A*, which carries it through the annulus between the outer driven cylinder *M* and the inner sensing cylinder *N*. *N* is attached by lugs *L* to rod *R*, which is supported and aligned by taut wires W_1 and W_2. The torque transmitted to *N* by the viscous action of the gas in the annulus twists wires *W* through a few degrees, the angle being measured accurately by means of a light beam reflected from mirror *A*. The stationary cylinders Q_1 and Q_2 eliminate end-effects at the top and bottom of cylinder *N*

What is the physical explanation for such a large reduction in the viscosity of a gas when dust is suspended in it? Elementary kinetic gas theory states that the viscosity, η, is given by:

$$\eta = \tfrac{1}{3}\rho\bar{c}\lambda \tag{1}$$

where ρ is the gas density, $\bar{c}$ is the average molecular velocity, and λ is the mean free path. At constant temperature, as in the present experiments, $\bar{c}$ remains constant. The addition of dust can only increase the density, ρ; hence the observed decrease in viscosity is presumably due to a decrease in the mean free path, λ, which in simple kinetic theory is given by:

$$\lambda = \frac{1}{\sqrt{2}\pi N\sigma^2} \tag{2}$$

where N is the (Loschmidt) number of molecules per cubic centimetre and σ is the diameter of the molecules.

If the dust cloud is regarded as a second 'gas' mixed with the true gas in which it is suspended, the expression for λ_g, the mean free path of the gas molecules in the dust-laden gas, becomes[3]:

$$\lambda_g = \frac{1}{\sqrt{2}\pi(N_g\sigma_g^2 + N_d\sigma_d^2\sqrt{1 + m_g/m_d})} \tag{3}$$

where N_d is the number of dust particles per c.c., σ_d is the diameter and m_d the mass of a dust particle (assumed the same for all). The g subscripts designate similar symbols for the gas molecules. m_g is negligible compared to m_d, so the final radical reduces to 1. Also, $N_d\sigma_d^2$ is small compared to $N_g\sigma_g^2$, and unless this circumstance can be changed, equation (3) reduces to equation (2). The only way that $N_d\sigma_d^2$ could be made comparable in magnitude to $N_g\sigma_g^2$ for the dust concentrations used in the present experiments is to assume that σ_d is an 'effective' diameter, which might be appreciably larger than the actual diameter of the dust particles. Since σ_d is squared, an effective diameter equal to, say, 50 times the actual diameter, increases $N_d\sigma_d^2$ by a factor of 2,500, and this is sufficient to make its value comparable to $N_g\sigma_g^2$, thus causing equation (3) to indicate a reduction in λ_g of about 50 per cent, which, from (1), would account for a reduction in the viscosity of about 50 per cent, as observed.

Taking the experimental data and reversing the calculation above, one concludes that if the dust particles were all 1μ in diameter, their 'effective' diameter, σ_d must be about 150μ. This suggests that the explanation of the observed effect may be that each dust particle is surrounded by a 'boundary layer' of gas adhering to it. This is not a new concept; indeed, the derivation of Stokes law for the settling of dust particles in a gas[4] presupposes the existence of such a boundary layer. An expression for the approximate magnitude of its thickness is[5]:

$$\delta = \sqrt{\frac{\eta\sigma}{\rho v}} \tag{4}$$

where v, in the case of a suspended dust particle, would be the difference in the gas velocities on opposite sides of the particle. From the known velocity gradient in the viscosimeter annulus, one may calculate v for the case of a 1μ diameter particle, and thus evaluate δ from equation (4). The result is about

100–500μ, thus agreeing reasonably with the value calculated for σ_d.

Thus, one is left with a picture of the suspended dust particles each surrounded by a gaseous boundary layer thick enough to make its 'effective' diameter some 50 or 100 times as great as its actual diameter. This increases the 'cross-section' of the particles sufficiently to cause the gas molecules to collide with these boundary layers just about as often as they collide with unattached molecules (that is, molecules not making up a boundary layer for some dust particle).

A modification of this picture may also be worth considering. The velocity gradient in the viscosimeter annulus causes a force in one direction on one side of any dust particle suspended therein, and a force in the opposite direction on the opposite side of that same particle. This couple should cause the particle and its boundary layer to rotate somewhat like the balls between the races of a ball bearing. For a typical velocity gradient such as 50 cm./sec. per cm., the rate of rotation of the suspended particles should be roughly 8 or 10 rev. per sec. This 'ball bearing' concept may also help account for the observed reduction of viscosity.

The effect discussed in this article may have some significance in meteorological theories of rainstorms, etc., and may even be worth considering in cosmological theories of the evolution of stars and planets from dust-laden gases. It is also possible that this phenomenon might be applied to develop a practical instrument for measuring dust concentration in industrial smoke-stacks and flues.

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BIOCHEMICAL CHANGES ACCOMPANYING IMPAIRED RESPIRATION IN STAPHYLOCOCCI

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IN a recent discussion of the cancer problem[1], mutants of yeasts with impaired respiration have been compared with cancer cells which also have a respiratory deficiency. In this connexion, mutants of staphylococci with oxidation defects which can be observed in cultures of some strains are of great interest[2,3].

A new method has been developed for the isolation of staphylococci with respiration deficiency from parent cultures, which is based on the selective inhibitory action of some fluorinated pyrimidines (Table 1), recently prepared as a new class of tumour-inhibitory compounds. The growth of staphylococci with impaired respiration (mutant *uv*-3) is approximately sixty times more vulnerable to the inhibitory action of 5-fluorouracil than the growth of the initial parent culture (*S. aureus* 209). In both cultures the inhibitory action is reversed by uracil, but not by thymine. On the other hand, 5-fluoro-2-deoxyuridine inhibits growth of the parent culture of staphylococci much more effectively than 5-fluorouracil, and this inhibition is reversed by thymine but not by uracil.

It therefore appears that 5-fluorouracil and 5-fluoro-2-deoxyuridine are blocking different metabolic pathways in staphylococci. In accordance with present views, 5-fluoro-2-deoxyuridine blocks the formation of thymine which is required for the synthesis of deoxyribonucleic acid[4]. It is very significant that 5-fluoro-2-deoxyuridine does not inhibit the growth of mutants with impaired respiration either in liquid or solid media, even at the maximum concentration tested, namely, 1,000 μgm./ml. This points to the possible synthesis of deoxyribonucleic acid thymine by different pathways in mutant and parent strains. Another possible explanation for this phenomenon is that it may reflect the relative impermeability of the mutant cell to 5-fluoro-2-deoxyuridine, so that the drug does not reach the site of its action.

Table 1. CONCENTRATION OF SOME FLUORINATED PYRIMIDINES REQUIRED TO INHIBIT THE GROWTH OF STAPHYLOCOCCI IN LIQUID BROTH

Compound	Micro-organism	μgm./ml.
5-fluorouracil	*Staph. aureus* 209	125
,, + thymine (100 μgm./ml.)	,,	125
,, + uracil (100 μgm./ml.)	,,	**500**
5-fluorouracil	Mutant *uv*-3	2
,, + thymine	,,	2
,, + uracil	,,	**25**
5-fluoro-2-deoxyuridine	*Staph. aureus* 209	25
,, + thymine	,,	**100**
,, + uracil	,,	25
5-fluoro-2-deoxyuridine	Mutant *uv*-3	> 1,000
,,	Mutant *uv*-2	> 1,000

The observations given above were used for the development of liquid nutrient medium, selectively inhibiting the growth of parent staphylococci and favourable for the multiplication of mutants with impaired respiration. For this purpose a nutrient broth containing peptone (0·5 per cent), glucose (1 per cent), sodium chloride (0·5 per cent), Hottinguer digest (30 ml./l.), corn steep liquor (0·2 per cent), and 5-fluoro-2-deoxyuridine (100 μgm./ml.) was distributed into test-tubes, and seeded with suspensions of the parent cultures of staphylococci (strains 209, *D*, *G*, and 121). The staphylococci were previously grown on meat-peptone agar for 24 hr. at 37° C., washed using physiological saline solution, diluted to a concentration of 2 × 10^8 cells/ml. and added to the selective medium in the amount 0·10–0·05 ml. per 1 ml. of broth. Parent staphylococci did not grow on this medium due to inhibitory action of 5-fluoro-2-deoxyuridine, but any mutants with impaired respiration which were present in the parent inoculum multiplied. If, after 5–7 days of incubation of such tubes at 37° C., their contents were plated on nutrient agar containing 5-fluoro-2-deoxyuridine (25 μgm./ml.), in some cases pure cultures of mutants were observed instead of the parent culture. These mutants were observed more frequently in the strains *D* and 209; in the strains 121 and *G* they were not recorded. Numerous experiments have shown that the quantity of inoculum added to the selective medium is of great importance. If one adds too many parent cells, these acidify the medium and inhibit the growth of mutants, which require a neutral or slightly alkaline medium for their multiplication. If the parent cells are too few, the probability of occurrence of mutants among them is negligible.

Mutant staphylococci isolated by the new technique show the same changes in respiratory activity which have been previously reported[2,5]. The respiratory coefficient is decreased by 40–60 per cent as compared to parent values, while the respiration is less sensitive to cyanide. The cytochrome pattern is also altered in the new mutants. Whereas normal staphylococci reveal three bands of cytochromes, a_2(630 mμ), a_1(600 mμ), and b_1(557 mμ), in mutants the band a_2 is not visible, either under usual conditions of observation or when liquid nitrogen with the subsequent devitrification is employed. Instead of band b_1, two new bands are observed in mutants, at 563 and 553 mμ, which are easily oxidized and reduced in the presence of succinate. In respect of splitting of b_1 band of cytochromes, the mutants of staphylococci with impaired respiration resemble the respiratory-deficient mutants of *E. coli*[5].

Further investigations have shown that mutants with respiratory deficiency in staphylococci differ from the parent culture in a number of important biochemical characters. It may be suggested that respiratory defects, as well as other hereditary metabolic alterations, can be related to some disturbances in the synthesis of deoxyribonucleic acid in mutant staphylococci.

Pigment. A characteristic feature of staphylococci with defective oxidation is their rose-orange intracellular pigment, which is lacking in cells of the parent culture. Mutants were cultivated on the nutrient agar and extracted by ethanol. The absorption spectra of the ethanolic solutions of pigments so obtained from nine different respiratory-deficient mutants of staphylococci were studied, and found to be very similar. Fig. 1 presents the data for pigment from the mutant No. 16, revealing a maximum optical density at 475 mμ. This observation points to the possibility that respiratory defects in mutant staphylococci are accompanied by some disturbances in the metabolism of flavins.

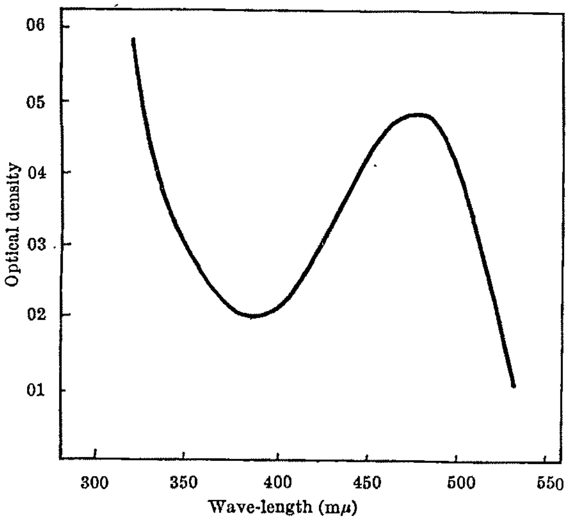

Fig. 1. Absorption spectrum of the intracellular pigment of mutant staphylococci with impaired respiration, strain No. 16

Synthesis of vitamin B_{12}. Theoretical considerations put forward by Woolley[6] suggest that alterations in flavins may affect the biogenesis of vitamin B_{12}. Experimental observations by the same author[7] indicate that respiratory defects and dedifferentiation of cells in some strains of spontaneous tumours of mice are accompanied by the ability of these cells to synthesize vitamin B_{12}.

The content of vitamin B_{12} in the cells of parent staphylococci as well as of a number of respiratory-deficient mutants, which were grown on the liquid medium under identical conditions, was measured with the aid of chromatographic and bioautographic techniques[8,9], using the culture of *E. coli* 113–3. The results of these measurements are presented in Table 2.

Table 2. CONTENTS OF VITAMIN B_{12} IN THE CELLS OF PARENT STAPHYLOCOCCI AS WELL AS IN RESPIRATORY-DEFICIENT MUTANTS

Strain	μgm./gm.	Strain	μgm./gm.
Parent 209	0·3	Mutant 21	18
Mutant 3	18	Mutant 1	26
Mutant 22	20	Mutant 2	17
Mutant 16	28	Mutant 20	23
Mutant 15	18	Mutant 14	16

It can be seen that the content of vitamin B_{12} in the cells of mutants may be increased almost a hundred-fold as compared to parent cells.

Catalase. Suspensions of staphylococci adjusted to 20 mgm./ml. dry weight were disrupted using a Mickle disintegrator at 0° C. and Ballotini glass balls No. 12. The catalase activities of the cell extracts were estimated spectrophotometrically[10]. Table 3 shows that respiratory-defective mutants contain much less catalase activity than the cells of the parent strain.

Table 3. CONTENTS OF CATALASE (IN ARBITRARY UNITS) IN THE CELLS OF PARENT STAPHYLOCOCCI AND IN THE RESPIRATORY-DEFICIENT MUTANTS

Strain	Catalase
Parent 209	4·02
Mutant 2	0·50
Mutant 21	0·56
Mutant 16	0·83

Amino-acid composition of the cell wall. Preliminary experiments carried out in collaboration with Drs. H. Rogers and H. R. Perkins showed a difference in cell wall composition between parent and mutant cells of staphylococci[11]. This observation has been confirmed using the new Park and Hancock[12] procedure, which was found to be very efficient. In hydrolysates of cell wall material of parent staphylococci, in accordance with the published evidence[13], the following components were recorded: alanine, glutamic acid, glycine, glycosamine, muramic acid and lysine. In mutants with impaired respiration (*uv*-2 and *uv*-3) only traces of lysine were recorded while diaminopimelic acid was observed. Since many bacteria possess the capacity to decarboxylate diaminopimelic acid into lysine[14], the substitution of lysine by diaminopimelic acid in the cell wall of respiratory-deficient mutants of staphylococci might indicate the loss by mutants of this specific decarboxylase. In the cell wall material of mutants were recorded also alanine, glutamic acid, glycine, glucosamine, muramic acid and aspartic acid.

Selective inhibition of the growth of mutants. In extension of earlier observations[15], a new series of alkylating agents, antimetabolites of nucleic acids,

Table 4. CONCENTRATIONS OF COMPOUNDS REQUIRED TO INHIBIT THE GROWTH OF PARENT STAPHYLOCOCCI AND RESPIRATORY-DEFICIENT MUTANTS ON NUTRIENT AGAR (μGM./ML.)

Compound	Parents 209	Mutants *uv* 2 and 3
Miracil *D*	500	25
Triethylenemelamine	> 1,000	400
6-Mercaptopurine	> 1,000	30
6-Chloropurine	15,000	1,500
Mitomycin *C*	0·2	0·002
Actinobolin	100	12
Actidione	> 10,000	5,000
Streptomycin	2	10
Chlortetracycline	8	8
Tetracycline	2	2

and antitumour antibiotics was tested in relation to their possible selective inhibition of growth of the respiratory-deficient mutants (Table 4).

It can be seen that antitumour agents (in contrast to the antibiotics streptomycin and tetracyclines) selectively inhibit the growth of mutants with impaired oxidation.

It is interesting to record that the antitumour antibiotic mitomycin C inhibits the growth of mutants a hundred times more effectively than that of parents. The mechanism of action of this inhibitor on bacteria has been related to the selective damage to the synthesis of deoxyribonucleic acid[16].

In view of the fact that respiratory-deficient mutants of staphylococci differ from the parent organisms in a number of biochemical characters, a further analysis of the mechanisms of the selective inhibition of the growth of these mutants by antitumour compounds would be desirable.

Some of the observations reported in this article were started at the time of tenure by one of us (G. F. G.) of the World Health Organization fellowship at the National Institute for Medical Research, London. The senior author is grateful to Sir Charles Harington for hospitality and to Mr. J. W. Lightbown for many helpful discussions.

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AN INVESTIGATION OF CLOSELY RELATED GAMMA-MYELOMA PROTEINS AND NORMAL MOUSE GAMMA-GLOBULIN BY PARTIAL ENZYMIC DEGRADATION AND STARCH-GEL ELECTROPHORESIS

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GAMMA-GLOBULIN as normally found in the serum of mammals is highly heterogeneous in its physico-chemical characteristics. Investigation of its chemical structure is hampered by the difficulty of obtaining γ-globulin fractions consisting of single molecular species. The serum γ-globulin is derived from a large number of cells in many sites throughout the body[1]. The γ-myeloma proteins produced by one of the transplantable plasma cell tumours, by contrast, although very similar to normal γ-globulin in most respects, appear to be limited to a small number of molecular species, probably produced by a single plasma cell clone and may therefore provide a simpler model for structural studies of γ-globulin.

The serum of C_3H mice bearing one of the transplantable plasma cell tumours (*X*5563) contains a high concentration of γ-myeloma protein[2]. By analysis on starch-gel electrophoresis, this γ-myeloma protein was found to consist of five distinct components which are indistinguishable immunologically and had the same molecular size, but varied in electric charge[3]. In an attempt to discover a basis for the difference between these closely related γ-myeloma proteins produced by a single plasma cell tumour and possibly to throw light on the complexity of normal γ-globulin, we had recourse to partial degradation of the proteins with papain and cysteine. Such treatment has been shown by Porter[4] to split rabbit antibody into fragments with sedimentation constants of 3·5 and molecular weights of 50,000–70,000.

Purification of proteins and papain treatment. Normal γ-globulin was prepared from concentrated C_3H mouse serum by zone electrophoresis on blocks of 'Geon 425' (British Geon, Ltd.) by the method described in ref. 5. This preparation contained γ-globulin, migrating more slowly than β-globulin[6]. The 5563 myeloma proteins were prepared from serum of tumour-bearing mice by chromatography on columns of DEAE cellulose[7] using a slow elution gradient from 0·015 *M* to 0·05 *M* potassium phosphate buffer, *p*H 6·5 (ref. 6). Since the components of the myeloma proteins vary by only small differences in charge, only partial separation of the globulin molecules can be achieved and fractions of mainly one component contained also small amounts of adjacent components.

The characteristics of the purified proteins on starch-gel electrophoresis are seen in Fig. 1. The striking spread of the normal γ-globulins over a wide area is an indication of their heterogeneity whereas the γ-myeloma proteins form well-defined bands varying in mobility. Fig. 1 shows the myeloma protein components (*a*–*e*) in serum from a mouse

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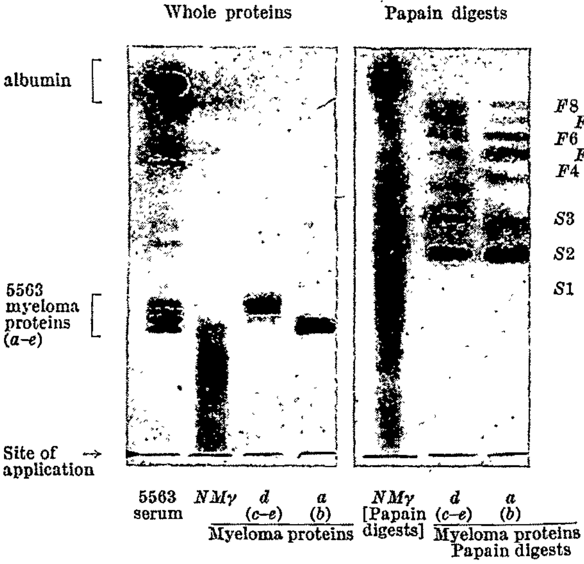

Fig. 1. Analysis by starch-gel electrophoresis. *NMγ*, normal mouse γ-globulin was prepared by zone electrophoresis; myeloma proteins : 5563 myeloma protein components were prepared by chromatography on DEAE (ref. 6); components *a*(+*b*) have a slight β-globulin contamination (band migrating faster); papain digests: purified *NMγ* and 5563 myeloma protein components shown on left photograph were treated with cysteine and papain by the method of Porter (ref. 4)

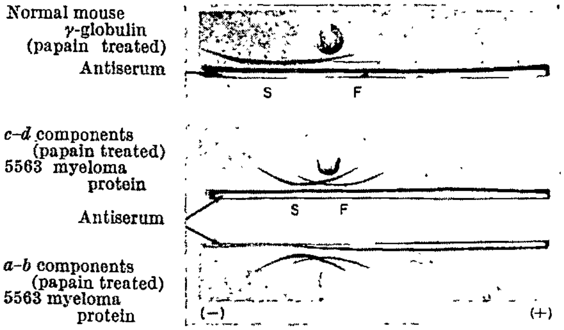
Normal mouse γ-globulin (papain treated)
Antiserum
S
F
c–d components (papain treated) 5563 myeloma protein
Antiserum
S
F
a–b components (papain treated) 5563 myeloma protein
(−)
(+)

Fig. 2. Immunoelectrophoretic analysis of papain-treated proteins. The antiserum used was a mixture of antiserum to normal mouse γ-globulin and the 5563 γ-myeloma protein. The proteins had been treated with papain and cysteine by the method of Porter (ref. 4)

bearing the 5563 tumour and the purified myeloma protein components $c + d$ and $a +$ some b. The myeloma proteins and γ-globulin sedimented at the same rate ($S_{20,w} = 6{\cdot}5$) in the ultracentrifuge.

The purified proteins were treated with papain and cysteine as described by Porter[4], except that incubation with the enzyme at 37° was continued for 1 hr. only. The reaction went to completion in this time with more than 85 per cent, of the protein recovered on weight basis, whereas longer periods of incubation resulted in losses of the split 5563 globulin. Analysis by ultracentrifugation was kindly carried out by Dr. P. Charlwood and showed that, as in the case of rabbit antibody[4], mouse γ-globulin and myeloma proteins were split into smaller molecules of S_{20} around 3·6.

Characterization of fragments in papain digests of purified proteins. The papain digests of normal γ-globulin and 5563 myeloma proteins were examined by immunoelectrophoresis against a mixed rabbit antiserum to normal γ-globulin and 5563 myeloma protein (Fig. 2). Treatment with papain and cysteine results in two types of fragments with distinct antigenic characteristics. Each digest gives two antigen–antibody precipitation arcs which cross. Similar results have been obtained with human γ-globulin (Edelman *et al.*[8]) and, for simplicity, these authors' nomenclature will be followed for the mouse globulin. The slowly migrating fragments (which move towards the cathode from the point of application) and the fast migrating fragments (which move towards the anode) have *S*- and *F*-antigenic grouping respectively. The observation that the precipitin arcs cross without interaction is taken to indicate that the *S* and *F* fragments have different structures and do not share any antigenic groupings.

Analysis by starch-gel electrophoresis revealed marked physicochemical heterogeneity of globulin fragments in the papain digests of these proteins (Fig. 1). The multiple well-defined protein bands, seen in the papain digests of the myeloma protein, are again in sharp contrast to the diffuse heterogeneity of the papain digest of normal γ-globulin. It also can be noted that the different myeloma protein components ($a(+\,b)$, $c + d$) yield fragments, some of which are identical and some of which differ qualitatively or are present in different quantities.

The papain digests were fractionated by chromatography on DEAE cellulose using gradient elution with potassium phosphate buffers of *p*H 8 (Fig. 3). Four protein peaks were obtained. These were concentrated by ultrafiltration and analysed by starch-gel electrophoresis and by gel diffusion in an Ouchterlony plate against antiserum to 5563 myeloma protein. As a result it was possible to classify the chromatographic protein fractions and the many physicochemically different fragments seen on starch-gel electrophoresis into two major groups (*S* and *F*) on the basis of their antigenic characteristics. Peaks S_1, S_2, S_3 (see chromatographic elution pattern, Fig. 3) were found to have the same antigenic groupings, giving reactions of identity when tested in a gel diffusion plate with antiserum to 5563 myeloma protein and normal mouse γ-globulin. This is shown for S_2 and S_3 in Fig. 4. The chromatographic peak *F*, however, had distinct antigenic grouping. Its antibody–antigen precipitation line crossed that of the S_3 fragment on an Ouchterlony diffusion plate (Fig. 4). Starch-gel electrophoresis revealed that chromatographic peaks S_1, S_2 and S_3 contained

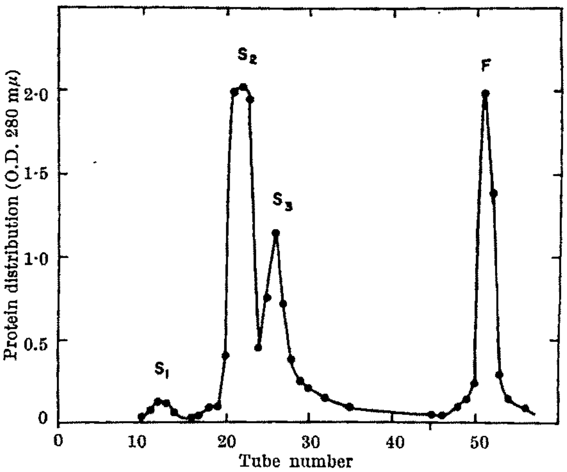

Fig. 3. Chromatography of papain digest of 5563 myeloma protein. Chromatography was carried out on columns of DEAE ion-exchange cellulose (ref. 7), using gradient elution with potassium phosphate buffer *p*H 8 as indicated on the graph. 3-ml. fractions were collected

Table 1. Sedimentation Constants

	S_{20} Whole protein	S_{20} Papain digest
Normal γ-globulin	6·5	3·86
5563 Myeloma protein (components *a–d*)	6·6	3·62
Papain digest 5563 myeloma protein		
S_2		3·73
S_3		3·71
F_5–F_8		3·51

The sedimentation constants were kindly determined by Dr. P. Charlwood on samples extensively dialysed against saline-phosphate buffer, *p*H 7·4 at concentrations of 0·3–0·6 per cent (w/v).

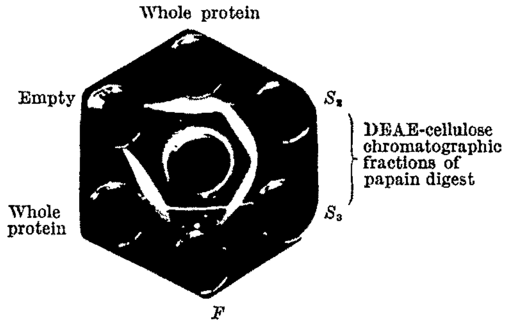

Fig. 4. Antigenic comparison of papain fragments of 5563 myeloma protein by agar diffusion. Centre cup, rabbit antiserum against 5563 myeloma protein; antigen was present at a concentration of 0·2 mgm. protein/ml. Whole protein refers to 5563 myeloma protein purified by DEAE exchange cellulose (ref. 3). Fractions S_2, S_3 + F were prepared from 5563 myeloma protein treated with papain and cysteine by chromatography or DEAE (Fig. 3)

starch-gel bands S_1, S_2 and S_3, respectively. Peak F, however, consisted of a number of fragments in the F_5–F_8 positions on starch-gel electrophoresis. Table I shows that the fragments S_2, S_3 and F had very similar sedimentation constants in the ultracentrifuge (S_{20} = 3·5–3·7).

Comparison of different 5563 *myeloma proteins*. It was mentioned earlier that plasma cell tumour 5563 secretes γ-myeloma proteins consisting of five components (*a–e*) which have slightly different mobilities on starch-gel electrophoresis. The difference in electrical charge between 5563 myeloma molecules *a* and *d* must be at least 3 single or multiple charge units in view of the regularity in increase of mobility. The separated components on treatment with papain and cysteine break down to fragments giving different patterns on starch-gel electrophoresis (Fig. 1). For purposes of discussion only fragments composing more than 5 per cent of the total protein will be considered. Papain digest of 5563 myeloma protein *a* (+ *b*) will be considered first. The starch-gel pattern shows a strong band in position S_2—there is a weak band migrating more slowly than S_3 which is due to contamination of the original myeloma protein with β-globulin. There are four bands, F_4–F_8, with F-antigenic grouping which migrate faster than the S bands. An approximate estimate of the quantity of different fragments may be obtained by making the starch-gel transparent and staining in glycerol by the method in ref. 9. The colour intensity of the bands is measured by a recording microdensitometer. This method tends to under-estimate the relative amount of fragment S_2 present in high concentration and over-estimate the minor components (F_4–F_8). Similar estimates were made by calculating recovery of different protein fragments from the chromatographic elution patterns (Fig. 3). The combined estimations are seen in Table 2. Both methods are very approximate, but indicate that fragment S_2 represents about two-thirds of original globulin molecule and fragments F_5–F_8 one-third. Myeloma protein *a*(+*b*), on treatment with papain and cysteine, thus splits into a single fragment (S_2) with S-antigenic grouping (two-thirds of total protein) and a number (four) of fragments with F-antigenic grouping representing roughly one-third of the original protein.

The papain digest of myeloma proteins *c* + *d* shows a slightly different pattern on starch-gel electrophoresis (Fig. 1) and yields two fragments with S-antigenic grouping, one in the same position as S_2 and a fragment with greater mobility, S_3. In addition, four or five fragments with F-antigenic grouping can be observed. They are present in different quantities from those in the papain digest of myeloma protein *a*(+ *b*). A quantitative estimate of the relative amounts of papain fragments shows that we have about 36 per cent of S_2 and 20 per cent of S_3. Fragment S_2 and fragment S_3 with S-antigenic grouping represent about two-thirds of the original myeloma protein, whereas the F-fragments (F_5–F_8) represent about one-third (Table 2). Table 2 also shows the relative distribution of fragments when myeloma proteins *b* + *c* with intermediary electrophoretic mobilities are treated with papain and cysteine. In this case 15 per cent of fragment S_3 is formed. The ratio S_2/S_3 thus decreases, when myeloma proteins *a*,*b* + *c*, and *c* + *d* are broken down, but the yield of total fragments with S-antigenic groupings stays constant and represents roughly two-thirds of the original protein (65–72 per cent by our estimations). Once more the relative amount of total fragments with F-antigenic grouping stays constant representing 28–35 per cent and being about one-third of the original protein molecule. The relative concentrations of F-fragments with different mobilities vary—when the faster migrating myeloma proteins are degraded higher concentrations of faster migrating F-fragments appear. The mobility of the original myeloma proteins thus appears to depend on variation in electric charge in both the fragments with S- and F-antigenic groupings.

In general these results agree with the results obtained by Porter[4], which suggested that rabbit antibody globulin split into three fragments roughly one-third the size of the original molecule. The 5563 myeloma protein, on treatment with papain and cysteine, appears also to split into thirds, two fragments with S-antigenic grouping and one fragment with F-antigenic grouping. The slowest migrating myeloma protein yields a single S-fragment (S_2) two-thirds of the original protein, but a number

Table 2. Estimate of Relative Amounts of Protein Fragments resulting from Papain and Cysteine Treatment of 5563 Myeloma Proteins

Myeloma proteins treated with cysteine and papain	*a* (+*b*)	*b*+*c*	*c*+*d*
Papain digest fragments	Per cent of total protein		
S_2*	52·5	46·5	33·5
S_3*	5·6	19·5	22
F_5*	21	14·6	7·3
F_6*	10·4	13	13·6
F_7*	7·3	3	14·2
F_8*	2·7	3	9·5
S_2†	67	55	36
S_3†	5	15	29
Total S†	72	70	65
Total F†	28	30	35
Ratio total S/F†	2·5	2·3	1·9
Ratio S_2/S_3†	13·4	3·6	1·2

* Values are approximate and based on measurement of colour intensity of stained protein bands after electrophoresis on a starch-gel block stained in glycerol (ref. 9). This method is known to under-estimate the components present in high concentration compared with those present in smaller amounts.

† Values based on protein elution pattern resulting from chromatography of papain digests on DEAE cellulose (Fig. 3). This method is quantitatively more reliable, but does not distinguish the different F-components.

of *F*-fragments (varying in mobility on starch-gel electrophoresis). Myeloma proteins with increasing electrophoretic mobility yield S_2 and increasing amounts of fragment S_3, which migrates faster than S_2, but the total amount of $S_2 + S_3$ remains constant. A quantitative variation in the *F*-fragments is also observed when myeloma proteins with increasing mobility are degraded. A single difference between S_2 and S_3 could not account for five original myeloma components, and it is therefore likely that there are also variations in the *F*-portion of the molecule. It remains to be seen whether the multiplicity of the *F*-fragments resulting from myeloma protein $a(+b)$ are a consequence of progressive enzymic digestion of a single *F*-fragment or whether a single myeloma protein band can contain different molecular fragments with *F*-antigenic grouping to start with. The gradual loss of fragments with *F*-antigenic grouping, if incubation with cysteine and papain is continued for more than 1 hr., may support the first possibility. This point and the difference between S_2 and S_3 are being investigated further.

We are indebted to Dr. P. Charlwood for determinations of sedimentation coefficients by ultracentrifugation, to Dr. M. Potter for sending us the 5563 plasma cell tumour line, and to Dr. R. R. Porter and Dr. J. W. Goodman for a gift of papain. We should like to thank Dr. R. R. Porter and Dr. J. H. Humphrey for helpful discussions, and Mrs. Marion Perryman for her assistance.

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RESPIRATORY EFFECTS OF PHOSPHORYLATION

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OXIDATIVE phosphorylation is the main mechanism for transfer of energy from the respiratory system by converting adenosine diphosphate (ADP) to adenosine triphosphate (ATP)[1,15]. The reaction

$$\mathrm{ADP} + \mathrm{PO_4} + \text{energy} \rightleftharpoons \mathrm{ATP} \qquad (1)$$

is controlled by a number of enzymes, enabling ATP to transfer energy to many synthetic processes in the protoplasm. In oxidative phosphorylation the supply of energy on the left side of equation (1) is delivered by the stream of electrons moving from an enzyme of lower oxidation potential to an enzyme of higher potential (the difference is 0·17 V. for a two-electron process). ATP and similar compounds are thus serving as the main energy-distributing system, the inhibition of which leads to rapid degradation of the living structure.

The mechanism of oxidative phosphorylation is still incompletely known. It has been claimed that the energy from a flow of electrons between a donor (*B*) and an acceptor (*A*) may be transferred to ATP over an intermediate reaction[2-4,15]. Phosphate may, at a low expense of energy, be combined with one of the enzymes, for example, as $B - \mathrm{PO_4}$, and then, on oxidation by *A*, be transferred to the high-energy state of ATP. Another possibility is phosphorylation by means of an intermediate substance, for example, a quinone[2]. It is postulated in both cases that the electrons are transferred from *B* to *A* through intermediate reactions. Even if other more direct pathways of transfer between *A* and *B* exist, phosphorylation would consequently serve as an accelerator of the stream of electrons through the respiratory system. This suggests the possibility of observing the effect of phosphorylation and the possible sites of this process by measuring the apparent reaction velocities of the enzymes involved.

A suitable technique has been developed on the basis of new spectrophotometric methods for accurate measurement of the time-course of reduction and reoxidation of all respiratory enzymes[7,10,11]. These methods may be applied to live intact tissues or cell suspensions. Owing to the inevitable partial invalidation of the respiratory unit, particulate fractions of homogenates are less suitable for these studies. The present article deals with oxidative phosphorylation in bakers' yeast. The experimental technique was based on the reversibility of the steady-state reaction (1). It was shown[8] that, in wheat-roots, supply of inorganic phosphate promotes an increasing reduction of the cytochromes, whereas the level of oxidation is raised by addition of ATP (ref. 8). These results were interpreted in terms of a displacement of the steady-state equation (1) by an increase or a decrease of concentration of the reactants. Nucleotides penetrate the protoplasm easily. Application of ADP + phosphate or of ATP to a yeast suspension affects the course of electron transfer in less than 3 min.

The experiments were conducted as follows: Bakers' yeast (from Svenska Jästfabriks AB) is thoroughly washed and 16 ml. of a 5–10 per cent suspension is introduced into a quartz circulation vessel[9,11]. The recipient is placed immediately in front of an *EMI* quartz photomultiplier tube 6256 connected to a Philips oscilloscope *GM* 5666. By means of a special electromechanical device[11], which moves the wave-length screw of a Bausch and Lomb grating monochromator rapidly between two end-intervals[11,12], a suitable portion of the spectrum is recorded in a Zeiss Frequentophot camera. The bands of cytochromes *c* (550 mμ) and *b* (562 mμ) are directly visible on the record[12]. Other band-peaks (diphosphopyridine nucleotide (DPNH) 340, flavoprotein (FP) 465, cytochromes a_3 444 and 598, c_1 554, and *a* 604 mμ) are caught by means of a mechanical device which interrupts the oscillographic image at these wave-lengths[11].

Precise measurements of band-heights postulate intimate knowledge of spectral coincidences and of the spectra of purified enzymes[9,11,12]. The time-course of the reduction of the enzymes at stopped aeration (or shifting over to nitrogen) and the reoxidation in air (or oxygen) may be varied, owing to the degree of exhaustion of respiratory substrates in the washed yeast, and the level of temperature, etc. Reduction of the respiratory enzymes is completed in a few seconds at 18° C., but reoxidation may sometimes take only 1–2 sec. Experience has shown that a speed of 4 spectrograms per sec. in all circumstances secures a reliable calculation of the half-times (ht = 50 per cent reaction) of reduction and reoxidation. The apparent velocity is calculated as c/ht (c = reacting portion of the enzyme).

A new technique was introduced for measuring the intensity of respiration during the single experiments. It was shown in this laboratory that the time-lag between turning off the air stream and the start of reduction of the cytochromes $c - a_3$ is inversely proportional to the velocity of respiration, provided that the periods of aeration are held constant. This demand is fulfilled by the introduction of a timer which, by means of electromagnetic valves, regulates the intervals of aeration (15 sec.) and stopped air-flow (mostly 75 sec.). Processes which are not directly linked to the respiratory chain are eliminated by the intermittent aeration.

At the start of the single experiments the apparent velocities of reduction and reoxidation in distilled water were recorded. Immediately following the start, the test substance was added to the recipient. The response was automatically recorded in 4, 6, 10, 16 min.

Results

Addition of ADP, with or without phosphate (the latter is always present in yeast cells), results in an increase of the respiration (Fig. 1), the maximum of which is attained in 3–4 min. As shown in Table 1, the respiratory effect is caused by an acceleration of the stream of electrons, namely, an increased velocity of reduction of the group $a_3 - c$ and of DPN. This increased reduction conveys a corresponding retardation of the reoxidation, because the off-reaction of cytochrome oxidase, namely, the transition $a_3 \xrightarrow{e} O_2$ remains approximately unaffected. Measurements show this to be the case (cf. Figs. 1, 2). As a consequence of the increased internal supply of electrons at constant velocity of the transference $a_3 \xrightarrow{e} O_2$, the cytochromes c and a_3, and flavoprotein are 20–40 per cent more reduced in the aerobic state whereas cytochrome b and DPN remain more unaffected. The balance between oxidized and reduced enzyme is determined by the relation k_1/k_2 in the sequence:

$$\xrightarrow[k_1]{e} \text{enzyme} \xrightarrow[k_2]{e}$$

on-reaction off-reaction

k_1 and k_2 represent the velocities of the on- and off-reactions.

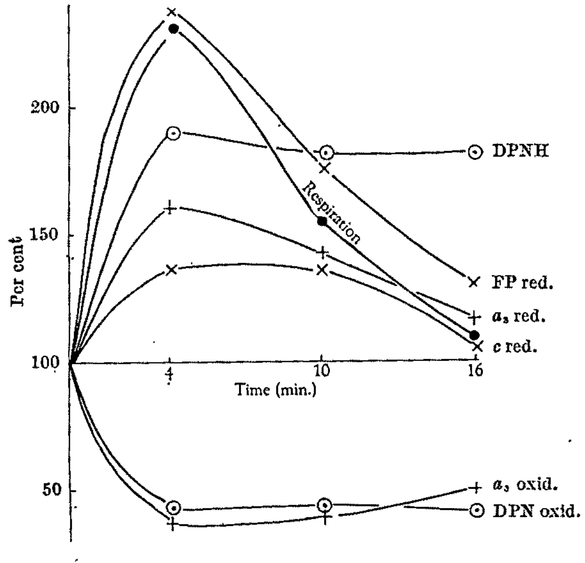

Fig. 1. Response of respiration and apparent velocities ($1/ht$) of reduction and reoxidation on addition of 0·01 M ADP + phosphate to a yeast suspension

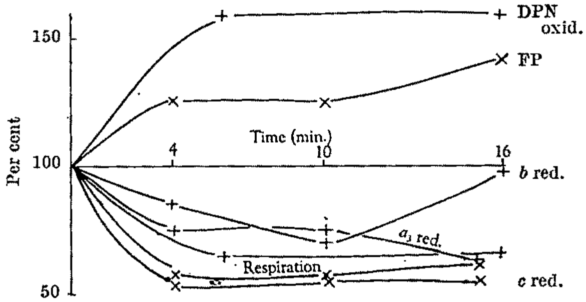

Fig. 2. Response of respiration and apparent velocities ($1/ht$) of reduction and reoxidation on addition of 0·01 M ATP to a yeast suspension

The fact that the step $a_3 \xrightarrow{e} O_2$ does not respond to ADP leads to the conclusion that this step is not involved in phosphorylation. The only known case of an actual acceleration of the stream of electrons from cytochrome oxidase to oxygen is the salt effect, the nature of which has been discussed elsewhere[10,13,14]. Another important difference between the effects of ADP and salts is the response of cytochrome b. In contrast to the uniform response of the total chain of enzymes to salts, glucose and alcohol, ADP reveals a segregation inasmuch as the reduction of cytochrome b is not accelerated.

This individual behaviour of cytochrome b does not mean, however, that it is bypassed by the electrons. An intensification of the stream of electrons between b and $c - a_3$ creates a 'vacuum' at b, appearing as a relatively retarded reduction. The observed facts are leading to the conclusion that the transition $b \xrightarrow{e} c$ is one of the main sites of oxidative phosphorylation. Cytochromes c and a_3 are, as previously shown[12], closely linked together kinetically and the observed identical response of these two enzymes (Table 1) leaves little room for intermediate reactions.

The response of flavoprotein to variations in the ADP/ATP balance is not identical with that of

Table 1. Change of the Relative Velocity of Reduction (at Stopped Aeration) 3–6 min. after Addition of 100 mgm. ADP of ATP (Disodium Salt), corresponding to Concentration of 0·01 mole/l. ht = time of 50 per cent Reaction (sec.)

	Cyt. a_3	Cyt. c	Cyt. b	Flavoprotein	DPN
$1/ht$ in water	0·36	0·38	0·22	0·23	0·06
Response on ADP	0·61 (+ 68 per cent)	0·60 (+ 57 per cent)	0·13 (− 42 per cent)	0·29 (+ 26 per cent)	0·13 (+ 84 per cent)
Response on ATP	0·23 (− 37 per cent)	0·27 (− 30 per cent)	0·20 (− 9 per cent)	0·13 (− 42 per cent)	0·04 (− 31 per cent)

Similar values are obtained 10–12 min. after addition of the reagents.

cytochrome b. It was shown earlier that flavoprotein sometimes acts simultaneously with cytochrome b and that one fraction of it may be linked structurally to the latter[12]. The total flavoprotein is, on the other hand, distributed over at least two enzyme systems, of which one is identical with succinodehydrogenase. This fact may explain the rather varying response of flavoprotein in the present experiments. The results are, however, not opposed to a possible site of phosphorylation near flavoprotein (for the effect of succinate oxidation on phosphorylation see ref. 16).

DPN is known to participate in a large number of enzyme reactions. Table 1 shows a strong acceleration of the step substrate $\xrightarrow{e}$ DPN under the influence of ADP and a corresponding retardation by ATP; these facts illustrate the well-known role of substrate oxidation by DPN in oxidative phosphorylation.

The theoretically expected reversal effect of ATP, as compared with ADP, is illustrated by the steady-state equation (1) (Table 1 and Fig. 2). Addition of ATP retards the transport of electrons from substrates to DPN and from cytochrome b to c. Consequently, the aerobic respiration declines. Values of only 50 per cent of that in distilled water were observed (Fig. 2). The steady-state oxidation – reduction of respiratory enzymes in aerated yeast, treated with ATP, shows an increasing oxidation, primarily, of DPN and the cytochromes b and a_3.

2,4-dinitrophenol (DNP) inhibits phosphorylation, but it acts, nevertheless, similarly to ADP. It accelerates the electron transfer from cytochrome b to the group $c - a_3$ (Table 2 and Fig. 3). This fact indicates a competitive effect of DNP, namely, this substance is obviously capable of transferring electrons between b and $c - a_3$, as a consequence of which the respiration may be raised above the level held in distilled water.

It is interesting to note that the 'vacuum' at cytochrome b is observed also with DNP, a fact emphasizing the probable competitive interaction of this substance in the phosphorylation mechanism. Attention is here again directed to the fundamentally different effect of salts.

Table 2. Effect of 1·3 × 10^{-5} *M* 2,4-Dinitrophenol (DNP) and further addition of 1·3 × 10^{-2} *M* Potassium Chloride (DNP + Potassium Chloride) on Respiration and Kinetics of Enzyme Reactions

A. Reduction at stopped aeration

Medium	Respiration (relative)	Apparent velocities (1/*ht* sec.)		
		a_3	c	b
Water	100	0·36	0·38	0·22
DNP 10 min.	125 (+ 25 per cent)	0·45 (+ 25 per cent)	0·51 (+ 34 per cent)	0·28 (+ 27 per cent)
DNP + potassium chloride 10 min.	167 (+ 67 per cent)	0·63 (+ 75 per cent)	0·57 (+ 50 per cent)	0·39 (+ 77 per cent)

B. Reoxidation in air

Medium	Apparent velocities (1/*ht* sec.)		
	a_3	c	b
Water	0·63	1·00	1·00
DNP 10 min.	0·78 (+ 23 per cent)	1·07 (+ 7 per cent)	0·85 (– 15 per cent)
DNP + potassium chloride 10 min.	1·30 (+106 per cent)	1·26 (+ 26 per cent)	1·55 (+ 55 per cent)

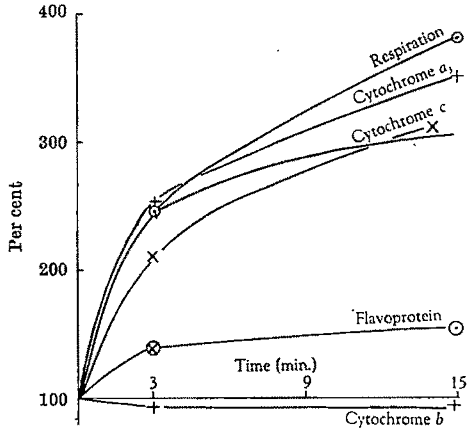

Fig. 3. Response of respiration and apparent velocities of reduction (1/*ht*) on addition of 1·3 × 10^{-5} *M* 2,4-dinitrophenol

Salts are obviously promoting an unspecific electron transference through the respiratory chain, primarily by facilitating the passage of electrons from a_3 to oxygen. Table 2 illustrates the important fact that the salt effect may be superimposed on the DPN effect, resulting in an over-powered electron transfer, which is suggested by a further rise of the respiration.

The unspecific stimulation by salts (electrolytes) produces no vacuum at cytochrome b (Table 3). Salts obviously stimulate both the more direct transfer of electrons and, by indirect stimulation, of $a_3 \xrightarrow{e} O_2$, also the oxidative phosphorylation. This was concluded in previous investigations on the mechanism of absorption and accumulation of salts[10].

Table 3. Effect of Inorganic Phosphate as compared with ADP + Phosphate on the Apparent Velocities of Reduction
Values in percentage of those observed with water (cf. Tables 1 and 2)

Medium	a_3 (per cent)	c (per cent)	b (per cent)	Flavoprotein (per cent)
Potassium phosphate (0·02 *M*; *p*H 6·2)	+ 68	+ 41	+ 98	+ 50
0·01 *M* ADP + phosphate	+ 68	+ 57	– 42	+ 26

Discussion

The results are in agreement with other work[2-4,15] on phosphorylation by means of intermediate reactions between two respiratory enzymes. Because these reactions are transferring electrons, oxidative phosphorylation acts similarly to a lowering of the internal resistance to the stream of electrons flowing through the system. The organized respiratory unit is envisaged here as a semiconductor, in which electrons are forwarded by means of exchange reactions between atoms and groups of atoms. Reversal of the concentration gradient ADP + phosphate > ATP acts similarly to an increase of the internal resistance, because the intermediate reactions are slowed down according to the law of mass action.

It has sometimes been claimed that two respiratory enzymes which are co-operating in oxidative phosphorylation do not react directly with one another[4]. This idea is at variance with the present experiments.

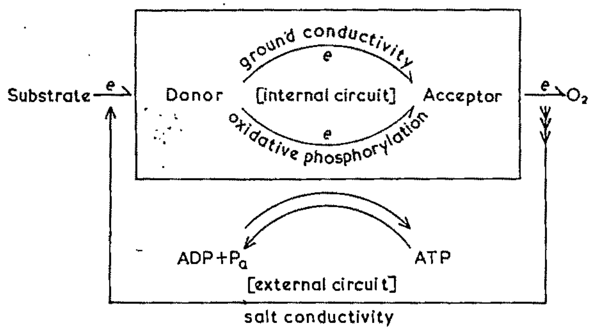

Fig. 4. Scheme of the inner and outer electric circuits in which the flow of electrons is in part controlled by the intensity of oxidative phosphorylation, namely, the activity relations of ADP and ATP, and by the activity of salts in the area surrounding the respiratory unit

My results point to the existence of other eventually more direct pathways, also, which, however, are insufficient for the maintenance of a maximum rate of respiration. There is, of course, a possibility that energy-saving mechanisms other than phosphorylation are co-operating in this 'ground conductivity' of the space between two respiratory enzymes.

The kinetic characteristics of the respiratory unit suggest the conclusion that the enzymes are organized as multimolecular sub-units which, owing to the structurally guided electron transference, are reacting at special points ('reaction centres') or surfaces[12]. This means that the electrons may be sluiced between two enzymes via intermediate processes ('factors') which are mediating the contact. When the supply of oxygen is stopped, the cytochromes c and a_3 start reduction (Table 1). The reduction of cytochrome b starts only after cytochrome c is almost reduced.

It was assumed that the multimolecular sub-unit $c - a_3$ is attached to cytochrome b at only one point or surface and that this area of contact remains oxidized until the $c - a_3$ complex is nearly saturated by electrons[12]. Switching on oxidative phosphorylation accelerates the process. My results convey the conclusion that the transference $b \overset{e}{\rightarrow} c$ is one of the main sites of oxidative phosphorylation, whereas the step $a_3 \overset{e}{\rightarrow} O_2$ does not participate directly.

The characteristic qualities of oxidative phosphorylation and of salts (electrolytes) in promoting the electron transfer through the respiratory system are shown in Fig. 4. Unlike DNP, which to some extent can substitute ADP in the intermediate electron transfer, salts do not compete with phosphorylation. Salts, on the contrary, act indirectly as powerful promoters of phosphorylation, mainly because the step $a_3 \overset{e}{\rightarrow} O_2$, which responds promptly to an total flow of electrons through the system.

The respiratory unit operates similarly to an electric generator[13], built on the basis of semiconductivity. An internal stream of electrons flows from the substrate to oxygen. The overall intensity (electronic density) of the stream is controlled by the electric conductivity of the outer circuit, namely, primarily the concentration (activity) of easily movable salt anions in the surroundings of the system. Salts accelerate non-specifically all internal pathways of electrons, hence the total aerobic respiration. The readiness of the substrate to deliver electrons is, of course, a fundamental regulator of respiration too, but it does not, unlike salts, affect the end-oxidation, namely, the velocity of the step $a_3 \overset{e}{\rightarrow} O_2$.

Salts thus regulate the external resistance to aerobic respiration, and oxidative phosphorylation implies an important regulator of the internal resistance. According to the scheme in Fig. 4, salts also accelerate the electrons which enter the respiratory system from the substrate side. Whereas yeast and animal cells are characterized by only one main exit of electrons, namely, at cytochrome a_3, there are at least two points of admission, but facts as to the biochemical pathways are here still incomplete. We know that the respiratory enzymes are more or less intimately bound to the cell structure, a_3 and b, very firmly, and c and flavoprotein somewhat more loosely. DPN is one of the most versatile co-enzymes. It is possibly synthesized in the nucleus[6] and moves around freely. My results on the acceleration of electrons from the substrate to DPN by ADP + phosphate illustrate the well-known fact that sites of phosphorylation are located between DPN and the substrates.

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COLICINE FACTORS AS FERTILITY FACTORS IN BACTERIA

Salmonella typhimurium strain *LT2*

SALMONELLA TYPHIMURIUM strain *LT*2 does not form and is not killed by colicines *E*1, *E*2, *I* or *K*[1,2]. However, it can be made colicinogenic for any of these colicines by growth in mixed culture with an appropriate colicinogenic strain of Enterobacter, presumably as a result of the transfer of a genetic element (colicine factor) controlling the production of a particular colicine. Colicine factor *I* (determining production of colicine *I*), when introduced into *S. typhimurium*, strain *LT*2, can be transmitted by cell contact to other sub-lines of strain *LT*2; and it also potentiates the transmission of colicine factors *E*1, *E*2 and *K* which, in *S. typhimur-*

ium, are not otherwise transmissible by cell contact (or, in the case of factor *E*1, only at a very low frequency). We report here that in *S. typhimurium*, strain *LT*2, factor *I* also makes possible recombination of chromosomal characters following cell conjugation, although the frequency is very low (10^{-6}–10^{-8}) compared with the frequency of transfer of colicinogeny (10^{-1}).

In a broth culture of an *LT*2 sub-line which has carried factor *I* for some time, only a small fraction (1/6,000) of the cells can transmit colicinogeny by cell contact[1]. However, Stocker and Smith[3] found that just after acquiring factor *I*, most cells transmit not only this factor but also any other factors the cell may possess. The increased ability to transmit persists for only 3–7 generations. In recombination experiments, therefore, the bacteria of the parent strain intended as 'donor' of colicinogeny had to be newly infected with factor *I* by growing them with cells possessing this factor. For example, when the donor strain was to be *cys D*–36 (an auxotrophic sub-line of strain *LT*2 requiring cysteine), it was infected with factor *I* by inoculating broth with about 10^6 cells/ml. of *cys D*-36 and about 5×10^4 cells/ml. of *cys D*-36 (*I*), that is, the same strain already carrying factor *I*. After overnight incubation at 37° C., the mixture was diluted 1/10 in fresh broth and incubated for 2 hr. at 37° C.; during this period, about 40 per cent of *cys D*-36 cells newly acquired factor *I* and, consequently, were all able to transmit it.

This 'donor mixture' was then incubated for 1 hr. at 37° C. with an equal number of acceptor cells (for example, *ath C*-5, a sub-line of strain *LT*2 requiring adenine + thiamine) and washed samples plated on unsupplemented glucose–ammonium sulphate medium to detect prototrophic (*cys* D^+ *ath* C^+) recombinants. About one prototrophic colony was formed for 10^8 cells plated, whereas control platings (*cys D*-36 alone; *ath C*-5 alone; and mixture of non-colicinogenic *cys D*-36 and *ath C*–5) gave either no colonies, or a significantly smaller number attributable to reversion. If the *cys D*-36 component of the donor mixture was replaced by the same strain carrying colicine factor *E*1 (*cys D*-36(*E*1)), the yield of recombinants was increased about one hundred-fold. The presence of factor *E*1 in the acceptor strain, however, has little or no effect on the yield of recombinants. Also, factor *E*1 did not permit recombination in the absence of factor *I*. Thus, the presence of factor *E*1 in the donor strain increases the rate of recombination when factor *I* is being transmitted, but does not by itself permit recombination.

The *cys D*-36 (*I*) minority component of the donor mixture, used to introduce factor *I* into the majority component *cys D*-36 (or *cys D*-36 (*E*1)), could be replaced by strain *ath C*-5 (*I*) without reduction of the yield of recombinants. This and other experiments showed that the *ath* C^+ genes of the recombinants came from the strain of the donor mixture initially lacking factor *I*; presumably from bacteria which had just acquired this factor.

Recombinants were obtained, at about the same frequencies, from crosses involving acceptor strains carrying many different contra-selective markers (*ade A*, *B* and *C*; *ath C* and *E*; *ara*; *cys A*, *B*, *C* and *D*; *gly; his; isol; leu; pro; thr; try A* and *D*; *str-s*). In fact, positive results were obtained for all loci tested. Pairs of genes which can be simultaneously transduced by phage *PLT*22, for example, *try D* and *cys B*, remain coupled more frequently than they do during phage-mediated transduction.

Differing donor alleles occupying two or more loci which cannot be simultaneously transduced by phage *PLT*22 are found together in recombinants formed during transfer of colicine factors too frequently for them to have been transferred separately to the same donor cell in different mating events. For example, either *ara* or *isol* occurs separately at a frequency of 10^{-7}, while together, they occur at 10^{-8}. This suggests that long portions of the bacterial chromosome can recombine; perhaps, at least in some cell-pairs, the entire chromosomes of the two pairing bacteria.

Colicine factor *I* was introduced into our sub-lines of *S. typhimurium*, strain *LT*2, by growth with *Shigella sonnei*, strain *P*9, and factor *E*1 by contact with *Escherichia coli*, strain *K*12–30 (both strains were kindly supplied by Prof. P. Fredericq, of Liège). Furness and Rowley[4] found that most wild strains of *E. coli* which produced colicine *I* carried *F* (fertility) factors which, however, could be separated from their colicine factors. We considered the possibility that our colicinogenic *S. typhimurium* strains could recombine because they had acquired *F* factors at the same time as colicine factors; but our colicinogenic *LT*2 strains do not recombine like *LT*2 strains carrying an *F* factor from *E. coli* (Zinder[5] and personal communication).

All the recombinants which have been analysed have been stable, and are thus presumably haploids produced by segregation after chromosomal recombination in a (transient) zygote or merozygote. All the recombinants tested were colicinogenic, nearly all producing colicines *I* and *E*1 in crosses where both factors were present in the donor mixture, whereas only about 25 per cent of acceptor bacteria acquire colicinogeny during the time allowed for mating. We suppose, therefore, that a zygote (or merozygote) results from a conjugation in which colicine factors are transferred.

It is not yet completely certain that gene transfer occurs only from the colicinogenic donor to the acceptor strain, and not also the other way about. However, this is probably so since the result of a cross is affected according to which parent is colicinogenic. For example: (1) in some crosses at least, the unselected markers of the strain accepting colicinogeny are much more frequent in recombinants than are their alleles from the donor strain; (2) colicine factor *E*1 greatly increases the frequency of recombination when it is present in the donor strain, but not when it is present in the acceptor strain; and (3) exposure of the mating mixture to streptomycin for 90 min. much reduces the yield of recombinants when the acceptor strain is streptomycin-sensitive and the donor strain is streptomycin-resistant; but has little effect when the acceptor of colicinogeny is streptomycin-resistant and the donor is sensitive.

Recombination in this system may be fundamentally similar to that mediated by the *F* factor in *Escherichia coli*. The presence of colicine factor *I* in *Salmonella typhimurium*, like that of the *F* factor (in either the *F* + or the *Hfr* form) in *E. coli*, enables the bacteria to pair, although the effect of factor *I* is very small except in bacteria which have recently acquired it; in factor *I*-induced pairing, as in pairing induced by the *F* factor in its *F* + state, chromosomal recombination occurs at a very low rate. The effect of factor *E*1 in raising the rate of recombination in factor *I*-induced pairing is more obscure; one may speculate that it facilitates the opening of the (presumed) closed-loop chromosome of the donor bacterium, so encouraging its transfer, in whole or in part, to the acceptor bacterium (compare the effect of the *F* factor in *E. coli* on passing from the *F* + to the *Hfr* state).

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Escherichia coli K-12

THE transfer of genetic material from one strain of *Escherichia coli K*-12 to another is dependent on a fertility factor, termed *F*, which determines the donor state; two *K*-12 strains devoid of *F* (F^--strains) are normally non-fertile when mixed together[6,7]. The *F* factor has been classed as an *episome* by Jacob, *et al.*[8], existing in the autonomous (cytoplasmic) state in the F^+ cell, and in the integrated (chromosomal) state in the *Hfr* cell. The integrated state of *F*, and of other episomes, appears to be related to the ability to promote the genetic transfer of other stably chromosomal characters. Colicinogeny (the heritable ability to produce an antibiotic-like protein called colicin) has also been shown to be determined by factors which have certain episomal characteristics[8].

Two lines of evidence have pointed to the implication of colicinogeny as a genetic vector in *K*-12. Furness and Rowley[9] reported that certain naturally occurring strains of *E. coli*, although showing no direct evidence of fertility with *K*-12 F^- strains, could, nevertheless, transfer to them a fertility factor which rendered these *K*-12 strains fertile with other *K*-12 strains. Unlike *E. coli K*-12, most of these wild-type coliform strains were found to be colicinogenic, the majority for colicin *I*, as were the infected *K*-12 F^- derivatives. However, colicinogeny and fertility do not appear to be determined by the same factor in these strains. This conclusion was reached after treatment with either cobalt chloride[9] or acridine orange, which have been shown to be effective in 'curing' or eliminating the *F* factor from F^+ strains[10,11]. After treatment, the fertile, colicinogenic *K*-12 strains lose their fertility but preserve their colicinogeny.

More recently, Ozeki *et al.*[12] have demonstrated the transfer by conjugation of what appear to be large segments of chromosome from a donor strain to a recipient strain of *Salmonella typhimurium*. The donor state in this case is conferred by the *recent* transfer of colicinogeny from a third strain which is specifically colicinogenic for colicin *I*. If the donor strain is in addition colicinogenic for colicin *E*1, the frequency of transfer is increased about 100-fold, to yield one recombinant/approximately 10^6 recipient cells. These colicinogeny determinants, *I* and *E*1, can be transferred to *E. coli K*-12 by mixing a culture of this strain with a culture of *S. typhimurium* which carries them[13]. An investigation was therefore undertaken of the fertility state of F^- *K*-12 strains subsequent to the transfer of these particular colicinogeny determinants. Two auxotrophic F^- strains of *E. coli K*-12 were employed: 58–161 F^-, which requires methionine, and *W*945 F^-, which requires threonine, leucine and thiamine for growth. In the first experiment, the method of Ozeki *et al.* was followed closely. *S. typhimurium* carrying colicinogeny *I* (col. I^+) and 58–161 F^- were grown overnight in mixed broth culture; the mixture was then diluted 1/50 in fresh broth and reincubated for 2 hr. A culture of the recipient strain *W*945 F^- was then added and the mixture of these three strains incubated for a further hour before washing and plating on minimal medium supplemented with thiamine. After incubation, prototrophic recombinants resulted at the low rate of about one in 10^8 *W*945 F^- cells plated. None of the individual strains, alone, nor in any combination of pairs, gave rise to any prototrophic colonies.

In contrast with the *S. typhimurium* system, the use of the presumptive donor strain 58–161 F^-, made stably colicinogenic for *E*1, failed to increase the frequency of recombinant formation. Another point of difference is that, in *E. coli K*-12, the donor state conferred by colicinogeny *I* is stable and does not depend on recent colicinogenization. When a strain of 58–161 F^-, stably colicinogenic for *I* (isolated from mixed culture with *S. typhimurium* (col. I^+)), was used in a simple bi-parental cross with *W*945 F^-, prototrophic recombinants again arose at the same low rate as before. All the recombinants tested from these experiments were colicinogenic, and fertile as donors in crosses with F^- recipients. It is, therefore, concluded that colicinogeny *I* confers on *K*-12 F^- strains the capacity for concomitant chromosomal and colicinogeny transfer to other F^- strains. Since *Salmonella* apparently cannot readily harbour *F*, it seems possible that in this system, as distinct from that reported by Furness and Rowley, the fertility factor is the colicinogeny determinant itself. This is supported by the fact that no recombinants arose when *Salmonella* strains, differing only in harbouring colicinogeny *E*1 or *E*2 instead of *I*, were used in experiments similar to those reported above.

A comparison was made of unselected marker transfer among a number of recombinants from a *col I*-mediated cross, and the comparable *F*-mediated cross, using 58–161 in the colicinogenic or F^+ state respectively as the donor parent with *W*945 F^- as the recipient (Table 1). Crosses were plated on minimal medium supplemented with thiamine so that selection was made for the ability to synthesize threonine (thr^+) and leucine (leu^+) from 58–161, and methionine (met^+) from *W*945. The two strains differed in seven other markers; the ability to synthesize thiamine (*thi*); to ferment the sugars arabinose (*ara*), lactose (*lac*), galactose (*gal*) and xylose (*xyl*); and resistance to the drug, sodium azide (*azi*) and the coliphage T6 (*T*6), A variety of recombinant types

Table 1. FREQUENCY OF RECOMBINANTS CARRYING GENETIC MARKERS OF *E. coli* STRAIN 58–161 WHEN CROSSED WITH *W*945 F^-

State of 58–161	No. of recombinants	Fraction of the recombinants carrying the 58–161 marker† thi	*thr	ara	*leu	azi	lac	*T*6	gal	xyl	Fraction of 'double-crossover' types
(Col. *I*)$^-$*F*$^+$	55	0·04	1·0	1·0	1·0	0·90	0·37	0·24	0·11	0	0·94
(Col. *I*)$^+$*F*$^-$	70	0·18	1·0	1·0	1·0	0·91	0·56	0·49	0·20	0·11	0·91

* Selected markers.

† Markers arranged in order of linkage as determined by *Hfr* and F^+ crosses (ref. 14).

resulted, as indicated in Table 1. Although the *col I*-mediated cross was only one-hundredth the fertility of the *F*-mediated cross, the table shows similarities in marker transfer from both types of crosses. Assuming a one-way transfer of genetic material (the colicinogenic strain acting as a donor as in the case of the F^+ strain), and assuming the order of markers on the *K*-12 chromosome as determined by *Hfr* or F^+ crosses[14], the recombinants from both types of crosses can be accounted for by a double crossing over in more than 90 per cent of the recombinants. It appears, therefore, that the genetic fragments transferred in the *col I* cross are of the same order of size as those involved in the F^+ cross, in which, for example, the distance between *thr-gal* has been shown to be about 15 per cent of the total chromosomal length[14].

Episomal systems determining certain types of colicinogeny appear, therefore, to resemble other episomal systems such as temperate phage and more particularly *F*, in the ability to mediate genetic transfer. It is tempting to imagine that if mutation were to occur in such colicinogenic strains so as to remove the lethal effect of the colicin, it would be difficult to distinguish them readily from F^+ strains. Other possible similarities of the colicinogenic and *F* systems are being examined.

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ANALYSIS OF IRRADIATED *DROSOPHILA* POPULATIONS FOR MEIOTIC DRIVE

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THE existence of chromosomes or alleles that are represented in the gametes of a heterozygote with a frequency greater than the expected 50 per cent is now well established for a variety of species. The immediate population result of introducing such a chromosome or allele must be a sharp increase in its frequency, along with any other genes associated by linkage, independent of or, indeed, in spite of, their phenotypic effects[1,2]. For this reason such phenomena have been referred to as cases of 'drive' and, more specifically, as 'meiotic drive' when the basis is found in some aberration of meiosis[3]. Such instances would seem to provide an unparalleled opportunity for the study of population dynamics, for example, the efficacy of response of natural selection to unfavourable genes. It seemed worth while, therefore, to determine whether such chromosomes or alleles characterized by drive might be induced in the laboratory.

We expected that such an event would occur with a very low frequency, and would probably not be detectable in an X-ray experiment conventionally designed. Instead, chromosomes were tested that had received light doses of radiation each generation for about 200 generations. These chromosomes had been derived from the population cages kept at the Long Island Biological Laboratory at Cold Spring Harbor by Dr. Bruce Wallace, to whom we are indebted for making samples of these populations available to us.

The genetic tests consisted simply of back-crossing heterozygotes for chromosomes derived from the irradiated populations and for stock chromosomes carrying mutant genes to the mutant stock in order to detect any deviation in the progeny from the expected 50 per cent of each type. Seven different populations were tested, and the standard mutants chosen marked the distal ends and centromere regions of the three major chromosomes. Furthermore, since it could not be anticipated in which sex any disturbances of segregation might be effective, both male and female heterozygotes were tested. Of 1,654 tests there were two instances in which the chromosomes derived from the irradiated population were recovered in significantly more than half the progeny, and where this deviation persisted after repeated testing.

One of these cases involved an irradiated second chromosome, which appeared in the F_1 of male heterozygotes, with frequencies ranging from 53 to 80 per cent (Table 1). The second chromosome showed this peculiarity over six consecutive generations and in combination with a variety of different

Table 1. CHROMOSOMES RECOVERED FROM MALES HETEROZYGOUS FOR THE IRRADIATED SECOND CHROMOSOME AND VARIOUS NORMAL SECOND CHROMOSOMES COMPARED WITH A CONTROL

Irradiated	Normal	Per cent
192	135	58·7
1,148	866	57·0
421	104	80·2
2,210	1,394	61·3
2,554	2,078	55·1
5,457	4,755	53·4
3,218	2,421	57·1
4,703	3,458	57·6
3,026	1,370	68·8
1,907	448	81·0
1,048	664	61·2
Controls:		
1,327	1,354	49·5
960	895	51·7

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stock second chromosomes. The bottle stock carrying this chromosome was left unattended for a period of several months, and subsequent re-testing failed to uncover it again. In the meantime the population from which this was originally derived was unfortunately discarded. It is not clear why we should not have been able to recover it again in the unselected population if it were in fact a bona fide case of drive.

In the second case, an irradiated X chromosome when present in a parental male, appears in 56–64 per cent of his progeny (Table 2). This has persisted over 20 consecutive generations; in fact, the mean recovery is now 67 per cent, presumably because of the persistent selection exercised in each generation for genes which accentuate the discrepancy. As in the preceding case, this chromosome behaves normally in the heterozygous female.

Table 2. NUMBERS OF X AND Y CHROMOSOMES RECOVERED, IN FEMALE AND MALE PROGENY, RESPECTIVELY, FROM MALES CARRYING THE IRRADIATED X, WITH A CONTROL SET FOR COMPARISON

Male	Female	Per cent female
1,267	1,675	56·9
962	1,327	58·0
797	1,192	60·0
491	799	61·9
329	535	61·9
656	973	59·7
655	838	56·1
428	762	64·0
234	409	63·6
Controls:		
973	1,077	52·5
520	524	50·2

Evidence that inequality of recovery of the two homologues (in this case the X and Y chromosomes) is not simply differential mortality of the male progeny caused by this particular X chromosome may be found in the following observations:

(1) When matings are made to attached-X females instead of ordinary females with free-X chromosomes, the deviation persists but with reversed direction, as one might expect if more eggs were being fertilized by X- than by Y-bearing sperm.

(2) Pair matings were made of females with males which carried the irradiated X chromosome. After eggs were collected and carefully counted, the sex of the adults emerging was scored. The overall mortality was simply the difference between the eggs laid and the total emerging adults. A χ-square test was made of the deviation in sex ratio, but to ensure the most conservative test, the overall mortality was added to the least frequent class, the male class. Even with this correction, which would tend to minimize the magnitude of the difference between the two classes, the P value was less than 0·005. This refutes the possibility that the difference between the numbers of the two sexes recovered may be explained by mortality and supports the alternative that it is caused by some pre-zygotic mechanism, as meiotic drive.

(3) At lower temperatures (18° C.) the magnitude of the effect is depressed, 56 per cent recovery, as opposed to 67 per cent at 25° C. However, if a male develops at 18° C. and is then mated in a culture bottle at 25°, which is then kept at the higher temperature during the development of the progeny, the resulting ratio in the progeny is that characteristic of the low temperature, not the higher, as would be expected if zygote mortality were responsible.

Those three lines of evidence speak strongly for an action before fertilization and possibly during meiosis, which would qualify this case for inclusion under the heading of meiotic drive. Preliminary cytological examination has revealed nothing unusual, but unless the basis for this effect were something quite obvious (as, for example, the formation of anaphase bridges as in 'segregation-distorter'[4]), one would not hope to have much success in this effort with a species the meiosis of which is as intractable cytologically as is that of *Drosophila melanogaster*.

We wish to acknowledge the contribution of technical assistance of Mrs. R. D. Wilkerson, the innumerable helpful suggestions of the genetics group at the Oak Ridge National Laboratory, and the invaluable assistance of Dr. Allyn Kimball, who constructed criteria based on sequential analysis, which expedited the testing enormously.

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CHROMOSOME FRAGMENTS AND MUTATION OF THE INCOMPATIBILITY GENE

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IN a study in *Petunia inflata* of changes in the self-incompatibility system, Brewbaker and Natarajan[1] found self-compatible plants among the selfed generation after X-radiation of the pollen mother cells. This is similar to results obtained in *Oenothera organensis*, *Prunus avium*[2] and *Trifolium* species[3], which have been interpreted on a basis of mutation of the self-incompatibility S gene. Brewbaker and Natarajan discovered, however, that their self-fertile plants had a small centric chromosome fragment extra to the normal diploid set of chromosomes. Their analysis supports very fully their interpretation that the fragment carries the S gene, and that a self-compatible plant has two alleles, say S_1 and S_2, in the normal pair of chromosomes, and either S_1 or S_2 in the fragment. Such a plant will produce 50 per cent pollen grains with one normal S chromosome, and the fragment. Half these fragment-carrying pollen grains have a different S allele in normal and fragment chromosomes.

It has long been known that a diploid pollen grain with two different, but not two like, S alleles has a weakened incompatibility reaction or has lost it altogether. This effect has been called competitive interaction between the S alleles so that neither allele produces its specific protein, which is responsible for the incompatibility reaction[4]. When the incompatibility reaction has been completely lost by competitive interaction, a plant with diploid pollen grains is fully self-compatible. When the incompatibility is only weakened, then the pollen tubes are less inhibited, but they are still unable to effect fertilization and produce seed when growing on their own style. The plant is therefore still self-incompatible.

This competitive interaction is the basis of Brewbaker and Natarajan's interpretation of the fragment and self-compatibility in *Petunia*. A plant which produces pollen grains with a normal chromosome with S_1 and a fragment with S_2 will exhibit competitive interaction between the alleles, and will be self-compatible.

The finding of a chromosome fragment and the behaviour of the self-compatible plants both as male and female parents in *Petunia inflata* proves conclusively that the change to self-compatibility induced by the X-rays and the selective method of self-pollination is due to the presence of two competing alleles in disomic pollen grain and not to mutation of the *S* gene itself. This is certainly true of the five self-compatible plants analysed. It may or may not be the explanation for the remaining 16 self-fertile plants which have not been reported in detail.

The extra *S* fragment in these self-compatible plants is precisely what would have been expected on the hypothesis of competitive interaction in a genus such as *Petunia*, where competitive interaction results in a complete loss of the pollen incompatibility reaction. This was shown by the data of Stout and Chandler[5] and interpreted by Lewis[4].

Brewbaker and Natarajan claim that a chromosome fragment and competitive interaction can explain results obtained by me in mutation studies in *Oenothera organensis*.

I propose to dispose of the *S* fragment explanation of self-compatible plants in *Oenothera* only. The arguments and findings given apply also to many of the self-compatible plants in *Prunus* and *Trifolium*, but these will not be discussed.

First, the competitive interaction in diploid pollen grains with two different *S* alleles in *Oenothera* has been analysed in detail in the combinations between *S* alleles used in the mutation studies. None of the pairs of different alleles showed competition strong enough to result in self-compatibility. Longer pollen tubes on selfing tetraploids were produced, but there were no seeds except in a tetraallelic plant $S_{2\cdot3\cdot4\cdot6}$ which gave only eleven seeds from 15 flowers pollinated, which is less than 1 per cent of the compatible seed set. The plants are therefore not self-compatible[4].

In *Oenothera organensis* the progeny obtained after X-ray treatment and self-pollination contained two classes: 50 per cent of heterozygotes S_1S_2' and 50 per cent of 'homozygotes' S_2S_2', where S_2' is a mutated form of S_2. This S_2' mutant confers full self-compatibility on the plant. These two genotypes can be unambiguously identified by their reaction as female parent in test crosses. The S_1S_2' plants as female inhibit both S_1 and S_2 pollen, S_2S_2' plants inhibit only S_2 pollen. Homozygotes of the type $S_2'S_2'$ were found in the progeny from selfing the S_2S_2' plants.

In *Petunia*, on the other hand, all the progeny from X-ray treatment and self-pollination were identical, and reacted in the same way as the parent, that is, as S_1S_2 when used as females in test crosses. Furthermore, the progeny from selfing again the self-fertile plants contained only the one class.

In *Oenothera*, even if competitive interaction were strong enough to have resulted in self-compatibility, the presence of homozygotes of the types S_2S_2' and $S_2'S_2'$ in their expected proportions on a stable gene mutation hypothesis excludes the possibility of an *S* fragment and competition as a basis for the self-compatibility. Such homozygous plants could not produce pollen grains with two different alleles as required by the fragment explanation.

Further evidence for the mutational origin of the self-compatible plants is not necessary, but it has been found recently in the reaction of diploid pollen grains in artificially produced tetraploids[6]. Not all pairs of *S* alleles show competitive interaction; some show dominance and some show independence. In a diploid pollen grain, S_2 and S_4 are independent in their action so that both are expressed. A diploid pollen grain with a normal S_2 and a mutant S_4' is incompatible on a style with S_4' or S_4, whereas a pollen grain with S_4' alone is not incompatible on such styles. In this case the activity of S_4' in the pollen, which has been lost by mutation, has been restored by the presence of S_2', so that S_4 protein is produced. The allele S_4' has not been permanently changed by the presence of S_2, but only its activity. Such a result cannot be explained on a fragment basis.

Accepting that a class of self-compatible plants in *Petunia* after X-ray treatment is due to the presence of a fragment bearing an *S* gene, this explanation does not apply to *Oenothera organensis*, and it would be surprising if all self-compatible plants found in *Petunia* could be explained on this basis. In *Petunia*, where competitive interaction is strong enough, and the fragment is transmitted by the pollen, both *S* fragmental, *S* mutational or *S* recombinational and possibly *S* trisomic self-compatible plants should be found. In *Oenothera* only mutational or recombinational *S* plants are expected.

The sieve operating in the incompatibility system will allow different classes of mutations to pass in different species. In *Oenothera* the sieve is more discriminating than in *Petunia*. In *Petunia* a wider spectrum of mutational changes will be recovered. It is possible that, if more pollen grain were tested for mutation in *Petunia* then the S_2' type of mutant in *Oenothera* may still not be found in *Petunia*. The tests in *Petunia* were on 3,100 pollinations and this represents about 3×10^6 viable pollen grains. If the X-ray induced mutation-rate is the same in *Petunia* and *Oenothera*, only 3 self-fertile mutants of the S_2' type would be expected.

A further point of interest is that in *Petunia* self-fertile plants were not obtained after X-ray treatment and self-pollination of homozygotes of the type S_1S_1. This has led to the suggestion that the self-fertility may be due to some rare equal or unequal gene recombination[7]. In *Oenothera* this is a possible explanation in some cases but not in all, because self-fertile mutants of the S_2' type have been obtained from *S* homozygotes (Lewis and Dowrick, unpublished).

In two of the most completely analysed self-compatible mutants induced in *Oenothera* the explanation rests on the mutation of an activator part of the gene or of a separate activator gene contiguous with the *S* specificity gene[6].

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LETTERS TO THE EDITORS

PHYSICS

A Notable Growth Spiral on Synthetic Diamond

IN previous communications[1] it was reported that a few growth spirals of step height of some 100 Å. were observed on what were otherwise very smooth cubic faces of synthetic diamonds. These spirals were found only among one batch of crystals (1958) made by General Electric (U.S.A.). Of several thousand faces examined only two very clear-cut single-step spirals were found, together with another eight irregular, shallow, imperfect examples.

A stock of selected (1960) General Electric crystals was secured later, and, despite careful search, which indeed uncovered many smooth cubic faces, no spirals were detected, although a considerable number of crystals were studied.

I have been engaged for some time past in examining microcrystal diamonds recently made by de Beers of Johannesburg. These crystals are of interest in that they show numerous highly perfect faces, both octahedral and cubic. They are being examined with a high-sensitive phase-contrast microscope and with two-beam interferometry, using, for both techniques, a 4-mm. objective, with magnification × 750. The crystals are tiny, the largest being 0·1 mm. across, and on these some thousands of faces have already been examined.

Among them another single growth spiral has been found, but it is indeed striking. It appears on an irregular face, which must be an incompleted cubic face. The phase-contrast picture (× 500) is shown in Fig. 1. Although of an apparent partial triangular outline, this face is certainly not octahedral (no angles of either 60° or 120° occur between edges). It is an incompleted cubic face.

The beautifully defined spiral is clearly of cube face origin, sweeping round with marked rectangular outline. Thus once again our original finding is confirmed in that this too fits the observation that all spirals found to date on diamond appear only on smooth cubic faces of synthetic diamonds and nowhere else.

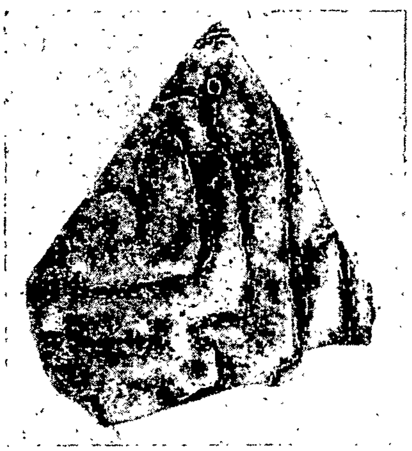

Fig. 1

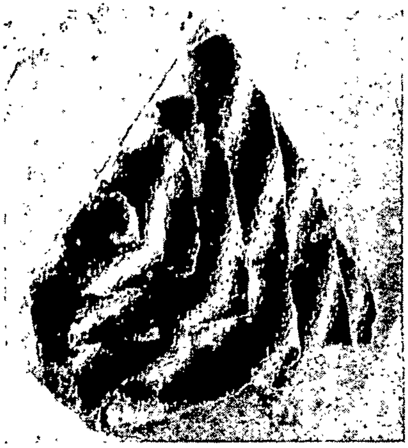

Fig. 2

This spiral is much more perfectly defined than any yet seen by us, and that it is one of considerable step height is shown by the two-beam interferogram (Fig. 2, × 500), taken with green mercury light. The height of the spiral step is about 1300 Å., which is surprisingly large.

The definition of the micrograph of Fig. 1 is so good on the original that it can be clearly seen that the spiral edge is double, and this can be detected too on the interferogram. It is worthy of note that on both the best of our earlier examples as well as on this one the complete face is covered by one single spiral.

It is once more re-emphasized that spiral growth is a quite rare phenomenon in diamond. It is certainly not the normal method of growth, either of synthetic or natural diamond, as the rarity of appearance proves.

The phase-contrast technique in use in this laboratory is capable of revealing spiral step edges as shallow as 2½ Å., and it is unlikely that any spirals have been missed on the thousands of faces studied by us.

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An Upper Limit for the Resistivity of a Superconducting Film

SEVERAL experiments have been carried out to check whether the resistance of a bulk metal in the superconducting state is truly zero. The best known is that carried out by Collins[1], in which a persistent current of several hundred amperes was maintained in a lead ring for two and a half years. At the end of this time there was no measurable decrease in the

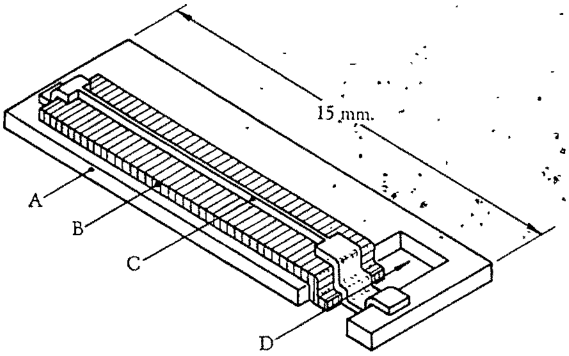

Fig. 1. Arrangement of superconducting films. Vertical dimensions not shown to scale

magnitude of the current. The resistance of the ring is quoted by Crowe[1] as less than 10^{-21} ohm, but no figure for the resistivity of the lead is given.

The question of whether the resistance of a superconducting film of thickness comparable with the depth of penetration is truly zero is of interest both from a fundamental point of view and because of the possibility of using persistent currents for storing information in computers. The present experiment was carried out to investigate this problem.

In principle, the experiment consisted of inducing a persistent current in a superconducting film, then measuring the resultant magnetic field with a bismuth Hall-effect probe of the type described by Kronick[2]. The presence of any resistance in the film would be indicated by a decay in the magnetic field. To increase the sensitivity of the measurement the L/R time-constant of the film in the normal state was made as short as possible by adopting the arrangement shown in Fig. 1. A lead film A, 1000 Å. thick, was evaporated on to a glass substrate, and a silicon monoxide insulating layer B, 2500 Å. thick, deposited on the lead in the position shown. A third film C, 800 Å. thick, composed of an alloy of lead containing 2 per cent indium, was deposited over the insulation, making contact with the lead film A at each end. The narrow section of C had a resistance of 50 ohms at a temperature just above the critical temperature ($\simeq 7°$ K.), which is about ten times higher than if the film had been made of pure lead. However, because of the small separation between it and the 'ground plane' A, its contribution to the inductance of the loop is quite small. Measurements on a scale-model showed that the total inductance of the loop was 5×10^{-9} H., and hence the time constant $L/R_N = 10^{-10}$ sec. The bismuth Hall-effect probe was placed near the centre of the area D enclosed by the films and the arrangement immersed in liquid helium at a temperature of 2·10° K. A persistent current was induced in the loop by means of an electromagnet and the magnitude of the magnetic field produced as a result of that current was measured before and after an interval of 3 hr. ($\simeq 10^4$ sec.). The accuracy of measurement of the magnetic field was limited by noise in the probe to 1 part in 50, but no decay in the persistent current outside this limit could be observed. Therefore the time constant in the superconducting state must be greater than 50×10^4 sec. and the ratio $R_S/R_N = (L/R_N)/(L/R_S)$ is less than 2×10^{-16}. The corresponding resistivity in the superconducting state is less than 10^{-21} ohm cm. This is quite close to the upper limit obtained by Collins, but due to the use of an alloy my figure for the ratio R_S/R_N is probably about one order of magnitude smaller.

I thank Dr. E. H. Rhoderick of this Laboratory for suggesting the experiment and for his helpful advice, and also the Admiralty for permission to publish this communication.

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Flow of Visco-Elastic Liquids from Tubes

It is frequently observed that when a visco-elastic fluid issues from a tube that the diameter increases as the fluid moves away from the tube exit.

To eliminate gravitational effects, consider the fluid flowing horizontally from a tube. It may be shown that if horizontal momentum is conserved, the diameter of the emerging jet should decrease as the material moves away from the tube exit. Since the diameter often increases with a visco-elastic liquid, it is deduced that momentum is not conserved and that there should be a force acting on the fluid. If this is the case, there should be an equal and opposite force acting on the apparatus from which the fluid flows.

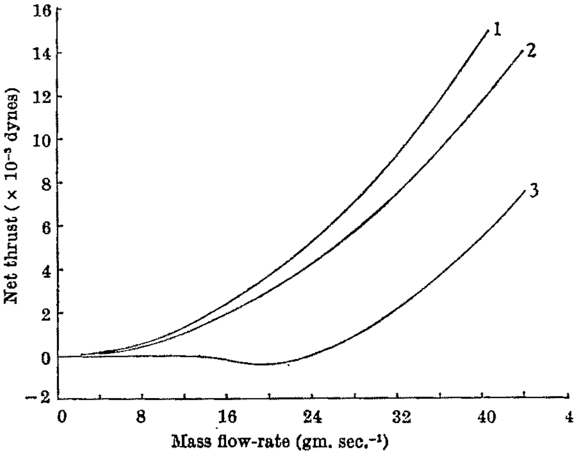

Fig. 1. Graphs of net thrust versus mass flow-rate for octal in paraffin 3/16-in. diam. tubes. Curve 1, calculated from cone and plate viscometer data; curve 2, calculated for flat velocity profile; curve 3, observed experimental results

Results of some experiments to test these ideas are shown on Fig. 1, on which is plotted the net thrust exerted by the flowing liquid on the apparatus versus the mass flow-rate. Curve 3, the observed net thrust, is unusual in that no net thrust is noted below a certain mass flow-rate. The net thrust then becomes negative and finally positive, but is much less than the calculated value.

It is well known that these fluids have a non-Newtonian distribution of normal stresses, and the results appear to show a measurable tensile force acting along the stream lines.

The same type of force seems to be present in the flow of solutions of aluminium laurate.

It was interesting to note that the apparatus moved in the same direction as the motion of the fluid when the net thrust was negative.

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Inelastic Scattering of Conduction Electrons in Dilute Silver–Manganese Alloys at Low Temperatures

THE method of Gruneisen–De Haas was recently employed by me[1] for the analysis of our thermal and electrical resistivity data at helium temperatures[2] on dilute silver–manganese rods (containing, respectively, 0·55, 0·32 and 0·14 atoms per cent of manganese in random solid solution) in transverse magnetic fields ranging from 0 to 25 kilogauss. This method of analysis yielded information regarding the variation of the electronic Lorenz parameter L_e with the magnetic field and the temperature, while, at the same time, effecting the separation of the measured thermal conductivity into its electronic and lattice components.

I wish to direct special attention to a result of that analysis: namely, that L_e, at liquid helium temperatures, dipped below the normal Sommerfeld value L_n ($= 2{\cdot}45 \times 10^{-8}$ watt-ohm-deg.$^{-2}$), attained a minimum, and approached the value L_n at temperatures close to 0° K.

In order to explain their own results (following those of Gerritsen and Linde[3]) on the electrical resistivity maximum in the case of dilute alloys of a noble metal with a small amount of a transition metal, Schmitt and Jacobs[4] discussed the effects of inelastic impurity scattering of the conduction electrons[5,6]. Since a co-operative magnetic transition is known to occur[7–9] in such alloys, in this temperature region, the spin degeneracy of the impurity ion is thereby removed and the scattering process involving the spin flips of the conduction electron and the ion becomes inelastic[4,10].

The critical test for the existence of such an inelastic scattering would be in the thermal resistivity[11]. The 'vertical' transitions of the electrons, to which heat conductivity is very sensitive, would result in an anomalous contribution to the electronic thermal resistivity. My results for the electronic thermal resistivity (w_e) of these alloys do exhibit this behaviour. The plots of w_eT against T show this in the form of a pronounced 'peak'.

Taking $f = [(\rho_m/\rho_0) - 1]$, (where ρ_m stands for the value of the electrical resistivity maximum and ρ_0 for the value at $T \rightarrow 0$), as a measure of the maximum fractional contribution of inelastic impurity scattering, I obtain an estimated $f \approx 0{\cdot}1$, in my silver–manganese rod specimens. This ignores any temperature-dependent contribution from elastic scattering by the impurity ions[10].

For making the corresponding estimates in the case of the electronic thermal resistivity in these specimens, I write, $f' = [\{(w_eT)_m/(w_eT)_0\} - 1]$. Here, $(w_eT)_m$ and $(w_eT)_0$ stand for the values at the 'peak' and at $T \rightarrow 0$, respectively. From my plots of w_eT versus T, we obtain $f' \approx 0{\cdot}5$, $0{\cdot}6_5$ and $0{\cdot}8$, in the order of decreasing manganese content.

The fact that inelastic impurity scattering makes such a large contribution to the electronic thermal resistivity of these alloys, while making only a small contribution to the electrical resistivity, accounts for the 'dip' in the value of L_e with respect to the normal value L_n. The further result that L_e seems to approach L_n at temperatures close to absolute zero (below $\approx$ 1° K.) is explained by the fact that this inelastic scattering cannot occur at 0° K. Scattering, which would involve loss of energy by the electron, is not permitted since there are no lower electronic states available; scattering which would involve gain of energy by the electron is also not possible since there are no ions in the excited states at 0° K. to transfer energy to the electron.

Full details will be published elsewhere.

Grateful acknowledgment is made of the benefit of discussions with Sir K. S. Krishnan, Director of the National Physical Laboratory of India.

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Breakdown Strength of Cæsium Iodide

Two crystal structures, both cubic, are found in the alkali halide group of compounds: (1) the 'sodium chloride type' of interpenetrating face-centred cubes, and (2) the 'cæsium chloride type' of interpenetrating simple cubes. All the group except cæsium chloride, cæsium bromide and cæsium iodide resemble sodium chloride at ordinary temperatures and pressures[1]. Measurements of the breakdown strength of halides of the sodium chloride type have shown[2] that there exists a simple relationship between the breakdown field strength, F, and the interionic distance, a, given by $Fa^3 = 28(\pm 25$ per cent) where F is measured in MV./cm. and a in Å. No previous work on the cæsium chloride type of halide has come to our notice.

In the course of some work on electro- and photo-luminescence of crystals, the opportunity was taken to establish whether cæsium iodide obeyed this relationship. The crystals used contained about 0·1 per cent thallium (to make them better scintillators). This presence of foreign ions may raise the breakdown strength slightly above that of the pure crystal[3], but it is unlikely that the increase will be greater than experimental error.

Two single crystals of cæsium iodide were used, each approximately 0·01 cm. thick. For this thickness, it is to be expected that 'intrinsic' breakdown will take place[4]. In one method of procedure, an aluminium point contact was used as one electrode and a metal plate the other, while in a second method a metal sphere of diameter 3 mm. was used as the first electrode. A film of water usually forms on cæsium iodide crystals and results in discharges around the edges. This was prevented by polishing the crystals in oil and keeping an oil film on the crystals during the experiments.

Over a series of experiments at room temperature using the first method, breakdowns occurred at fields which varied from experiment to experiment, due probably to the presence of local flaws in the crystal structure, the minimum and maximum fields at which breakdown occurred being 0·29 and 0·36 MV./cm.

With the second method, the corresponding limits were 0·20 and 0·40 MV./cm., and the average value was 0·32 (±0·05) MV./cm.

The interionic distance for cæsium iodide is 3·95 Å. (ref. 5). Then for this crystal $Fa^3 = 20 \pm 3$, in reasonable agreement with the value found for the sodium chloride type of crystal structure.

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ASTROPHYSICS

Trajectories of Chromospheric Disk Surges

As a result of the use of rapid sequence *H*-alpha cinematography, Lockheed Solar Observatory has collected a large quantity of data on disk surges during 1959 and 1960. Since January 1960, the band pass of the birefringent filter has been displaced 0·5 Å. to the blue or red of *H*-alpha. The increased contrast between active absorption features and the background in the wings of *H*-alpha has resulted in a greater ability to detect disk surges. The percentage of flares of all classes associated with observed surges increased from 11·4 per cent in 1959 to 20·4 per cent in 1960. Approximately 94 per cent of the total flares were sub-flares. During the two years, a total of 953 flare surge events were photographed at the Observatory.

The study of surges observed in the wings of *H*-alpha has permitted the identification of a new class of disk surges which are characterized by extremely intricate structures. Unlike the 'classical' disk surges which appear as straight brush-like extensions from flares[1], these dark surges, which we term 'complex surges', appear on single frames as irregular, dark mottles. Although material is clearly being ejected with typical velocities of 100 km./sec., the complex surges show little preferential direction of flow and have trajectories seemingly dominated by local force fields. Table 1 lists three large complex surges observed in 1960.

Table 1

Date 1960	Time Start (U.T.)	Time Max.	Extension	Mean velocity (km./sec.)	Associated position	Flare importance
May 9	0030	0052	0·13 R	69	S. 11 E. 04	1
Sept. 25	1817	1855	0·30 R	92	S. 18 W. 22	1
Nov. 20	1640	1655	0.19 R	77	N. 23 W. 45	1–

While viewing the off-band films of these events, particular attention was paid to the motion of the complex surges relative to the neighbouring bright coarse mottling. It was found that whenever the direction of flow was altered the dark surge material appeared to avoid the bright mottling. The surges with very complex structure were deflected many times as the surge material appeared to flow along the lanes between the bright mottling.

The complicated trajectories of these surges may perhaps be understood in terms of magnetic fields in the coarse mottling. The kinetic pressure of surge material moving with a speed of 100 km./sec. with a density of 10^{11} protons per cm.[3] would be balanced by the magnetic pressure of a field of about 20 gauss. The correspondence between magnetic fields of such magnitude and CaII plages was first established by the Babcocks[2], and has recently been corroborated by Howard[3] and Leighton[4]. The boundaries of CaII plages appear to be contained by 10 gauss contours and the fields in such plages may reach as high as 200 gauss. The wings of *H*-alpha are not formed at the same height of the chromosphere as the centre of the CaII *K* line; however, bright mottling in the wings of *H*-alpha and CaII plages may well correspond to different heights in the chromosphere along continuous vertical magnetic columns.

Detailed work on the association between magnetic fields and the coarse mottling in the wings of *H*-alpha is needed; but it does appear that bright mottling is adjacent to large field gradients. Chromospheric filaments apparently lie between regions of opposite polarity[2], and when filaments are visible in the wings of *H*-alpha, they are generally bordered by bright mottling apparently identical to that which deflects the surges. Further work on the trajectories of disk surges is continuing as the observations reported here suggest that surges may be utilized as 'test particles' for the mapping of chromospheric and coronal magnetic fields.

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GEOLOGY and MINERALOGY

Origin of Ignimbrites

A FIELD and laboratory study of the Ordovician ignimbrites of North Wales that has been in progress since 1953[1–3] has revealed certain limitations in Marshall's original hypothesis[4] regarding the origin of this interesting category of rocks. These limitations have been apparent to workers elsewhere[5–8]. The evidence from North Wales, and much of the published evidence from North and South America, is categorically opposed, however, to the hypothesis of origin now proposed by Steiner[9]. As this new hypothesis is based on a petrological study of the type ignimbrites from the North Island of New Zealand, and involves the far-reaching conclusion that liquid immiscibility plays a large part in the petrogenesis of acid igneous rocks, a critical appreciation of Steiner's views is urgently required. A detailed petrological criticism is in preparation, and will be published in full elsewhere. In the meantime, it is appropriate to state that Steiner's conclusions appear to be based on a misinterpretation of the evidence afforded by the New Zealand rocks.

Steiner correctly observed the presence of two contrasting glasses in fresh New Zealand ignimbrites—a feature of certain ignimbrites mentioned by other authors[10,11]. Ancient ignimbrites, such as those from North Wales, cannot contain unaltered glass, but they do retain, in many instances, the textural evidence of its former presence. It is on the textural evidence derived from a comparison of North Welsh rocks with unaltered New Zealand specimens that Steiner's hypothesis of liquid immiscibility is rejected. The presence of two, and, rarely, of three, distinct glasses appears to be an accidental feature of certain ignimbrites, and not of universal importance. In some fresh New Zealand ignimbrites, fragments and shreds of juvenile glass can be seen to enclose rounded and partly resorbed xenoliths of earlier glassy ignimbrites and vitric tuffs, along with globular masses of a similar shape and size of another glass of higher refractive index. The conclusion that this second glass did not originate by liquid immiscibility, but is present as small xenoliths, is amply confirmed by the occasional presence of much larger angular xenoliths of the same glass, containing resorbed phenocrysts. In other instances the accessory xenolithic origin of the small globular masses of the second glass is not so readily obvious, but, once its characteristic features have been defined by study of unambiguous examples, the general identity of origin of the second glass can be confidently accepted. The peculiar groupings and matching shapes, within patches of small globules of this second glass, are clearly related to the partial resorption and separation of the perlitic fragments of an earlier obsidian. Vent-rocks from North Wales, and large fragments contained within ignimbrites, both from New Zealand and North Wales, often have very intricate textural patterns, produced by the plastic distortion of hot obsidian containing numerous gas vesicles and dissolving xenoliths of earlier glass. It is the texture shown by rocks of this type that has misled Steiner.

Steiner observed that the second glass has a higher refractive index than the juvenile glass. This can be confirmed, and indeed is to be expected, if the second glass were present as reheated and partially dissolved fragments of an earlier obsidian that consolidated within or below the volcanic vent, for this would be derived from either an earlier volatile-poor fraction of the present magmatic phase, or from a magma less advanced along the differentiation sequence.

Much of the textural evidence described by Steiner from New Zealand ignimbrites can be studied equally well in North Welsh rocks. The phenocrysts of ignimbrites, for example, cannot be described as generally showing multiple resorption features. The vast majority of the felspar phenocrysts are obviously broken and abraded by gas-generated expansion, and only the rare quartz phenocrysts preserved within xenolithic obsidian lumps show that intricate resorption outline regarded by Steiner as evidence for gentle flow rather than a violent expansion of gas. Any resorption of phenocrysts occurred mainly before eruption.

The conclusion of Hausen that ignimbrites are more akin to lavas in their mode of origin than to pyroclast-showers is one that most workers in the field would support, but the field and petrographic evidence is definitely opposed to the view that they are true lavas. There may be, in fact, every gradation from the
'boiling milk' gas-liquid emulsio
Lacroix[12] to a *nueé ardente* of gas
clasts, but the majority of welde
between these two extremes, and, a
ted, their textures indicate grav
mutual adherence of hot plastic gla
sizes, deposited from suspension i
gas stream. (The analogy with
deposition in marine sedimentation
many flattened glass bubbles seen
Zealand and Welsh rocks confirm th
flattened lenticles common in man
mostly accessory obsidian xenolit
largest and most widespread flows
be entertained that any of the in
posed of collapsed glass-froth.
ignimbrite of the Pass of Llanberis
one such example.

With the further accumulation of
acid volcanism it seems likely that
the work of Jagger[13] on basic mag
possible. Welded ignimbrites a
product of a fluent gas-generating
basic pyromagma, the lenticles c
being consolidated fragments of an
basic bench—or epimagma. The d
sillars and welded ignimbrites ma
the difference between pahoehoe an
principal difficulty facing workers
that nowhere has the deposition o
brite been observed. The evidenc
microscopic examination of these
doubt, however, that the differe
average ignimbrite flow and the
ardentes is principally one of degree
state and quantity of the erupting
there is overwhelming evidence tha
agent—liquid immiscibility finds
mechanism of ignimbrite formation

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Skiddaw Slates proved in t Inlier

The small inlier of Lower Palæoz
Teesdale crops out beneath Carbo
the banks of the River Tees below C
the hamlet of Langdon Beck, Co
inlier is poorly exposed, but consis
belt of soft greenish and light-colour
slates, until recently regarded as co
liferous, and a western area in w
lava occur. The slates are best e
workings near the Pencil Mill (*NY*

at one time the softer beds were worked up into slate-pencils.

The Teesdale Inlier was discovered in the latter part of the last century when the highly cleaved slates were described as probably Skiddaw Slates, Lower Ordovician, by Dakyns[1] in 1877. A further account of the inlier in 1878 by Gunn and Clough[2] referred the slates tentatively to the Silurian Stockdale Shales. Later authors followed Gunn and Clough in regarding the slates as probably Silurian until Kendall and Wroot[3] revived the view that they were better placed in the Skiddaw Slate Series, and this latter view was supported by Dunham[4]. Five mica-lamprophyre dykes are intruded into the slates in the vicinity of the Pencil Mill and have been described by Williams[5]. The lavas at the western end of the Inlier are tentatively referred to the Borrowdale Volcanic Series.

The first fossils to be found in the slates of the inlier were collected near Pencil Mill by Mr. G. F. Lea during a Durham undergraduate geological mapping exercise in the spring of 1959. The specimens consisted of poorly preserved graptolites which were not found *in situ*. Several subsequent visits to the inlier in search of further graptolites were unsuccessful, but some small elongated thin-shelled fossils were obtained which are believed to be phyllocarid crustacean carapaces and tentatively referred to ? *Caryocaris* sp. On a further recent visit to the inlier three more graptolites were obtained from slate scree in the Pencil Mill workings and the preservation of these specimens is more definite. It is noteworthy that all the fossils found in the slates of the inlier are inconspicuous to the naked eye, and it is undoubtedly owing to this that they have remained undiscovered until the last few years.

The graptolites found in the Teesdale Inlier have been examined by Prof. O. M. B. Bulman, who has made tentative identification of the specimens as follows: ? *Cryptograptus* sp.; *Glyptograptus dentatus* (Brongn.); *Didymograptus* sp., the proximal end of a pendent didymograptid, possibly *D. climacograptoides* Holm MS. (Bulman).

This fauna, particularly the presence of pendent didymograptid in association with *G. dentatus*, is sound evidence of Lower Ordovician age and shows that the beds belong to the upper part of the Skiddaw Slate Series. Prof. Bulman suggests that the slates of the Teesdale Inlier may in fact be an easterly extension of the Ellergill Beds, the central division of the Skiddaw Slates of the Cross Fell Inlier. The presence of ? *Caryocaris* sp. in the Teesdale slates is supporting evidence for Skiddaw Slates Series age. Thus the long-disputed question of the age of the slates of the Teesdale Inlier is finally solved by the discovery of a graptolite fauna.

The advice and assistance of Prof. O. M. B. Bulman and Mr. J. D. D. Smith, who kindly examined the graptolites and other fossils from the Teesdale Inlier, are gratefully acknowledged.

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Ekanite, a New Metami
Ceylon

SINCE 1953, several specime
44 gm., of a green transparent
which when cabochon-cut as a
some asterism, have reached F
occurs in the gem pits of Ehe
district, Ceylon, and was origi
Mr. F. L. D. Ekanayake, who su
be a new species. This has no
chemical and physical study, o
results are given here.

The mineral is metamict, an
D. I. Bothwell gave: SiO_2 55·6,
Fe_2O_3 0·5, CaO 13·7, PbO 0·8 pe
Al_2O_3, MgO, MnO, total 100·3 p
formula:

$$(Th,U)(Ca,Fe,Pb)_2$$

The refractive index and dens
found are 1·5969 and 3·280 respe
ing at 510° C. for 24 hr. these co
and 3·276.

At temperatures between 650
mineral re-crystallizes to a ph
powder and single-crystal diffrac
indexed on the basis of a body-ce
having dimensions *a* 7·46, *c* 14
after heating at 1,000° C. for 24 h
the mineral becomes opaque putt

Heating at higher temperature
ing leads to the development of
huttonite. If the mineral is ful
re-crystallized at 1,000° C., the p
a thorium analogue of the britho
group[1].

Besides some microscopic inclu
directions at right angles, ekanite
larger opaque inclusions not
certainty.

The mineral is named after the
account will be published elsewh

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R.

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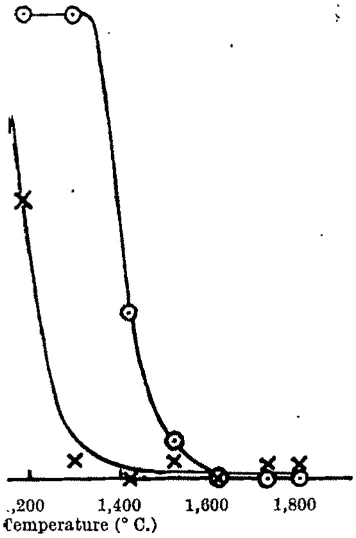
METALLURG

Arc Initiation on Heated
Hydrogen Discha

AN undesirable phenomenon
experimental investigations of to
discharges is the formation of unipo
at metallic parts of the system expo
Previous work[2,3] has indicated th
played by insulating inclusions in
cess of arc initiation under these c
investigating the arcing characteri
metals, exposed to a toroidal d



function of temperature for molybdenum
⊙, Temperature increasing; ×, tem-
erature decreasing

tion of temperature. The results
ort the hypothesis that arcs are
agency of second phases.
ten, tantalum and molybdenum,
of commercial purity, are biased
ect to the plasma at up to 2 kV.
nsities of up to 16 amp./cm.[2] are
charge for a period of 200 μsec.
the wires can be varied by direct
There is, in addition, some heating
(due mainly to ions drawn during
od) which sets a lower limit to the
ing on the biasing voltage (730° C.
t 2 kV.). It is found that arcing
the wires are cool but not when
e temperature is sufficiently high
sion. Fig. 1 shows the arcing rate
ges in which an arc occurs) as a
ature for molybdenum at 2 kV.,

marked transition from an arcing
g state occurs during both heating
ansitions are attributed to: (*a*), on
n of second phases; (*b*), on cooling,
This interpretation is supported
riments on molybdenum. If the
to the non-arcing state and then
radiation) it does not arc initially
ture (970° C.), but de-conditions to
er a further period of discharges.
ing treatment retains a super-
of impurities which, however,
te at the ambient temperature.
he arcing characteristics change
ourse of experiments on a particu-
ds to occur less readily in the later
particularly at low voltages. This
uted to the loss from the wire of
hases. The mechanism by which
eved to be essentially that which
A second-phase particle on the
up by the incoming positive ions
breakdown occurs. The energy
e breakdown is sufficient to evapo-
rtion of the particle. The puff of
l can initiate an arc if of sufficient
size and if the electric field is of sufficient strength. If, however, these requirements are not met, a simple loss of impurity occurs at the surface. Thermal cycling will, however, tend to replenish the depleted surface layer by diffusion of impurities from the interior. This hypothesis is supported by experiments on molybdenum at less-severe electrical conditions than those mentioned above. Thus at 1·7 kV., 8 amp./cm.[2], the arcing-rate passes through a maximum at about 1,050° C., during heating and again on cooling. At low temperature, therefore, the competition between surface depletion and precipitation is won by the former due to low diffusion-rates.

We have made use of the depletion process to prepare molybdenum wires which are virtually non-arcing at any temperature in the range 700–1,800° C. The procedure adopted is to cool the wire slowly from 1,800° C. while at the same time exposing it to an uninterrupted series of discharges at the maximum electrical conditions of 2 kV., 16 amp./cm.[2]. Very little arcing occurs during this process if the cooling-rate is sufficiently slow; the surface particles are therefore evaporated before they grow big enough to start an arc. After several such operations no arcing can be induced by extended heat treatment aimed at homogenizing the wire to encourage surface precipitation. It seems likely therefore that the wire is depleted throughout its volume by this method.

These observations suggest that the conditioning of various metals against arcing at room temperature observed by Craston *et al.*[1] is due to the depletion at the surface of insulating inclusions.

A detailed report is to be published elsewhere.

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CHEMISTRY

Prevention by Cysteamine of Radical Formation in Bovine Serum Albumin by γ-Rays

CYSTEAMINE is one of the most effective substances for the protection of animals against ionizing radiations, and *in vitro* has been shown to reduce radiation damage of macromolecules by energy transfer in solid polymethylmethacrylate[1], by capture of OH· radicals for serum albumin in dilute solution[2], and by hydrogen transfer to an organic radical for polymers in aqueous solution[3,4]. Gordy and Miyagawa[5] have found that the electron spin resonance spectrum of the protein zein is altered in magnitude and character if cysteamine is present, and have ascribed this to the combination of cysteamine with the protein by a disulphide exchange reaction. Earlier experiments[1] led us to believe that an energy-transfer process is a more probable mechanism of protection, and to test this hypothesis we studied the effect of cysteamine on the electron spin resonance spectrum of bovine serum albumin irradiated with cobalt-60 γ-rays at room temperature and at 77° K. (dose, 5 Mrads, dose-rate = 750 krads/hr.). The mixture of 90 per cent bovine serum albumin + 10 per cent cysteamine was produced by freeze-drying a solution. Although bovine serum albumin contains 17 disulphide groups per molecule, none of these reacts with cysteamine under isoelectric conditions because they are sterically inaccessible[6,7] and the possibility of protection by disulphide exchange is therefore excluded (human serum albumin combines with cysteamine at the isoelectric point because one disulphide bond is reactive[7]).

A 400 kc./s. magnetic field modulation X-band spectrometer was used. The samples were placed in an H_{014} transmission-type cavity. The source of microwave power was a low-voltage reflex $K302$ klystron, and crystal detection employing a $CV2154$ crystal was used. A maximum linear sweep amplitude of about 200 gauss was obtained using an electronic 'bootstrap' type linear sawtooth generator. Thus all spectra were recorded as the first derivative of the absorption intensity as a function of variation in magnetic field with the microwave frequency held constant. At maximum usable sensitivity, 7×10^{14} radicals in α,α-diphenyl β-picryl hydrazyl could be detected at room temperature.

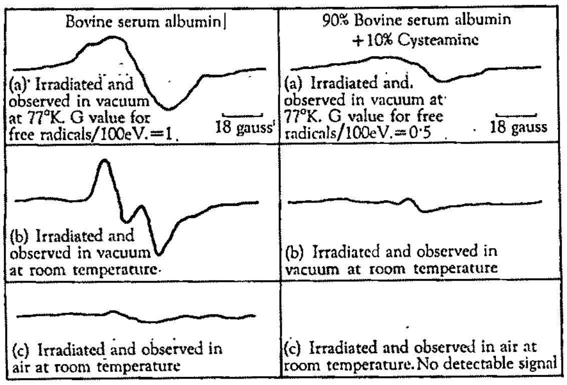

Fig. 1. Electron spin resonance spectra of bovine serum albumin and a mixture of 90 per cent bovine serum albumin + 10 per cent cysteamine under various conditions. All spectra recorded at the same spectrometer sensitivity

Fig. 1 shows that the presence of 10 per cent of cysteamine reduces the intensity of the electron spin resonance spectrum of bovine serum albumin whether the irradiation is carried out at 77° K., at room temperature *in vacuo*, or at room temperature in air. The nature of the electron spin resonance spectrum is different under these three conditions, but in every case cysteamine has the same effect, namely, to reduce to half or less than half the number of radicals that are formed. In each case the same weight of cysteamine on its own gives no signal. Measurements at 77° K. presumably reveal all the radicals that are produced on irradiation, while electron spin resonance at room temperature measures those radicals that for steric reasons have been unable to recombine.

The protection by cysteamine seen in the room-temperature measurements could be due either to energy transfer from the proteins to the cysteamine before a radiochemical reaction occurs, or due to hydrogen transfer leading to a reconstitution of the protein radical ($R^{\cdot}$) :

$$R^{\cdot} + \text{SH}\text{—} \rightarrow R\text{H} + \text{S}^{\cdot}\text{—}\ (2\ \text{S}^{\cdot}\text{—} \rightarrow \text{—S.S—})$$

The electron spin resonance signal of the mixture at room temperature is small and a choice between the two mechanisms is not possible.

This hydrogen transfer 'repair' mechanism (cf. refs. 3 and 4) cannot, however, apply at 77° K., since the radicals are effectively frozen and prevented from reacting, and the observed protection must therefore be ascribed to an energy-transfer process similar to that originally observed in synthetic polymers. Moreover, the electron spin resonance spectrum of the mixture at 77° K. is qualitatively the same as that of bovine serum albumin alone. This confirms that the bovine serum albumin has been protected by transfer of energy to the cysteamine rather than by a chemical reaction, which would probably give rise to a signal in the mixture different from that for bovine serum albumin alone. There seems to be no reason for limiting this interpretation to bovine serum albumin and we feel that the disulphide exchange reaction need not be involved in the protection of other proteins by sulphydryl compounds, in particular since our electron spin resonance experiments with irradiated cystine and bovine serum albumin (Libby, D., Ormerod, M. G., and Alexander, P., unpublished work) do not lead us to the conclusion of Gordy, Ard and Shields[8] that radicals induced by irradiation are chiefly associated with the sulphur atoms to which the energy deposited in other parts of the protein molecule migrate. The electron spin resonance pattern of bovine serum albumin irradiated at 77° K., when all the radicals are still present, bears no resemblance to that of irradiated cystine.

Our experiments lead us to question the hypothesis[9] that disulphide exchange plays a part in the protection of animals against radiation by cysteamine, since this mechanism need not be invoked to account for the protection of proteins *in vitro*, whether this occurs directly or by capture of OH· radicals formed in water[2].

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Behaviour of Magnesium Impurity in Gallium Arsenide

Anomalous behaviour of magnesium in gallium arsenide has been reported by Edmond[1] and Weisberg *et al.*[2], who found that magnesium behaves as a donor impurity, differing in this respect from zinc, cadmium and mercury. Edmond incorporated $5 \cdot 7 \times 10^{19}$ atoms/cm.^{3} of magnesium in his specimens and found it to be a donor impurity ; however, Weisberg found magnesium to be a donor when present at low concentration, but an acceptor at levels higher than $4 \cdot 4 \times 10^{18}$ atoms/cm.^{3}. The experimental methods employed by Weisberg are not known to me; however, Edmond prepared his specimens by heating gallium arsenide and magnesium together in sealed silica tubes for 1 hr. at 1,551° K., a small quantity of arsenic being added to prevent decomposition.

Two specimens were prepared in these Laboratories by the method described above. As they had similar properties, the calculations and work reported here relate to one of these samples only.

After cooling, a light grey stain was observed on the inner surface of the tube. This stain, which appeared over a small area only, was not removable by aqua regia and indicated reaction between the tube and its contents. The specimen was carefully etched to remove surface oxides and was compared spectrographically with the pure gallium arsenide from which it was prepared. The most sensitive lines of magnesium and silicon, barely visible for the pure arsenide, showed up strongly for the specimen. The ratio of silicon to magnesium was estimated to be somewhat greater than unity.

In order to determine the true behaviour of magnesium, a further specimen was prepared in a different manner. Gallium arsenide and magnesium were placed in a carbon container with a well-fitting lid. This container, together with the correct quantity of arsenic, was sealed within a silica tube and heated to 1,551° K. for a short time.

Spectrographic examination indicated a much smaller silicon content than in the other specimens. This specimen was p-type with $6 \cdot 1 \times 10^{18}$ acceptors/cm.^{3} compared with $5 \cdot 6 \times 10^{19}$ magnesium atoms/cm.^{3} which were incorporated by weighing. Magnesium, therefore, is an acceptor in gallium arsenide and probably occupies gallium lattice sites.

Reaction between magnesium and silica proceeds according to the general equation :

$$2Mg + SiO_2 = 2MgO + Si \qquad (1)$$

This implies that in experiments of this kind, a thin layer of magnesium oxide forms on the inner surface of the tube. The rate of reaction is limited by the rate at which fresh silica can reach the surface (by solid state diffusion), and the final ratio of silicon to magnesium in the melt is determined by the composition of the oxide phase in equilibrium with the melt.

At 1,551° K., the system $MgO - SiO_2$ has one stable intermediate phase Mg_2SiO_4, and another phase $MgSiO_3$ of marginal stability. The extra stability of Mg_2SiO_4 is − 15,120 cal./mole and large variations in the activities of MgO and SiO_2 are to be expected for $MgO : SiO_2$ ratios close to 2 : 1 (compare the treatment of system CaO–SiO_2 [4]).

For equilibrium with oxide phases having $MgO : SiO_2$ ratios less than 2, it is best to use the equation :

$$2Mg(l) + 2SiO_2(s) \rightleftharpoons Mg_2SiO_4(s) + Si(l) \qquad (2)$$

$$\Delta G^0 = -83{,}980 + 2 \cdot 52\, T \log T - 4 \cdot 99 T$$

$$= -79{,}250 \text{ cal. at } 1{,}551° \text{ K.}$$

For equilibrium with oxide phases having $MgO : SiO_2$ ratios greater than 2, it is best to use :

$$2Mg(l) + Mg_2SiO_4(s) \rightleftharpoons 4MgO(s) + Si(l) \qquad (3)$$

$$\Delta G^0 = -53{,}740 + 2 \cdot 52\, T \log T - 4 \cdot 99 T$$

$$= -49{,}000 \text{ cal. at } 1{,}551° \text{ K.}$$

In order to proceed further, it is necessary to know values of the activity coefficients of silicon and magnesium in gallium arsenide. These are not available and, as this is only an approximate treatment, ideal solution behaviour has been assumed for the purpose of these calculations. Liquid magnesium and undercooled liquid silicon are adopted as the standard states.

In the experiment carried out in these Laboratories, the amount of magnesium incorporated in the specimen was $5 \cdot 25 \times 10^{19}$ atoms/cm.^{3}.

Combining this information with a ΔG^0 value of − 79,250 cal. and, using the expression :

$$\Delta G^0 = -RT \log_e \frac{x_{\text{silicon}}}{x^2_{\text{magnesium}}}$$

we obtain :

$$x_{\text{silicon}} = 1 \cdot 18 \times 10^{-3}$$

$$\text{ratio } \frac{x_{\text{silicon}}}{x_{\text{magnesium}}} = 1 \cdot 32 \times 10^4$$

$$x_{\text{magnesium}} = 8 \cdot 9 \times 10^{-8}$$

(where x is the mole fraction).

Alternatively, using a ΔG^0 value of − 49,000 cal. we obtain:

$$x_{\text{silicon}} = 1 \cdot 17 \times 10^{-3}$$

$$\text{ratio } \frac{x_{\text{silicon}}}{x_{\text{magnesium}}} = 95$$

$$x_{\text{magnesium}} = 1 \cdot 24 \times 10^{-5}$$

As silicon is a donor impurity in gallium arsenide, electrical behaviour, previously attributed to magnesium, may be explained by the process of chemical replacement described here. The specimens prepared in contact with silica were shown by thermal E.M.F. measurements to be n-type but the ratio $x_{\text{silicon}}/x_{\text{magnesium}}$ appeared to be less than the estimated value of 95.

Taking into account electronegativities of the atoms involved, and the stabilities of their compounds, it appears that the assumption of ideal behaviour is reasonably accurate for silicon but not for magnesium. Silicon has a structure similar to that of gallium arsenide and can substitute on either lattice site[5]. Each magnesium atom, by analogy with zinc or cadmium, should substitute on a gallium site and be surrounded tetrahedrally by four arsenic atoms. Magnesium also forms a stable arsenide, Mg_3As_2 (estimated heat of formation, − 90,000 cal./mole), in which each magnesium atom is bounded by four arsenic atoms as nearest neighbours. For this reason, the activity coefficient of magnesium in gallium arsenide should be considerably less than unity.

Equations (2) and (3) represent the limiting conditions likely to be encountered in experimental work. Ratios of $x_{\text{silicon}}/x_{\text{magnesium}}$ derived from them need to be reduced to allow for the non-ideality of magnesium. The extent of this reduction is not easy to estimate.

This work is being carried out under Admiralty contract *CP.4D*(2) 5298/58/60.2.

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Crystal Growth of Gallium Arsenide using Carbon Boats

GALLIUM arsenide is a semiconductor of great interest from the purely scientific point of view and as a device material. It is therefore desirable to be able to prepare single crystals of high purity. Because of the reactive nature of gallium liquid and arsenic vapour at high temperature, only a limited number of refractories may be used as boat materials for gallium arsenide (melting point, 1,511° K.). Among these are carbon, vitreous silica and alumina. In addition a sealed envelope of vitreous silica is generally employed to prevent the escape of arsenic vapour from the system.

It is observed that, in general, the use of carbon or carbonized silica boats facilitates single crystal growth, whereas ingots grown in silica boats exhibit various degrees of wetting and subsequent polycrystallinity. On the other hand, despite reduced wetting, material grown in carbon boats is the more strongly *n*-type. Typically, a crystal grown in a carbon boat yields an electron concentration of $1{\cdot}3 \times 10^{18}$/cm.^{3} and Hall mobility, $\mu_n = 2{,}500$ cm.^{2}/V./sec., compared with the usual 3×10^{16}/cm.^{3} and 5,000 cm.^{2}/V./sec. obtained for crystals grown in silica. It is worth adding that in all cases the observed mobility is less than that to be expected from the carrier concentration[1], implying a fair degree of compensation. There is, therefore, a considerable gain to be achieved by the removal of residual impurities.

Although the results above indicate that the presence of carbon increases the donor concentration in gallium arsenide, the absence of wetting suggests that this is not due to carbon being dissolved in the melt. It is suggested, therefore, that the prime effect of carbon is to bring about reduction of the silica envelope according to the equation:

$$\mathrm{SiO_2(s) + 2C(s) \rightleftharpoons Si(s) + 2CO(g)} \qquad (1)$$

Spectrographic examination indicates that silicon is present in fair amounts in gallium arsenide grown in carbon boats.

At the melting point of gallium arsenide, the standard free energy change for reaction (1) is $\Delta G^0 =$ 26,000 cal.[2]. Using the relation:

$$\Delta G^0 = -RT \log_e Kp = -2RT \log_e p_{\mathrm{CO}}$$

where p_{CO} is partial pressure of carbon monoxide, we obtain $p_{\mathrm{CO}} = 1{\cdot}3 \times 10^{-2}$ atmosphere. We can see, therefore, that starting with carbon and silica in a closed system, free silicon is produced until the pressure of carbon monoxide reaches the above value. In addition, the free silicon is able to dissolve in molten gallium arsenide to form a dilute solution; its activity is thereby reduced and the reaction can proceed further from left to right.

Since similar results are obtained for carbon as for carbonized silica boats, it seems that silicon must enter the melt from the vapour phase. The following reactions are reversible and provide mechanisms for the transport of carbon and silicon from one part of the system to another:

$$\mathrm{C(s) + CO_2(g) \rightleftharpoons 2CO(g)} \qquad (2)$$

$$\mathrm{Si(s) + SiO_2(s) \rightleftharpoons 2SiO(g)} \qquad (3)$$

At 1,511° K., the partial pressure of silicon monoxide is $9{\cdot}55 \times 10^{-4}$ atmosphere.

In practice, the equilibrium vapour pressure of carbon monoxide is not attained. It seems likely that since the partial pressure of arsenic in the system is high (0·9 atmosphere), the rate at which silicon enters the melt is mainly limited by gaseous diffusion and convection.

Carbon should be a satisfactory crucible material for gallium arsenide under conditions where it is unable to react with reducible oxides such as silica. Recent chemical analysis of gallium arsenide grown in contact with carbon has shown the carbon content to be less than $2{\cdot}7 \times 10^{17}$ atoms/cm.^{3}, which is the limit of detection of the method. One would expect the solubility of carbon in gallium arsenide to be comparable with that in germanium at the same temperature (about 10^{13} atoms/cm.^{3} at 1,511° K.).

This work is being carried out under Admiralty contract *CP.4D*(2) 5298/58/60.2.

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Methane Oxidation initiated by Electrical Discharge

AN apparatus for the mass-spectrometric study of free radicals occurring in gas-phase reactions has recently been completed in this Laboratory. While testing the equipment we have investigated the reactions in a methane–oxygen mixture, subsequent to electrical discharge in one of the gases.

The apparatus, which is illustrated in Fig. 1, is essentially similar to that of Foner and Hudson[1]. A flow of gas is maintained through a silica discharge tube, the discharge products being swept into the 'Pyrex' reaction vessel, where they may mix with another gas entering down a side tube. The mixing zone is sampled by a pinhole in platinum foil leading to the ion source of a Metropolitan-Vickers *M.S.*2 mass spectrometer. The ion source was modified by the makers to provide a higher pumping speed, differential pumping from the filament region, and line-of-sight access from the pinhole leak to the ionization chamber.

The electrical discharges were maintained by a 200-watt radio transmitter, working at 13,560 kc./s., which supplied a coil wound around the discharge tube, 2 ft. above the sampling pinhole. Pressures of 500–750μ and flow-rates of 500–700 ml./sec. were used in this work.

The detectable products from a discharge in methane were mainly ethane and hydrogen, with some acetylene and ethylene; approximately one-third of the methane was removed, but the $\mathrm{C_2}$ hydrocarbons detected could only account for 60 per cent of this. When oxygen was added (through the side tube) to the products of a discharge in methane the detectable products were scarcely affected; there was a slight decrease in the amount of ethane formed, and some water and carbon dioxide were detected, but 40 per cent of the carbon was still unaccounted for.

If the oxygen is submitted to the electrical discharge and the products mixed with undissociated

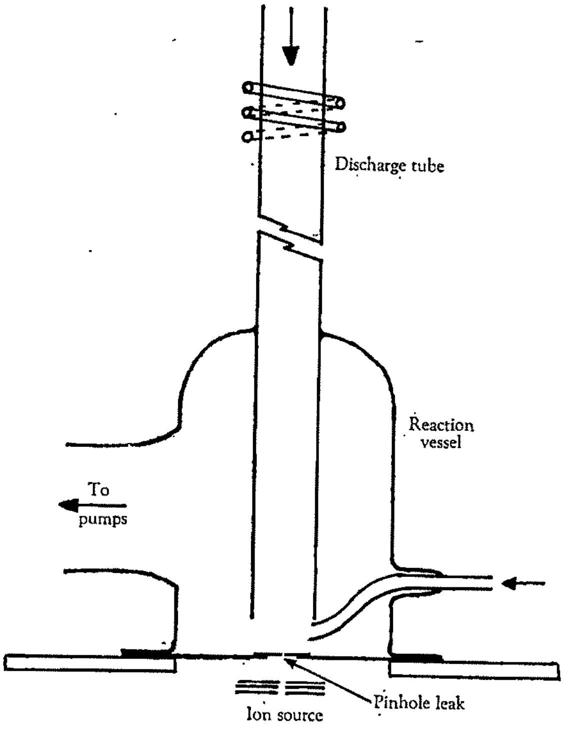

Fig. 1

methane, the detectable products of the reaction are very different. Within the limits of the present work there is a carbon balance, 80 per cent of that lost as methane appearing as ethane, and the remainder equally divided between ethylene and carbon dioxide; no acetylene is detectable. The remaining hydrogen appears mainly as water, only about 10 per cent being H_2.

A large residual peak exists at a mass/charge ratio of 17, its height being two and a half times that of the water peak at $m./e. = 18$. We are unable to explain this peak, except by assuming the presence of hydroxyl radicals.

Since we were not able to use low electron energies to confirm the presence of free radicals[2], the above conclusions must be regarded as tentative. Work is now in progress to use the low electron energy method, and also to coat the surface of the platinum foil so as to reduce the rate of recombination of radicals thereon; further results will be reported in due course.

We wish to thank Mr. G. Wilkinson for glass-blowing, and Dr. J. W. Linnett for the use of the mass spectrometer, which was provided by grants from the Department of Scientific and Industrial Research and Esso Research, Ltd. One of us (M. B.) is the holder of a Department of Scientific and Industrial Research maintenance grant, and the other (J. C.) is an Imperial Chemical Industries, Ltd., Research Fellow.

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Internuclear Distances of Methyl and Methylene Radicals

The experimental determination of the geometry of the methyl and methylene radicals is very difficult[1,2]. Hence it may be of some interest to report estimates of the internuclear distances of the methyl and methylene radicals. Comparisons were made of several hydride radicals and molecules of the first row of the Periodic Table as shown in Table 1 and Fig. 1. The arrangement indicates regularity in the differences of the internuclear distances of these structures.

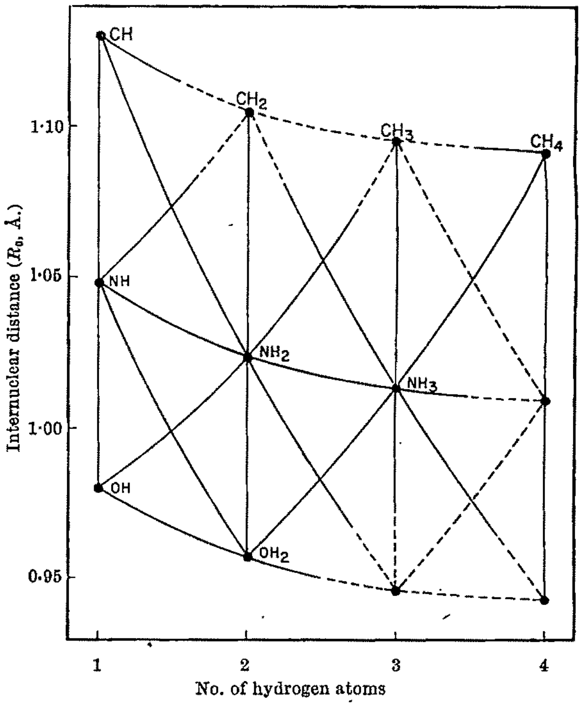

Fig. 1. Relation between internuclear distances of the hydrides of elements of the first row of the Periodic Table as a function of the number of hydrogen atoms

The internuclear distances of methyl and methylene radicals are estimated to be respectively 1·096 and 1·105 Å. It is interesting to point out the general utility of networks of the type shown in Fig. 1. A large grid of which Fig. 1 is a part has been used in another connexion[3]. A physically significant point of such a network must lie at the juncture of four lines as indicated in the diagram. The structures shown in Fig. 1 are both radicals and saturated molecules. Since the nitrogen hydrides fit into this simple scheme,

Table 1. Comparison of Internuclear Distances of First-Row Hydrides

	R_0		R_0		R_0		R_0
CH	1·130[a]	CH_2	1·105[b]	CH_3	1·096[b]	CH_4	1·092[f,g]
NH	1·048[a]	NH_2	1·024[c,d]	NH_3	1·014[e]		
	0·082		0·081		0·082		
NH	1·048[a]	NH_2	1·024[c,d]				
OH	0·980[a]	OH_2	1·957[d]				
	0·068		0·067				

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Table 2. GEOMETRY OF THE HYBRID METHYLENE RADICAL

CH or CD Å.	Fig. angle	I (CHD) × 10^{-40} gm. cm.^{2}	Deviation from y-axis
1·105	137° 20′	5·262	6° 50′
1·095	140°	5·252	6° 15′
1·071	147° 54′	5·230	4° 46′

it may be expected that the corresponding carbon hydrides should also be located within the same framework in similar manner.

From the rotational constants[2] $B''(CD_2) = 3{\cdot}96$ and $B''(CHD) = 5{\cdot}33$ cm.$^{-1}$, the principal moments of inertia of the CHD radical are found to be: $I(CD_2) = 7{\cdot}084 \times 10^{-40}$ gm. × cm.^{2} and $I(CHD) = 5{\cdot}247 \times 10^{-40}$ gm. × cm.^{2}. In the latter case, the figure axis inclines slightly from the y-axis of the co-ordinate system. The comparison shown in Table 2 indicates that neither the measured distance (1·071 Å.) nor the predicted value (1·105 Å.) is satisfactory. It can also be seen that a carbon–hydrogen distance of 1·095 Å. yields $I(CHD) = 5{\cdot}252 \times 10^{-40}$ gm. × cm.^{2}, which checks the experimental value. However, the results rest on the assumption that the experimentally determined rotation constants are correct. Unless the two radicals under discussion show some extraordinary electron configurations, it is expected that the present prediction and final experiment must ultimately agree. No detailed calculations have been made for the methyl radical.

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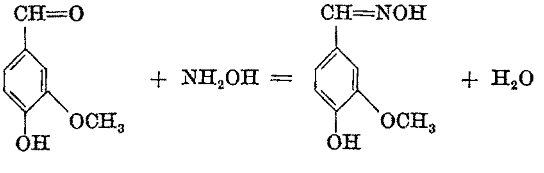
Polarographic Determination of Vanillin

VARIOUS physico-chemical and chemical methods have been used for the determination of vanillin. Of the volumetric methods[1], the oxime method has been modified in many ways, whereby either the quantity of the hydrochloric acid set free in the reaction of hydroxylamine hydrochloride with vanillin, or the quantity of the unreacted hydroxylamine, is titrated visually or potentiometrically. Colorimetric methods are based on the slight reducing capacity of phenols[2], the polarographic methods[3] on the reduction of the aldehydic group of vanillin.

I have devised a new method for the determination of vanillin by utilizing the condensation of the aldehydic group with hydroxylamine with formation of the oxime, according to the following scheme:



The resulting oxime is polarographically active. The condensation of the aldehydic group proceeds quantitatively and the resulting oxime is reduced at more positive potentials. The sensitivity of the method is twice that of the direct polarographic determination. The condensation as well as the reduction of vanillin oxime proceed best in a phosphate buffer of pH 5·9. Under these conditions, the height of the oxime wave is precisely double that of vanillin before condensation (Fig. 1).

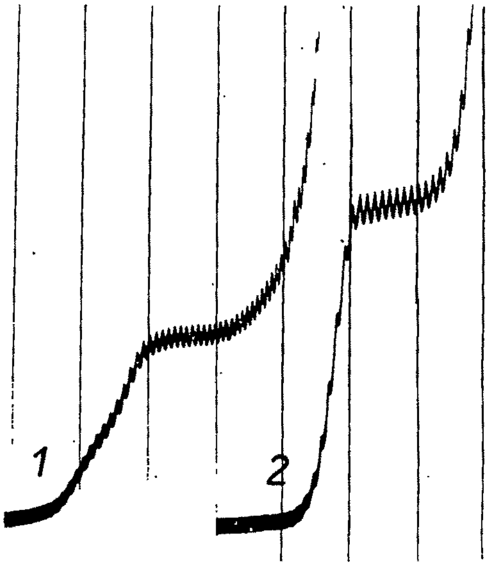


Fig. 1. Condensation of vanillin with hydroxylamine. Curve 1: 5 ml. 0·1 M ammonium chloride + 5 ml. 1 × 10^{-3} M vanillin solution, from −1·30 V. Curve 2: 4 ml. phosphate buffer, pH 5·9 + 5 ml. 1 × 10^{-3} M vanillin solution + 1 ml. 1 M hydroxylamine hydrochloride, from −0·78 V. Saturated mercurous sulphate electrode, sensitivity 1: 30, 195 mV./absc.

In phosphate buffer of pH 5·9, vanillin is reduced at a half-wave potential of − 0·93 V. (sat. calomel electrode) in a single well-developed four-electron wave. The dependence of the height of the oxime wave is a linear function of the concentration of vanillin. The wave shows a diffuse character, is easy to read and suitable for analytical use.

A detailed account of the reaction conditions and of the utilization of the method for the analysis of vanillin, both in the pure state and in foods, will be described elsewhere.

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Spectrophotometric Determination of the Electron Affinities of Some Diatomic Molecules

THE purpose of this communication is to present a calculation of the electron affinities of some molecules from charge transfer spectra. Mulliken's[1] theory for a charge-transfer process leads to the expression:

$$h\nu_{\text{max.}} = I_D - E_A - \Delta \qquad (1)$$

where $h\nu_{\text{max.}}$ is the energy corresponding to the most probable electronic transition, I_D and E_A are the vertical ionization potential of the electron donor and the vertical electron affinity of the electron acceptor. Δ represents the stabilization energy, relative to the ground-state, of the excited state in the nuclear configuration corresponding to the ground-state. This relation has been confirmed for some series of various donors with one acceptor[2,3] and for series of a particular donor with various acceptors[4].

By using spectroscopic data for charge transfer from one electron donor to various electron acceptors the electron affinities of these acceptors may be evaluated, provided that one absolute value of E_A is accurately known. It is assumed that the value of Δ is independent of the nature of the electron acceptor[4]. Thus, equation (1) leads to the relation:

$$h\nu_{\text{max.}}^{(1)} - h\nu_{\text{max.}}^{(2)} = E_{(2)} - E_{(1)} \qquad (2)$$

The magnitude of the vertical electron affinities of molecular halogens have not yet been established. These data were calculated by using the recent experimental results for the absorption spectra of iodine atom and aromatic hydrocarbon complexes[5] (Table 1).

Table 1. ELECTRON AFFINITIES OF MOLECULAR HALOGEN COMPOUNDS FROM CHARGE TRANSFER SPECTRA OF BENZENE COMPLEXES

Electron acceptor A	$\lambda_{\text{max.}}$	$h\nu_{\text{max.}}$	E_I—E_A	E_A	E_X—D_{X_2} (refs. 8 and 9)	E_A (previous estimation)
I	495 (ref. 5)	57·8	0	74·6 ± 1·5 (ref. 8)		
I_2	297 (ref. 6)	96·3	38·5	36	40 ± 1·5	41·5 (ref. 1)
Br_2	292 (ref. 7)	98·0	40·2	34	35·6 ± 1·5	
Cl_2	278 (ref. 7)	102·9	45·1	30	30·7 ± 1·5	≤39 (ref. 8)
ICl	287 (ref. 7)	99·6	41·8	33		

Wave-lengths given in mμ. Energetic data in kcal.

The lower limit for the equilibrium values of the electron affinities $E^e_{X_2}$ of the homonuclear molecules X_2 can be obtained from the relation:

$$E^e_{X_2} \geqslant E_X - D_{X_2} \qquad (3)$$

where D_{X_2} is the dissociation energy of the molecule. The values obtained for the vertical electron affinities of the halogens are smaller than the lower limits for $E^e_{X_2}$. As the stabilization energy of the excited state of the atomic iodine complex may be slightly higher compared with the corresponding state of the molecular iodine complex, the data of Table 1 present the lower limits for the vertical electron affinities of the halogen molecules. The vertical electron affinity of I_2 is expected to be lower than the corresponding equilibrium value[10]. However, our results indicate that in the benzene–iodine complex the repulsive part of I_2^- curve does not cross the I_2 curve at its minimum, as was indicated by the results of the dissociative attachments of the electrons in free iodine[10].

The same method was applied for the evaluation of the electron affinities of oxygen and of nitric oxide. Ultra-violet absorption spectra of oxygen in aromatic and aliphatic hydrocarbons[11-14] and of nitric oxide in alcohols[14] were observed. The continuous bands were assigned to charge transfer spectra[13,14], the oxygen and nitric oxide molecules acting as electron acceptors. The band onset energy $h\nu_0$ (arbitrarily chosen for $\varepsilon = 10$ litre mole^{-1} cm.$^{-1}$) was used for the specification of the transition energy. It is assumed that the vibrational broadening is independent of the electron acceptor. This relation is quite well fulfilled for the series of the I_2 aromatic complexes. The energetic data for oxygen and nitric oxide complexes are presented in Table 2.

Using the value of E_{I_2} we obtain $E_{O_2} = 17 \pm 4$ kcal. and $E_{NO} = 20 \pm 4$ kcal.

A recent theoretical estimation of the equilibrium electron affinity of oxygen[16] leads to a much lower value of 3·5 kcal. However, the result obtained for E_{O_2} from charge transfer spectra is in agreement with the equilibrium electron affinity of oxygen estimated from thermochemical data[8].

Electron attachment experiments in nitric oxide indicate that the electron affinity of this molecule is positive[8].

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BIOCHEMISTRY

Structure of Collagen

A TRIPLE helical structure for collagen with two systematic hydrogen bonds for every three residues was put forward from this laboratory in 1955 [1]. This structure was criticized by Rich and Crick[2] on the basis of a few short interatomic contacts occurring in it; they suggested that only structures with one hydrogen bond for every three residues will be free of bad contacts. Recently, we have examined the whole question carefully, and it has been found that

Table 2. DETERMINATION OF THE ELECTRON AFFINITIES OF OXYGEN AND NITRIC OXIDE

D \ A	I_2		O_2		NO		E_{I_2}—E_{O_2}	E_{NO}—E_{O_2}
	λ_0	$h\nu_0$	λ_0	$h\nu_0$	λ_0	$h\nu_0$		
Benzene	380 (ref. 6)	76	301 (ref. 14)	95	252 (ref. 14)	112	19	3
Cyclohexane	280 (ref. 3)	102	242 (ref. 12)	118			16	
Methanol	300 (ref. 15)	95	248 (ref. 14)	115·3			20	
Ethanol	300 (ref. 15)	95	251 (ref. 14)	114			21	

Wave-lengths given in mμ. Energetic data in kcal.

it is possible to build up a two-bonded structure (two hydrogen bonds for three residues) while retaining all contacts within permissible values. This has been possible mainly because a study of the observed interatomic contacts in various reported organic structures showed that contact distances actually occur which are much shorter than those mentioned by Rich and Crick[2], namely, C . . . C = 3·6 – 4·0 Å. and C . . . O = 3·2 – 3·4 Å. In fact, if Rich and Crick's criteria were adopted, some of the accepted structures like the α-helix will become unacceptable. Secondly, the study of the relation between the NH . . . O hydrogen bond distance and the infra-red frequency showed that the observed frequency (Ramachandran, G. N., Sasisekharan, V., and Thathachari, Y. T., unpublished work) in collagen, which is higher than that observed in other proteins, should correspond to a value of the order of 3·0 Å. The actual parameters of the minor helix of the collagen structure have also been re-determined. These gave a value of 2·95 Å. for the residue height and 3·28, rather than 3·33 (10/3), for the number of residues per turn. As a consequence, the twist for three residues in the triple helical structure should be 30° rather than 36°.

On the basis of these results, a satisfactory two-bonded structure has been worked out, and its co-ordinates are given in Table 1. The two hydrogen bonds which are formed, namely, $N_1H_1 \ldots O_2$ and $N_3H_3 \ldots O_3$, are of the same type as in the earlier structure proposed by Ramachandran and Kartha[1]. They are of the order of 3·00–3·05 Å. and the hydrogen-bond angles are less than 30°. The primary bond distances, bond angles and the deviation from planarity of the amide group are all well within the ranges observed in organic structures. Denoting the three chains by *A*, *B* and *C*, the only short contacts which occur are: (1) $O_2(A) \ldots O_3(A) = 2{\cdot}73$ Å.; (2) $C_1(B) \ldots O_2(A) = 2{\cdot}90$ Å.; (3) $C_4(A) \ldots O_2(B) = 3{\cdot}10$ Å.; (4) $C_4(A) \ldots O_3(B) = 3{\cdot}03$ Å.; and (5) $C_1(A) \ldots C_1(B) = 3{\cdot}50$ Å. The structure is thus seen to be satisfactory in every respect.

Table 1. ATOMIC CO-ORDINATES OF THE ATOMS IN ONE SET OF THREE RESIDUES OF THE PROPOSED STRUCTURE OF COLLAGEN

The cylindrical polar co-ordinates given are with respect to the axis of the major helix

Atom	*r* (Å.)	Degrees	*z* (Å.)
C_1	1·15	0·0	0·00
C_1'	2·27	12·0	1·00
O_1	3·26	–3·6	1·15
N_1	2·43	38·8	1·70
H_1	2·15	63·1	1·71
C_2	3·52	35·4	2·72
C_2'	2·93	23·0	3·96
O_2	1·75	17·5	4·16
N_2	3·79	15·8	4·82
H_2	4·78	18·1	4·70
C_3	3·47	2·0	6·00
C_3'	2·50	18·7	6·86
O_3	2·68	45·5	6·50
N_3	1·95	1·8	7·87
H_3	2·29	–24·4	8·04
C_4	1·15	30·0	8·85

The two-bonded structure is found to be highly specific and is satisfactory only for a coiled-coil structure with a twist close to 30° for three residues, and is definitely unsatisfactory for twists of 10° and 50°. It is also satisfactory only for a residue-height close to 3·00 Å. Thus, it is possible to explain stereochemically why collagen occurs with the particular structure actually found and why the parameters are the same for collagen obtained from widely different sources. The inextensibility of the collagen structure can also be explained on the basis of the steric hindrance produced by the proline and hydroxyproline residues.

Although, when the sequence gly-pro-hypro occurs, two bonds cannot occur systematically, the above structure can be slightly modified locally to incorporate this sequence. The detailed co-ordinates for such a modified structure have also been worked out.

Finally, it may be mentioned that the calculated Fourier transform of the new structure is in good agreement with observation, and its expected infrared dichroism is also consistent with the observations.

Thus we would expect that most of the residues in the regular collagen structure would take up a two-bonded configuration, which is highly specific. It should, however, be mentioned that other types of linkages such as ester linkages and gamma-peptide linkages have been reported for collagen, but the number of these is so small that the main structure is not likely to be affected by these.

We wish to thank Messrs. Y. T. Thathachari, B. R. Lakshmanan, and C. Ramakrishnan, who also collaborated in this project. The detailed results are to be published elsewhere.

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Effect of Temperature on the Concentration of Free Radicals in Melanins from *Allium cepa*

IN a previous communication we reported the presence of free radicals in the seeds of the onion *Allium cepa*[1]. Further investigations have shown that these free radicals are attributable to the melanins present in the black seed-coats.

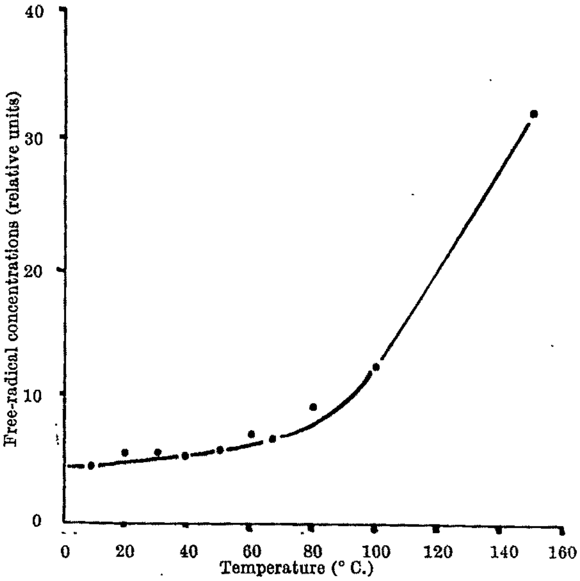

Fig. 1. Variation with temperature of free radical concentrations in onion seeds

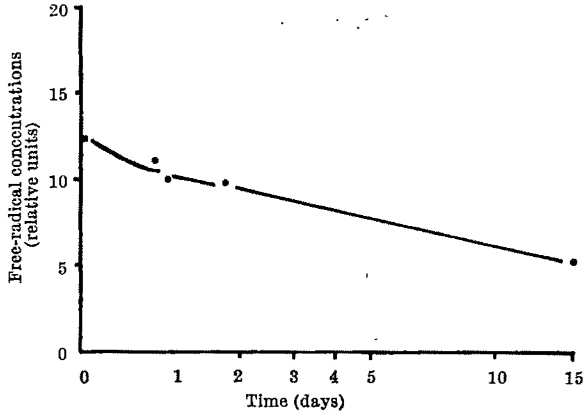

Fig. 2. Rate of decay in liquid oxygen (seeds heated to 100° C.)

Batches of 10 similar seeds were kept at a specified temperature for 15 min. and then placed in the liquid oxygen-surrounded cavity of a microwave spectrometer (125 kc./s. field modulation; x-band model, with automatic locking of klystron frequency to that of the cavity).

Fig. 1 shows the effect of temperature on the relative free-radical concentration, there being a marked increase in the seeds kept at 60° C. and above. The signal diminished in strength with time (the seeds being kept in liquid oxygen throughout the experiment). The fall was quite rapid over the first 3 hr. and slower thereafter. A similar effect of temperature on the signal strength was found to occur with seed-coats alone, or with melanin extracted from seeds by refluxing with concentrated potassium hydroxide. Melanins extracted from such widely different sources as *Calliphora* adults, *Rana* tadpoles, human hair and dark-coated seeds of *Dianthus barbatus*, *Delphinium* sp. and *Aesculus hippocastanum* gave results essentially similar to those described for onion seeds. Barley, marrow and French beans seeds without melanin in their seed coats gave no appreciable signal. Ingram, Allen and Mason[2] have described the presence of unpaired electrons in melanin extracted from *Sepia* ink and *Calliphora* pupæ.

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Adenosine Triphosphate- and Coenzyme *A*-dependent Decarboxylation of Oxalate by Extracts of Peas

Despite the wide distribution of oxalic acid in plants, and its high concentration in certain tissues[1], very little is known of its metabolism. Investigations with whole plants indicate that the acid is either metabolically inert[2,3] or metabolized slowly to glycollic acid[4]. Work with cell-free extracts, on the other hand, has demonstrated that the enzyme oxalate oxidase is widely distributed in higher plants. This enzyme has been shown to be associated with the 'particulate fraction' of sugar beet leaves[5,6] and roots[7].

The following report provides evidence for the presence, in extracts of germinating pea cotyledons, of an enzyme system which catalyses an adenosine triphosphate (ATP)- and coenzyme *A*-dependent conversion of oxalate to formate and carbon dioxide. The enzyme system is associated with the supernatant fraction obtained after centrifugation of a homogenate at 10,000g for 30 min., and does not require oxygen for activity.

Pea seeds (*Pisum sativum*, var. Telephone) were germinated in moist peat moss for five days. The cotyledons were homogenized with twice their weight of a solution containing 0·45 M sucrose, 0·2 M *tris* buffer of pH 7·2, and $5 \times 10^{-4} M$ 2,3-dimercapto-1-propanol. The homogenate was squeezed through cheese cloth, the filtrate centrifuged at 1,200g for 7 min., and the supernatant so obtained centrifuged at 10,000g for 30 min. This crude supernatant was used as a source of the enzyme system. To demonstrate co-factor requirements, the crude supernatant was adjusted to pH 6·0 with 1 M acetic acid and centrifuged at 10,000g for 10 min. The clear supernatant solution was brought to pH 7·2 with 1 M potassium hydroxide, and dialysed for 3 hr. against 0·01 M *tris* buffer at pH 7·2. All operations were carried out at 0–3° C.

Table 1 shows the co-factor requirements for the decarboxylation of oxalate-1,2-^{14}C. The reaction was carried out in a conventional Warburg vessel, $^{14}CO_2$ being absorbed by 0·2 ml. 20 per cent potassium hydroxide in the centre well. After 30 min. incubation at 30° C., the reaction was stopped by tipping saturated potassium bisulphate from the side arm. Carbon-14 labelled carbon dioxide was precipitated as barium carbonate and the counts/min. determined with a Geiger–Müller tube. The results demonstrate an absolute requirement for ATP and coenzyme *A*. Omission of glutathione or magnesium ions causes a significant decrease in the rate of decarboxylation. Omission of phosphate causes a slight stimulation in the rate, while omission of thiamine pyrophosphate has little effect. By treatment of the enzyme system with 'Deacidite *FF*' in the chloride form, it is possible to demonstrate a stimulation in the rate of decarboxylation by the addition of thiamine pyrophosphate.

Table 1. Co-factor Requirements for the Decarboxylation of Oxalate-1,2-^{14}C

Components	$^{14}CO_2$ Counts/min.
Complete	1,012
Minus ATP	8
,, coenzyme *A*	7
,, glutathione	888
,, thiamine pyrophosphate	929
,, magnesium chloride	570
,, phosphate	1,255

The complete reaction mixture contained 1 μmole oxalate-1,2-^{14}C (12,200 counts/min.); 0·5 ml. enzyme (approx. 5 mgm. protein); 100 μmoles phosphate buffer pH 7·2; 5 μmoles glutathione; 0·1 μmole thiamine pyrophosphate; 0·1 μmole coenzyme *A*; 5 μmoles magnesium chloride; 5 μmoles ATP. Final volume 2·0 ml., pH 7·2

The stoichiometry of the reaction was demonstrated by incubating 1·14 μmoles of oxalate-^{14}C (100,000 counts/min.) with the enzyme system under the conditions shown in Fig. 1. At fixed times the reaction was stopped and $^{14}CO_2$ was determined as described here. Each reaction mixture was then extracted continuously with ether for approximately 20 hr., the ether extract so obtained evaporated to a small volume under a flow of ammonia gas, and the concentrated extract made up to a known volume. An aliquot of each of the extracts was chromatographed in 95 per cent ethanol/concentrated ammonia, 100:1 (v/v)[8]. A radio-autograph of the chromatogram

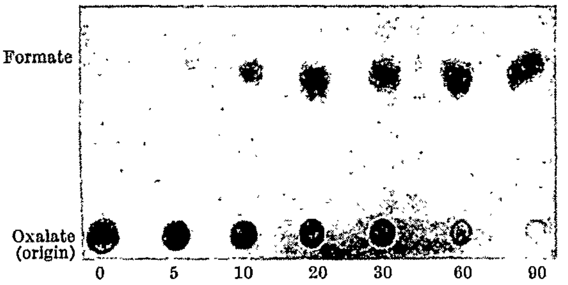

Fig. 1. Formation of formate-^{14}C during decarboxylation of oxalate-^{14}C. To each reaction vessel was added 1·14 μmoles oxalate-1,2-^{14}C (100,000 counts/min.); 1·0 ml. crude supernatant (approx. 27 mgm. protein); 5 μmoles glutathione; 0·1 μmole thiamine pyrophosphate; 0·1 μmole coenzyme *A*; 5 μmoles magnesium chloride; 10 μmoles ATP; 0·2 ml. 20 per cent potassium hydroxide in the centre well of a conventional Warburg vessel. Final volume 2·0 ml., *p*H 7·2. Temperature, 30° C.

(shown in Fig. 1) illustrates that the decarboxylation of oxalate-^{14}C is accompanied by the formation of a radioactive compound migrating ahead of oxalate-^{14}C. This compound was identified as formate-^{14}C by (*a*) two-dimensional co-chromatography with authentic formate, using 95 per cent ethanol/concentrated ammonia, 100:1 (v/v)[8], in the first dimension (R_F, 0·37), and absolute ethanol/16 per cent ethylamine, 4:1 (v/v)[9], in the second dimension (R_F, 0·65); (*b*) steam volatility under acid conditions; (*c*) degradation to $^{14}CO_2$ by mercuric acetate[10].

Aliquots of the above extracts were assayed for formate-^{14}C and oxalate-^{14}C. Formate-^{14}C was first estimated by degradation to $^{14}CO_2$ by mercuric acetate. Oxalate-^{14}C was then estimated by degradation to $^{14}CO_2$ by ceric sulphate[11]. This procedure was found to give satisfactory quantitative recoveries using mixtures of authentic oxalate-^{14}C and formate-^{14}C. The results shown in Table 2 demonstrate that the decarboxylation of each mole of oxalate-^{14}C results in the formation of one mole of formate-^{14}C and one mole of $^{14}CO_2$.

Table 2. STOICHIOMETRY OF OXALATE DECARBOXYLATION

Incubation time (min.)	Oxalate-1,2-^{14}C decarboxylated (μmoles)	Formate-^{14}C formed (μmoles)	$^{14}CO_2$ formed (μmoles)
0	0·00	0·02*	0·00
5	0·14	0·12	0·10
10	0·25	0·28	0·29
20	0·58	0·55	0·57
30	0·73	0·71	0·70
60	1·09	1·11	0·96
90	1·11	0·97	1·05

Conditions were the same as those described for Fig. 1. All samples were counted as barium carbonate. Each figure represents the mean of two determinations. The maximum deviation from the mean was ± 0·04 μmole.

* Separate experiments showed that up to 2 per cent of oxalate-^{14}C was consistently degraded to carbon-14 labelled carbon dioxide by mercuric acetate. The error is not known exactly for each estimation, but in every case represents a potential error less than the maximum deviation of ± 0·04 μmole.

It has not been possible to determine the stoichiometry of ATP in the reaction, since the enzyme system is quite crude. Coenzyme *A* is required only in catalytic quantities.

On the basis of the above evidence, the following scheme for decarboxylation of oxalate is suggested:

$$\text{Oxalate} + \text{coenzyme } A \xrightarrow{\text{Mg}^{++}\text{, ATP}} \text{oxalyl coenzyme } A$$
$$\text{Oxalyl coenzyme } A \longrightarrow CO_2 + \text{formyl coenzyme } A$$
$$\text{Formyl coenzyme } A \longrightarrow \text{formate} + \text{coenzyme } A$$

The reaction sequence is similar to that demonstrated by Jakoby *et al.*[12] for extracts of *Pseudomonas* grown on oxalate, and by Quayle *et al.*[13] for *P. oxalaticus* extracts. However, in both these systems oxalate was not activated directly, but required catalytic quantities of acetate[12] or succinate[13]. Treatment of the pea enzyme system with 'Deacidite *FF*' in the fluoride form failed to reveal a requirement for either acetate or succinate.

At present it is difficult to suggest a physiological role for this enzyme system in plants. The participation of ATP and coenzyme *A* suggests that the reaction may have some significance in the energetics of intermediary metabolism.

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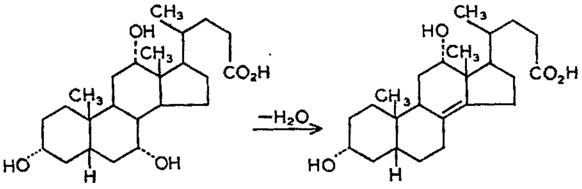
Carcinogenic Activity of Apocholic Acid

THE production of cancer by bile acids is a topic of far-reaching interest, because of the natural occurrence and biosynthesis of these substances in the body and of their molecular structure, which places them in the biologically essential family of steroids. In view of this, the two main bile acids, namely, cholic acid and deoxycholic acid, have often been tested for carcinogenicity, but results have so far been either negative or controversial. Thus, while both Ghiron[1], and Cook *et al.*[2], were able to produce sarcomas in mice by subcutaneous injection of deoxycholic acid, Shear *et al.* recorded negative results with both cholic and deoxycholic acid and attributed the sarcomas described in the literature to the possible influence of the solvent used[3]; more recently, Klein also reported negative results using the intramuscular route[4]. It was, therefore, evident that if these acids were carcinogenic, their activity was weak in degree and not constantly reproducible.

This communication reports experiments made with apocholic acid (II), a product of mild dehydration of cholic acid (I), and the results indicate a definite carcinogenic activity for this compound.

Forty-two 3-month-old male mice of strain *XVII nc/Z* (Radium Institute) were given subcutaneous injections in the right flank (10 times with 10-day intervals between each injection) of 0·5 mgm. of apocholic acid dissolved in 0·2 c.c. sterile neutralized olive oil. Fourteen mice which died between the 120th and 217th day following the start of our



(I) (II)

experiment showed no tumour at the site of injection. The first sarcoma to be observed *in situ* occurred on the 204th day, and the animal died on the 227th day; three other mice developed sarcomas on the 230th, 241st, and 370th day, respectively.

Spontaneous sarcomas of the soft parts have never been observed in our strain of mice; nor could the excipient be held responsible for the production of the tumours: every year since 1955 a control group of some 40–60 mice are given 10 injections of 0·2 c.c. of the oil referred to above, and in similar conditions, and out of a total of 350 animals thus treated (120 of which survived over 500 days and 66 over 700 days) none has ever shown a tumour at the site of injection.

It is worth mentioning that both deoxycholic and apocholic acid are powerful complexing and enolizing agents, giving stable addition-compounds ('choleic acids') with numerous organic molecules[5]. The present work adds experimental evidence to the proposition that certain steroids with an appropriate molecular structure are able to produce tumours *in situ*[6]; it also lends support to the theory which postulates an enolization of proteins through van der Waals forces as an early step in certain mechanisms of chemical carcinogenesis[7].

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Interaction of Proteins and Nucleotides in Buffered Aqueous Solutions

A NUMBER of reports have appeared in the literature on the binding of organic ions by proteins. Klotz and Luborsky[1] have discussed the influence of various solvents on the binding of such ions and the nature of the complexes which are formed. Kakuliya[2], studying the interaction of proteins with electrolytes, noted a decreased rate of specific conductivity and an increased *p*H when adenosine triphosphate (ATP) was added to a protein solution. Tonomura and Morita[3] observed the binding of pyrophosphate by myosin *A* and myosin *B*.

During the preparation of solutions containing ATP and albumin, Katz (personal communication) noted the formation of a precipitate. Since the solutions were not well buffered, we assumed that the precipitate was due simply to the formation of an insoluble un-ionized salt similar to protein precipitates formed by the action of trichloroacetic or phosphotungstic acid on aqueous solutions of proteins.

We have investigated this precipitation of nucleotides and proteins under rigorously controlled conditions of *p*H and of nucleotide and protein concentration and wish to report the results of our preliminary experiments.

In our first experiment the precipitation of albumin by ATP was investigated. All reactions were carried out in 7·0 ml. of McIlwain buffer at the *p*H's and concentrations shown in Table 1. All mixtures were centrifuged at 2,500 r.p.m. The supernatants were analysed for protein by a modification of the biuret reaction[4] and for ATP by measuring the optical density at 260 mμ in a Beckman *DU* spectrophotometer.

Table 1. PRECIPITATION OF ALBUMIN BY ATP

*p*H	Albumin (mgm.)	ATP (mgm.)	Albumin precipitated (mgm.)	ATP precipitated (mgm.)
2·2	4·0	21·2	4·0	9·7
	4·0	42·4	4·0	9·8
	4·0	84·8	4·0	9·8
3·4	4·0	21·2	4·0	7·7
	4·0	42·4	4·0	8·7
	4·0	84·8	4·0	6·6
4·6	4·0	21·2	0·25	0
	4·0	42·4	1·5	2·6
	4·0	84·8	4·0	5·3
5·6	4·0	21·2	0·25	0·2
	4·0	42·4	0·50	0
6·8	4·0	21·2	0·20	0
	4·0	42·4	0·40	0·4

It may be seen from Table 1 that there is complete precipitation of albumin at *p*H's below the isoelectric point of the protein and virtually no precipitation at *p*H's above this point.

Similar investigations were carried out using lysozyme, hæmoglobin, and human globulins. All these proteins were found to interact with ATP when the *p*H was on the acid side of the isoelectric point of the protein, but not when it was on the alkaline side. This could also be demonstrated when ATP was replaced by triphosphates of other purine or pyrimidine ribosides, but not when the corresponding diphosphates or monophosphates were used. We are at present trying to establish the quantitative nature of these reactions.

In another series of investigations the behaviour of the precipitate was examined. The precipitate formed by ATP and albumin was centrifuged and redissolved in distilled water. It was then lyophilized and dissolved in 0·05 ionic strength veronal buffer, *p*H 8·6. Paper electrophoresis of this solution yielded a single spot, the mobility of which was the same as that of the original albumin from which the precipitate was formed.

Similar results were obtained when albumin was replaced with hæmoglobin, lysozyme, and human globulins. In the case of lysozyme the enzymatic activity was found to be unaltered when the precipitate was redissolved.

The ability of ATP to react with serum proteins was also examined. To 0·5 ml. of human serum (dialysed against isotonic saline, *p*H 4·6) was added a solution of ATP buffered at *p*H 4·6. The precipitate was obtained by centrifuging and redissolved in isotonic saline at an alkaline *p*H. Paper electrophoresis of this solution yielded spots with mobilities equal to those of human globulins, but no spots with the mobility of albumin. Paper electrophoresis of the supernatant of the original precipitate resulted in a single spot which migrated with human albumin at *p*H 8·6 and *p*H 7·4.

Selective precipitation has been of limited value as a preparative tool because of possible alterations of the proteins, both those precipitated and those remaining in solution. However, our results indicate that little, if any, alterations occur when ATP is used as the precipitating agent. This may have some limited application in the separation of proteins. Furthermore, it is felt that this reaction may have a bearing on the transport and storage of high-energy phosphate compounds.

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Electrophoretic Mobility of Cultured Mesodermal Tissue Cells

AMBROSE *et al.*[1] have shown that individual tumour cells from hamster kidney possess a significantly greater electrophoretic mobility than normal homologous kidney cells. Later, Purdom *et al.*[2] obtained a correlation between the development of a mouse sarcoma and the progressive increase in the negative electrophoretic mobility of individual cells obtained from this sarcoma at various stages of its development. As the tumour cell acquired more malignant properties, evinced by the formation of early metastases, the negative electrophoretic mobility of individual cells was observed to have increased.

Recently, Ben-Or *et al.*[3] observed that the electrophoretic mobility of individual cells from adult rat liver during regeneration was appreciably greater than that of homologous non-regenerating liver cells. They suggested that the raised electrophoretic mobility of the former was an expression of growth in general rather than of malignancy in particular.

We have found that mouse fibroblasts cultured by conventional methods from embryonic tissue, mouse subcutaneous fibroblasts[4] strain *L*, clone 929 (obtained from Mrs. A. A. Newton, Department of Biochemistry, The University, Cambridge) and fibroblasts cultured from the hearts of adult mice show reproducible microelectrophoretic mobilities, which are the same for fibroblast cultures freed from the glass either mechanically (policemen) or enzymically (trypsin).

The embryonic fibroblasts possess a mobility significantly greater than the adult fibroblasts, with the *L*-strain fibroblasts exhibiting an intermediate electrophoretic mobility (Table 1).

All tissue cells were prepared for microelectrophoresis by the procedure used for human erythrocytes[5]. The electrophoretic mobility measurements on the cells were made in a cylindrical microelectrophoresis chamber at 37·0 ± 0·1° as described previously[6]. A mobility determination was based on the migration of about one hundred individual cells. No appreciable divergence in the electrophoretic mobility of individual batches of cells has been observed from one culture to another. Human erythrocytes washed in the same 0·145 *M* aqueous sodium chloride system were used as a reference standard[5].

Table 1. MOBILITIES OF VARIOUS CELLS IN 0·145 *M* SODIUM CHLORIDE: 3×10^{-4} *M* SODIUM BICARBONATE, at 37·0 ± 0·1°

System	*p*H	Mobility (μsec.$^{-1}$ V.$^{-1}$ cm.)
Embryonic mouse fibroblasts	6·0–8·0	−0·97 ± 0·05
Adult mouse fibroblasts	6·0–8·0	−0.82 ± 0·05
L-strain mouse fibroblasts	6·0–8·0	−0·88 ± 0·05

The *L*-strain fibroblasts, originally derived from Earle's 20-methylcholanthrene-treated strain *L* which produced sarcomata on injection into *C3H* strain mice, can probably be considered to be malignant. However, sub-lines of the *L*-strain may differ from the original in a number of ways, as a result of transformations during the long period since the initial cloning[7]. Any direct comparisons between the *L*-strain and the embryonic and adult fibroblasts (cultured only for about 100 hr.) may be misleading. The *L*-strain, however, does exhibit an electrophoretic mobility greater than that of the adult fibroblasts in keeping with the observations in ref. 1. Though any electrophoretic mobility measurements made on tissue cells after washing and removal from a natural or a culture medium must be viewed with caution in relation to the behaviour *in vivo*, it is probably permissible to compare the mobility of the adult and embryonic fibroblasts cultured *in vitro* (Table 1).

The higher electrophoretic mobilities of the embryonic fibroblasts as compared with the adult fibroblasts support the views expressed in ref. 3. A raised electrophoretic mobility is therefore probably associated with growth in general and thus it is not simply the higher net negative electrokinetic charge of malignant cells which is the significant factor in their invasiveness.

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Identification of 20β-Hydroxy-pregn-4-ene-3-one in Luteal Tissue from Pregnant Whales

USING biological methods of assay, several authors have reported gestogen activity in extracts of whale corpora lutea[1–4]. Prelog and Meister[5] were the first to isolate progesterone from this source by a chemical method and reported amounts of 3 mgm. per 100 gm. tissue. In addition to progesterone the biologically inactive 5α-pregnane-3β-ol-20-one was also found. These two substances seem to be the only steroids isolated from the reproductive organs of the whale.

Since either 20α- or 20β-hydroxy-pregn-4-ene-3-one, or both, have been shown recently to be naturally occurring gestogens in several mammalian species, it seemed of interest to make a search for these compounds in the whale as well.

In this investigation a combination of chromatographic and spectrophotometric methods was applied to extracts of luteal tissue from pregnant fin-whales (*Balaenoptera physialus* L.). The tissues were collected in the Antarctic and kept frozen until processed.

Portions of tissue were homogenized and extracted with ethanol/ethyl ether (3 : 1). After removal of fatty material by repeated precipitations in cold 70 per cent methanol, the methanolic solution was shaken with three times its volume of petroleum ether (boiling point 40–60° C.) and collected. The petroleum ether was then extracted with five additional volumes of 70 per cent methanol, the pooled extracts evaporated, the residue taken up in a small volume of absolute methanol and chromatographed on paper. Evaporations were made at reduced pressure below 60° C. For chromatography the ligroin–80 per cent methanol system of Zander and Simmer[6] (system 1), and the heptane–formamide system of Zaffaroni (system 2) were used.

Three compounds were easily detected by contact photography of chromatograms using light of 253 mμ. Their ultra-violet absorption spectra in methanol with a distinct maximum at 240 mμ and the positive soda fluorescence reactions indicated the presence of the Δ^4-3-keto-grouping in the steroid molecule. After rechromatography in system 1, one compound moved like progesterone. It was present in large amounts, and its infra-red absorption spectrum was shown to be identical with that of progesterone. The second compound showed chromatographic behaviour like 20β-hydroxy-pregn-4-ene-3-one in both system 1 and 2, both as the free and acetylated compound. More material was processed, and the substance was isolated in quantities sufficient for registration of the infra-red absorption spectrum, which was found to be identical with that of the authentic 20β-hydroxy-pregn-4-ene-3-one.

The third substance was chromatographically similar to 20α-hydroxy-pregn-4-ene-3-one in both the free and acetylated form in system 1. However, in system 2 it moved faster than this substance, both free and acetylated. Moreover, it showed a negative reaction to the iodine test, which is considered to be rather specific for the 20α-grouping. The identity of this substance remains to be established.

From the observations reported, it seems safe to conclude that both progesterone as well as 20β-hydroxy-pregn-4-ene-3-one are naturally occurring gestogens in the whale. This corresponds to those found in the horse[7] and the cow[8], while in man both the 20α- and 20β-hydroxy compounds are present in addition to progesterone[9]. In rat ovarian tissue 20α-hydroxy-pregn-4-ene-3-one and progesterone have recently been found to be reversibly interrelated[10]. Species variations in stereospecific enzymes in gestogen metabolism therefore seem to occur. The observations reported here for the whale add to our knowledge about such variations.

We are indebted to Prof. Å. Jonsgård, University of Oslo, for his aid in obtaining material from whales, to Prof. W. Klyne, London, for generous gifts of reference compounds, and to S. Veige, chemical engineer, AB Leo, Hälsingborg, Sweden, for recording and interpretation of infra-red absorption spectra.

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Antibodies blocking Neutralization of Penicillinase by the Homologous Antiserum

Immunization of rabbits with purified exopenicillinase of *Bacillus cereus* results in the appearance of antibodies which neutralize approximately 90 per cent of the activity of the enzyme[1,2]. The present communication describes the preparation and some of the properties of neutralization-blocking antibodies, evoked by injection of penicillinase-producing cells. Immune serum containing neutralization blocking antibodies will be referred to as 'blocking antiserum'.

The strains of *B. cereus* used in this work were the wild-strain 569, which forms penicillinase following induction with penicillin, and the mutant-strain 569/*H*, derived from strain 569, which forms penicillinase constitutively.

Neutralizing antiserum was prepared by immunizing rabbits with a highly purified preparation of exopenicillinase produced by strain 569/*H* of *B. cereus*[3]. 20 mgm. of the enzyme were administered with Freund's adjuvant in 3 intramuscular injections at 10-day intervals.

Blocking antiserum was prepared by immunizing rabbits with intact cells of *B. cereus* 569/*H*. The cells were collected from a 12-hr. old broth culture, washed with, and resuspended in, physiological saline. The immunization consisted of 7 subcutaneous injections over a period of 40 days. Each dose contained 100 mgm. bacterial dry weight.

Absorption of antibodies was carried out by mixing equal volumes of sera and packed bacterial cells and incubating for 2 hr. at 37° C. The mixture was kept for 20 hr. at 4° C. before cells were removed by centrifuging.

The effect of the blocking antiserum on the interaction of penicillinase with the neutralizing antiserum was assayed in the following way. 0·2 ml. aliquots of a purified penicillinase preparation (575 units/ml.) were incubated (30 min. at 28° C.) with 0·2 ml. of the appropriate dilutions of the blocking antiserum. A quantity of neutralizing antiserum in excess of that required for maximal neutralization (1,000 neutralization units in 0·2 ml.) was added and the mixture incubated for another 30 min. Normal rabbit serum replaced blocking antiserum, neutralizing antiserum or both in the respective control tubes. The activity of penicillinase was assayed by the iodometric method[4].

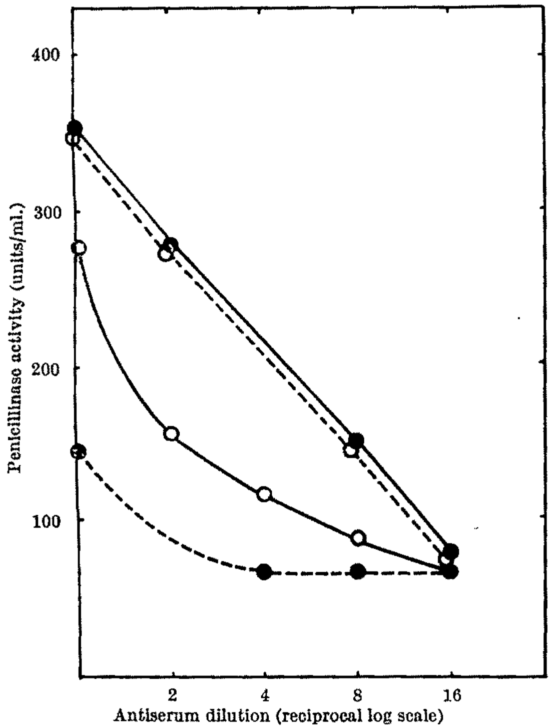

Fig. 1. Activity of penicillinase incubated with neutralizing serum, following exposure to serial dilutions of 'blocking antiserum'. The 'blocking antiserum' was treated as follows: ● - - - - ●, Absorbed with constitutive cells; ○——○, absorbed with induced cells; ○ – – – ○, absorbed with non-induced cells; ●——●, untreated

The results, presented in Fig. 1, are expressed as residual enzyme activity following incubation with varying dilutions of the blocking antiserum and excess of neutralizing antiserum. Also shown in Fig. 1 is the effect of absorption of the blocking antiserum by the homologous (569/*H*) cell suspension, and by both induced (that is, penicillinase forming) and non-induced cells of the parent strain 569.

It will be noted that the undiluted non-absorbed blocking antiserum interferes strongly with inhibition of penicillinase by the neutralizing antiserum. The inhibition decreases as the blocking antiserum is diluted. Blocking antibodies are removed largely by absorption with the homologous cells, and, to a lesser extent, by absorption with induced 569 cells. Absorption with non-induced 569 cells has no effect on the blocking titre of the antiserum.

The blocking antiserum agglutinates cells of the constitutive (569/*H*) strain and, to a lesser degree, induced cells of the adaptive strain 569. The agglutinating titre for non-induced cells is very low. Thus the susceptibility to agglutination by the blocking antiserum appears to be related to the level of penicillinase formed by the cell. A similar correlation has been found in cross-absorption experiments. Absorption of the antiserum with constitutive cells resulted in complete removal of agglutinins, whereas absorption with non-induced cells had no effect on the titre of agglutination of constitutive cells. Induction of penicillinase imparted to the adaptive cells a limited ability to absorb agglutinins. The results are summarized in Table 1.

The close correlation between the blocking and agglutinating properties of the antiserum suggests that both activities may be due to a single species of antibody, directed to cell-bound penicillinase.

Table 1. AGGLUTINATING TITRE OF THE 'BLOCKING ANTISERUM'

Serum absorbed with	Cell suspension agglutinated		
	Constitutive	Induced	Non-induced
(*a*) Constitutive cells	0	0	0
(*b*) Induced cells	1 : 640	1 : 40	0
(*c*) Non-induced cells	1 : 1,280	1 : 80	0
(*d*) Not absorbed	1 : 1,280	1 : 80	1 : 20

A difference in the immunological response to cell-bound and to extracellular penicillinase was to be expected on the basis of established antigenic differences between the two states of the enzyme[4,5].

Quite apart from the structural aspects of the bound and free forms of bacterial penicillinase, the present observations may bear on the problem of *in vivo* resistance to penicillin. The acquired resistance to penicillin of clinically important micro-organisms depends in most cases on their ability to form penicillinase[6]. Formation of penicillinase-neutralizing antibodies could thus be regarded as a specific component of the acquired defence mechanism of the host. Modification of the host response, along the lines described, might have a distinct survival value for the infecting agent.

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PHYSIOLOGY

Effect of Temperature on the Retinal Slow Potential of the Horseshoe Crab

IN a previous paper[1] we indicated that the retinal slow potential, a generator potential, of the horseshoe crab (*Tachypleus tridentatus*) decreased in its amplitude as the time in darkness before illumination is reduced, even if stimuli of equal intensity and duration were applied. This depressive effect of the short time-interval is compatible with the 'depression'[2] or 'refractoriness'[3] of the receptor potential of the Pacinian corpuscles. It was found that the grade of 'depression' as well as the amplitude of the retinal slow potential is affected by changes in temperature, though the resting potential seems to be little influenced.

The retinal slow potentials were recorded intracellularly from single ommatidia of the horseshoe crab by the same procedure as that described by another author[4]. The temperature of the bathing solution could be varied between 6° C. and 23° C. by controlling the inflow of the cold or warm oxygenated physiological solution for the horseshoe crab[5] or sea-water which was stored in a cylinder, and was measured by employing a thermocouple which was placed by the preparation.

The records illustrated in Fig. 1 are responses obtained after 60 (column *A*) and 15 (column *B*) sec. in darkness at various temperatures. As shown in

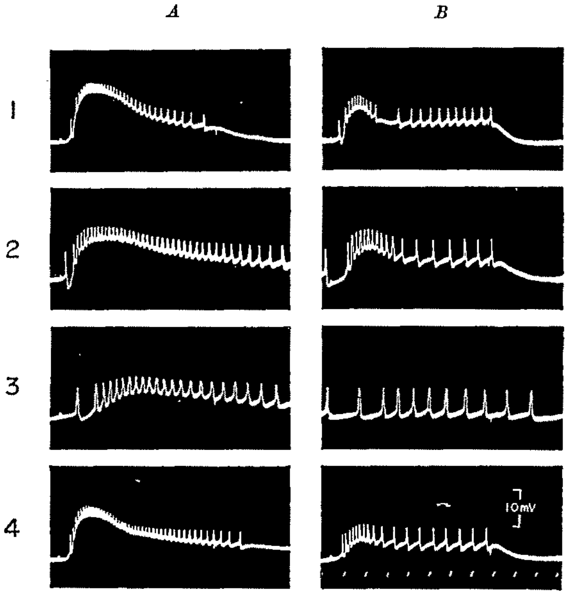

Fig. 1. Effect of temperature on the action potentials arising within a single ommatidium of the horseshoe crab. Records in column *A* and *B* were obtained by the same stimuli after 60 and 15 sec. in darkness at various temperatures. From the top records downwards, 12, 9, 6 and 12° C. The onset and turn-off of illumination are indicated as pips. Note the reverse effect of temperature on the amplitude of the slow potential and the spike potential. Time, 0·1 sec.

Fig. 1, differences in the shape of the slow potential due to its time in darkness before illumination were also found at different temperatures. In addition, the separation of the initial maximal and the decreased, sustained depolarization, which is usually clearer in the responses obtained by illumination of high intensity and after a shorter time in darkness or at high temperature, became indistinct at low temperature (records 3 in column *A* and *B*) as the result of the prolongation of the falling phase of the initial maximal depolarization. In most other experiments, flashes which are short enough to obtain simple responses without any additional decreased depolarization were applied to make the measurement of the falling phase of the slow potential easier.

The temperature coefficient of the amplitude of the maximal depolarization of the slow potential was much larger than that reported in the propagated action potential of nerve[6] and muscle[7] fibres. Its Q_{10} was positive and ranged from about 3 to 5 in the responses obtained after 60 sec. in darkness. It should be mentioned that the temperature coefficient of the duration of the rising phase was larger than that of the falling phase, being negative. On the other hand, the temperature coefficient of the amplitude of the spike potential generated by the retinal slow potential was negative, as it is in the giant axon of the squid[6].

The records of the bottom row of Fig. 1 were taken after the temperature had been raised once more to that of the top records. The amplitudes of the slow potentials were both smaller than their initial figures. This after-effect of cooling was often observed under our experimental conditions in which temperature was lowered at the rate of about 0·5° C./min., and raised at about 1° C./min. This seems not to be due to the depolarization of the membrane potential during the temperature run, since it could appear without any significant change in the resting potential in most cases. Thus the after-effect can be explained by saying that when the temperature is changed it takes time for the new functional level to establish itself.

These effects of temperature on the slow potential indicate that some chemical processes are probably involved in the generation of the retinal slow potential, one of them being suggested by Fuortes[8]. A detailed report on the effect of temperature on the electrical activity of the sensory cell in the lateral eye of the horseshoe crab is being prepared for publication.

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Binding of Calcium Ions by the Aorta

PREVIOUS work[1] has shown that the aorta has an inherent ability to bind calcium ions *in vitro*. This binding in turn had a marked influence on the stability of emulsions, causing precipitation. The distribution and solubility characteristics of the primary substance suggested that collagen was responsible for the calcium binding, although other possibilities were recognized. Because of the importance attributed to binding of calcium ions by the aorta in plaque formation[1], further work is reported here to add to the information concerning the identity of the binding agent(s). This was accomplished by noting the effect of various enzymes and extractive procedures on the aortic binding of calcium chloride labelled with calcium-45 which could be followed autoradiographically.

All experiments were conducted with human thoracic aortas of adult males. These were prepared as described previously[1], and used in the form of pieces 6 cm.^{2} in order to conserve material. Following decalcification with ethylenediaminetetraacetate at *p*H 7·2 in the cold and subsequent washing and extraction with acetone–petroleum ether[1], the strips were incubated overnight with the appropriate enzyme at 37° C. at optional *p*H. They were then washed free of enzyme, and incubated overnight with 3–4 μc. labelled calcium chloride in sealed vials. After washing and drying, the strips were exposed to dental film for 17 hr. within a plastic cassette. Conditions for radioautography were strictly controlled to permit comparison with control strips which were not enzymatically treated. All experiments were repeated at least twice and in duplicate. Summarized in Table 1 are the results of various treatments on the calcium-binding of the aorta. In all cases the physical appearance of the aorta strip remained intact following the experimental procedure.

These results indicate that several factors may be responsible for calcium-binding by the aorta. These are protein in nature as made evident by the fact that

Table 1

Treatment	Effect on calcium binding
(1) Hyaluronidase[2], bovine testes, 300 U.S.P. units/mgm. per 15 ml. saline-acetate, *p*H 6·0	None
(2) Elastase[3], 100 units/5 mgm., 15 ml. saline, *p*H 8·0	None
(3) Collagenase, 10 mgm./15 ml. distilled water, *p*H 7·2	Very slight reduction
(4) Alkaline phosphatase, intestinal, 180,000 Bodansky units/15 ml. saline, *p*H 8·4	Marked reduction
(5) Trypsin, twice crystallized, 10 mgm./15 ml. distilled water, *p*H 8·0	Complete elimination
(6) Extraction of soluble proteins with 0·25 *M* glucose[4]	None
(7) Extraction of chondroitin sulphate with 30 per cent KCl–1 per cent K_2CO_3[5,6]	Marked enhancement

they are completely eliminated by trypsin digestion. The present evidence would indicate that the factors are not hyaluronic acid, elastin or collagen, but probably an insoluble protein-phospholipid complex. Phospholipids, in particular the sphingomyelins, have been reported to increase in atherosclerotic aortas[7,8]. The extraction with potassium chloride–potassium carbonate appears to remove an inhibitor of the calcium binding which has the properties of a chondroitin sulphate.

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Effects of Certain Androgenic Steroids on the Increase of Endometrial Carbonic Anhydrase caused by Progesterone

IT has previously been demonstrated by Lutwak-Mann[1] that, as a result of treatment of œstrogen-primed rabbits with methyltestosterone, an appreciable increase in endometrial carbonic anhydrase occurred, and that when methyltestosterone and progesterone were given simultaneously, methyltestosterone appeared to prevent the activating effect of progesterone on the endometrial enzyme. The present investigation dealt with the question of how other androgenic steroids affect the endometrial carbonic anhydrase response to progesterone. The following steroids were tested: methyltestosterone, androstenedione, androstanolone, Δ^5-androsten-3β, 17β-diol and stigmasterol.

All experiments were performed on immature albino rabbits, weighing approximately 1·5 kgm. at the beginning of the experiments. Following Clauberg's method[2], the rabbits were injected subcutaneously once daily with 5 μgm. of œstradiol for six days. After this period, progesterone and androgen were given simultaneously in five divided doses at daily intervals by subcutaneous injection into the neck of the animal. The standard dose of progesterone was 2·0 mgm. and the androgen doses varied as indicated in Table 1. On the second day after the last injection, the rabbits were killed by breaking the neck and cutting the carotid artery. The uteri were isolated and rinsed with cold saline. The endometrium was dissected and homogenized with cold distilled water. The homogenate was centrifuged and the supernatant was used for the determination of enzyme activity. The enzyme activity of the extract was estimated colorimetrically by modifying the Philpot and Philpot method[3], one enzyme unit being defined as $(T_0 - T)/T$, where 'T_0' is the reaction time without enzyme and T is the same with enzyme.

As can be seen in Table 1, a dose of 2 mgm. of progesterone caused a marked increase in the activity of endometrial carbonic anhydrase. However, when various doses of androgen were given with 2 mgm. of progesterone, the combined effect of the two steroids on the enzyme activation was rather lower than that due to 2 mgm. of progesterone alone. In a dose of 0·02 mgm., considerable lowering of endometrial enzyme activity was demonstrated in methyltestosterone, androstenedione and androstanolone. There was significant difference between the endometrial enzymes with and without androgen. Percentage inhibition of carbonic anhydrase was 42 by methyltestosterone, 52 by androstenedione and 49 by androstanolone. However, 0·02 mgm. of Δ^5-androsten-3β,17β-diol and stigmasterol were not so pronounced as the other androgens, an inhibition of 34 per cent each being observed. A total of 0·2 mgm. proved exceedingly effective for the inhibition of the effect of 2 mgm. of progesterone, causing a 47 per cent inhibition by Δ^5-androsten-3β,17β-diol and a 42 per cent by stigmasterol.

The androgens used caused a significant fall in the increased enzyme activity produced by progesterone. It seems that the anti-progestational ability is practically the same for methyltestosterone, androstenedione and androstanolone, but somewhat weaker than the others in Δ^5-androsten-3β,17β-diol and stigmasterol.

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Table 1. EFFECTS OF ANDROGENIC STEROIDS ON ENDOMETRIAL CARBONIC ANHYDRASE INCREASE CAUSED BY PROGESTERONE

Group	Series of hormone	No. of rabbits	Dose of progesterone (mgm./animal)	Dose of androgen (mgm./animal)	Carbonic anhydrase content (enzyme units/gm. wet tissue)
a	Control	10	seasame oil		24 ± 3
b	Progesterone	11	2·0		557 ± 36
c	Progesterone + methyltestosterone	6	2·0	0·2	480 ± 44*
		5	2·0	0·02	333 ± 5
		5	2·0	0·002	447 ± 30*
d	Progesterone + androstenedione	6	2·0	2·0	385 ± 24
		5	2·0	0·2	367 ± 31
		4	2·0	0·02	280 ± 22
e	Progesterone + androstanolone	5	2·0	2·0	458 ± 37*
		4	2·0	0·2	378 ± 32
		6	2·0	0·02	294 ± 21
		5	2·0	0·002	366 ± 44
f	Progesterone + Δ^5-androsten -3β,17β-diol	3	2·0	2·0	599 ± 34*
		4	2·0	0·2	304 ± 35
		4	2·0	0·02	375 ± 27
g	Progesterone + stigmasterol	5	2·0	2·0	494 ± 41*
		3	2·0	0·2	332 ± 39
		5	2·0	0·02	377 ± 68

* $P > 0·05$ compared with group *b*.

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Effect of Allyl*iso*propylacetamide on Liver Metabolism

THE administration of allyl*iso*propylacetamide (AIA) causes porphobilinogenuria in rabbits[1], but it is not known whether this is caused by an increased synthesis of porphobilinogen (PBG) or a decreased utilization. PBG is synthesized in the liver via δ-aminolævulinic acid (ALA) from glycine and acetate or succinate and it seemed possible that a study of the effect of AIA on the metabolism of carbon-14-labelled glycine, acetate and succinate by rabbit liver might throw a light on the problem.

Four male rabbits (2·5–3·0 kgm.) each received 300 mgm. of AIA by stomach tube twice daily for 6 days. The urine from each animal then contained about 14 mgm./100 ml. of porphobilinogen (24 mgm./24 hr.) and 7·5 mgm./100 ml. of ALA (13 mgm./24 hr.). Four rabbits not treated with AIA were controls. The animals were killed, and chopped preparations[2] of the livers incubated with substrates labelled with carbon-14 as indicated in Table 1. The tissue was incubated for 3 hr. at 37° in air, killed and extracted with ethanol, and the ethanol-aqueous extracts chromatographed in two dimensions using phenol–water as the first solvent and *n*-butanol–propionic acid–water as the second. Radioactive substances on the chromatograms were located by radioautography and counted directly on the paper with an end-window counter[2]. The carbon-14 contained in each compound was expressed as a percentage of the total carbon-14 in the cell extract.

There was a decreased total incorporation of the isotope into the soluble intermediaries. Nevertheless, with labelled acetate and succinate as substrates considerably more carbon-14 was present in glutamic acid in the AIA-treated animals compared with the controls; this was also true for alanine when labelled succinate was used as substrate. When [2 : 3-^{14}C] succinic acid was the substrate, the radioactivity in glutamic acid and alanine was about twice as great in both groups of animals as with [1 : 4-^{14}C] succinate as substrate (Table 1). This is to be expected if these two amino-acids were derived from succinate via the tricarboxylic acid cycle and transamination. No other obvious differences in distribution of carbon-14 were seen between the tissues from AIA-treated and the control animals in the incorporation of carbon-14 into aspartic acid, glucose, proline, γ-aminobutyric acid, valine, lactic, malic and fumaric or citric acids. The lack of increased incorporation from labelled acetate and succinate into aspartate in the AIA-treated animals compared with the controls suggests that there was no increased passage of carbon atoms from the keto-acids into the corresponding amino-acids derived from them by transamination. The preferential incorporation of carbon-14 into the alanine and glutamic acid may indicate that there is an abnormality of the further metabolism of these amino-acids in the AIA-treated animals, but it seems unlikely that this is directly related to the mechanism responsible for the porphobilinogenuria.

It was impossible to interpret the experiments using [2-^{14}C] glycine because the material decomposed to a variable extent during the purification and chromatographic procedures.

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Inhibition of Emotional Reactions by Phenothiazine Tranquillizers

PHENOTHIAZINE tranquillizers inhibit conditioned responses of various laboratory animals. Since this action can be demonstrated by means of a great variety of methods, it is generally considered to be characteristic of these compounds. Some tranquillizers also diminish 'emotional reactions'[1–4]; but these effects have not been studied so much, mainly due to a lack of simple and adequate methods. Thus, views are still divided whether depression of the emotional reaction is a primary effect or an effect secondary to the sedative action of such drugs[3,4]. Any new technical procedure which permits the study of the influence of a drug on emotional behaviour independent of its general depressive effect, therefore, appears interesting.

Table 1. TOTAL INCORPORATION AND DISTRIBUTION OF CARBON-14 IN ALANINE AND GLUTAMIC ACID

Group of animals	Control			AIA-treated		
Substrate	[2-^{14}C]acetate	[1: 4-^{14}C]succinate	[2: 3-^{14}C]succinate	[2-^{14}C]acetate	[1: 4-^{14}C]succinate	[2: 3-^{14}C]succinate
Glutamic acid	11·1 ± 2·0 (6·7–16·3)	0·19 ± 0·05 (0·12–0·31)	0·39 ± 0·10 (0·30–0·67)	20 ± 3·7 (13–27)	0·73 ± 0·17 (0·50–1·2)	1·5 ± 0·30 (1·1–2·4)
Alanine	4·0 ± 0·75 (1·7–5·1)	2·6 ± 0·44 (1·4–3·4)	4·2 ± 0·6 (2·4–5·0)	5·2 ± 0·67 (0–1·7	4·6 ± 0·67 (2·8–5·8)	8·5 ± 2·0 (4·2–13)
Total carbon-14	95 ± 48 (35–240)	1,700 ± 280 (1,100–2,300)	1,600 ± 250 (900–2,000)	65 ± 9·2 (40–79)	1,500 ± 320 (990–2,400)	1,400 ± 270 (900–2,200)

Samples of rabbit liver (containing about 10 mgm. dry weight of tissue in a vol. of 0·25 ml.), derived from four control animals and from four animals treated with AIA, were incubated in air for 3 hr. at 37° with 5 μc. of either 1·01 μmoles [2-^{14}C]acetate, 0·46 μmole [1:4-^{14}C]succinate or 0·46 μmole [2: 3-^{14}C]succinate. The total carbon-14 incorporated into the sum of the soluble metabolic intermediates is expressed as 10^{-3} × counts/min./25 mgm. dry wt. of tissue. The radioactivity found in glutamic acid and alanine is expressed as a percentage of the total carbon-14 in the sum of the soluble metabolites. All results are given as the mean of 4 observations ± *S.E.* (range).

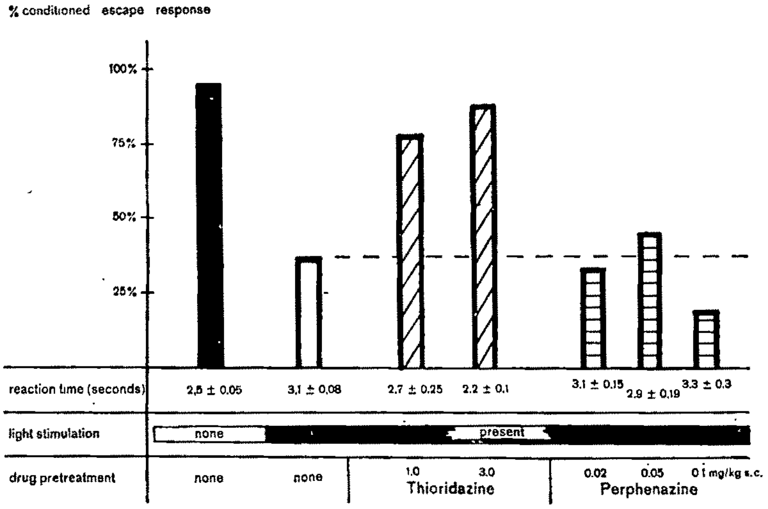

Fig. 1. An intense light stimulus prevents the normal course of a conditioned escape response in rats in a non-reinforced procedure. Thioridazine inhibits this disturbing influence. Perphenazine is ineffective in this respect, but depresses further the conditioned response

Based on frequent observations that novel stimuli which elicit an emotional reaction in rats regularly interfere with the correct performance of a conditioned response, the following test was devised: rats were trained to climb a vertical pole as soon as an acoustic signal (conditioned stimulus—tone of 500 c./s.) was sounded. The animals could thus avoid an electric shock delivered 5 sec. later through the grilled floor of the cage (conditioned escape response)[5]. Each rat was trained daily for 10 successive trials with a 1-min. interval between each trial. After an adequate training period, all rats performed the conditioned escape response correctly in 94 ± 1·1 per cent of all trials, the average reaction time being 2·4 ± 0·07 sec. During the subsequent trials, an intense light stimulus (100 W.) was applied 2 sec. prior to each acoustic signal. This stimulus terminated simultaneously with the acoustic signal either at escape or after 5 sec., that is, at the onset of the electric shock. The rats exposed to the light stimulus performed the conditioned escape response in only 63 ± 2·5 per cent of all trials with an average reaction time of 2·5 ± 0·08 sec. These results demonstrate that the light stimulus interferes partially with the conditioned escape response. Light stimulation elicited a higher degree of disturbance in a non-reinforced procedure in which no electric shock was applied, that is, conditioned escape response in only 37 ± 2·4 per cent of all trials within 3·1 ± 0·08 sec. (In control trials without light stimulation, the conditioned escape response was performed in 95 ± 0·9 per cent of all non-reinforced trials with an average reaction time of 2·5 ± 0·05 sec.)

In the circumstances, it appeared more suitable to study drug action on this disturbing influence by means of the non-reinforced procedure. In order to avoid any accommodation to the interfering light stimulus, this test was carried out only once a week, reinforced training procedure being followed on the other days. Each group alternately underwent the non-reinforced procedure alone and the non-reinforced procedure with prior light stimulation under drug therapy and saline. At least 20 trials in 10 rats were required to assess the action of one dose of drug. The compounds were administered subcutaneously 2 hr. before the test, controls receiving saline. Two phenothiazines were selected which were found in previous studies[4] to possess an opposite activity pattern: perphenazine and thioridazine. The results of these experiments are summarized in Fig. 1. Rats pretreated with thioridazine (1 and 3 mgm./kgm.) perform the conditioned escape response correctly despite the disturbing light stimulus (78 ± 7·2 per cent and 88 ± 1·9 per cent). Their reaction time is normalized or even slightly shorter than in rats not exposed to the light stimulus. Thioridazine, therefore, inhibits the disturbing influence of the light stimulus. Perphenazine, on the other hand, does not prevent the disturbing influence of the light stimulation; a minimal effect is noted with a low dose of 0·05 mgm./kgm. (45 ± 5·9 per cent), whereas higher doses (0·1 mgm./kgm.) further depress the conditioned escape response (19 ± 3·3 per cent).

It is generally assumed that an intense light stimulation elicits an emotional response in rats[6]; such emotional reactions (confusion, stupor, anxiety, etc.) can interfere with the normal operant behaviour of the animals[7] or—as shown in these experiments—with the performance of a conditioned response. The degree of depression of the motor behaviour serves as a measure of the extent of the emotional reaction. The finding that the thioridazine-treated rats perform the conditioned escape response correctly despite the disturbing light stimulus points to a primary action of this drug on the emotional response. This anti-emotive property is apparently not due to general sedation, as it is demonstrable in doses which do not inhibit the conditioned response. On the other hand, perphenazine does not inhibit the emotional reaction. However, this compound further inhibits, in relatively low doses (0·1 mgm./kgm.), the conditioned escape response.

These findings are in accord with those obtained in previous work[4,5] when it was found that thioridazine depresses selectively emotional defecation and has relatively little effect on the conditioned escape response in rats, whereas perphenazine inhibits predominantly the conditioned response and depresses emotional defecation only in higher doses. Apparently, the depression of emotional reactions on one hand, and inhibition of a conditioned escape response on the other, are, to a certain degree, two independent properties of the phenothiazine tranquillizers.

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Further Evidence for Phosphatidic Acid as the Sodium Carrier

We have presented evidence[1,2] before that phosphatidic acid acts as the Na^+ carrier in the membrane of the avian salt gland. The proposed scheme for Na^+ transport involves a cycle in which disodium phosphatidate is formed at the inner surface of the membrane from diglyceride, adenosine triphosphate (ATP), and 2 Na^+, and is hydrolysed to diglyceride, orthophosphate and 2 Na^+ near the outer surface of the membrane. One of the more important questions which we would like to test in relation to this scheme is whether the rate of renewal of phosphate in phosphatidic acid is compatible with the expected rate of Na^+ transfer across the membrane.

The time-courses of the responses in this tissue can give some indication as to whether this rate is compatible with the proposed scheme. Initially, albatross salt gland slices were incubated for 90 min. in the presence of orthophosphate-phosphorus-32; various concentrations of acetylcholine (plus eserine) were then added to the medium. With each concentration of acetylcholine, samples taken at 10-min. and 20-min. intervals after the addition of acetylcholine showed the same amount of radioactivity in the phosphatidic acid with respect to the radioactivity of the orthophosphate (and the ATP) of the tissue (Table 1). The radioactivity levelled off at different and successively higher values with increasing concentrations of acetylcholine. The incorporation of phosphorus-32 into phosphatidic acid was measured at shorter intervals of time after the addition of acetylcholine in goose salt gland slices. After addition of acetylcholine, the radioactivity of the phosphatidic acid showed a rapid increase; this radioactivity reached a maximum value 1–2 min. after the addition of acetylcholine to the medium, and then levelled off and maintained this same value relative to the specific activity of the precursor for the remainder of the period tested, which was up to 20 min. after the addition of acetylcholine (Fig. 1).

To test that the levelling off of the radioactivity in phosphatidic acid was not due to a cessation of response to acetylcholine, albatross salt-gland slices were incubated with acetylcholine (10^{-4} *M*) plus eserine (10^{-4} *M*) for 90 min. before addition of phosphorus-32 to the medium; the slices were removed from the incubation bath 30 min. after the addition of phosphorus-32. Five times as much radioactivity was found in the phosphatidic acid from stimulated slices as compared with the controls. These results show that whatever the mechanism responsible for the increased incorporation of phosphorus-32 into phosphatidic acid in response to acetylcholine, this mechanism was still operating in response to acetylcholine even after 90 min. of exposure to this agent.

Table 1. The Response of Albatross Salt-Gland Slices to Different Concentrations of Acetylcholine

Additions (molar concentration) Acetylcholine	Eserine	Radioactivity of phosphatidic acid (c.p.m. per milligram fresh weight of tissue)	
0	0	50	
		10 min. after acetylcholine added	20 min. after acetylcholine added
10^{-7}	10^{-4} *M*	107	109
10^{-6}	10^{-4} *M*	150	176
10^{-5}	10^{-4} *M*	200	217
10^{-4}	10^{-4} *M*	228	224

Slices of albatross salt gland were incubated in bicarbonate saline with glucose (1 mgm. per ml.) and phosphorus-32 for 90 min. before the addition of acetylcholine and eserine. The radioactivity is expressed as total counts per min. (c.p.m.) in the phosphatidic acid from 1 mgm. fresh weight of tissue, corrected to a radioactivity of 10^4 c.p.m. per mgm. fresh weight of tissue for the orthophosphate of the tissue. Under these conditions of pre-incubation the specific activity of the 7-min. acid hydrolysable-phosphate of the ATP of the tissue was the same as that of the orthophosphate of the tissue. The values for phosphatidic acid radioactivity given are the averages from two experiments.

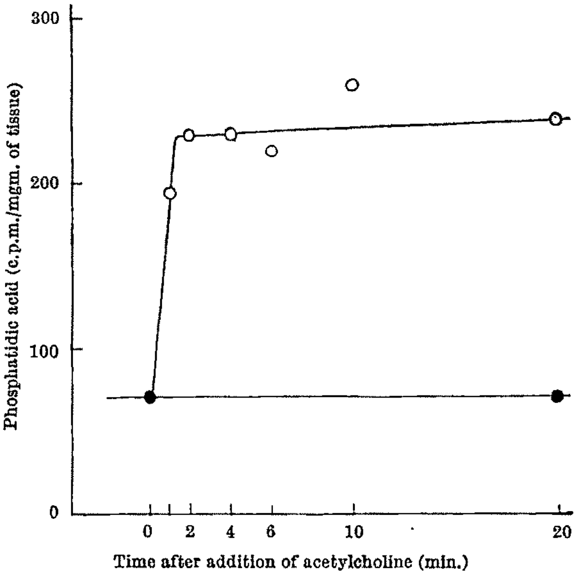

Fig. 1. Time-course of the incorporation of phosphorus-32 into phosphatidic acid on addition of acetylcholine in goose salt-gland slices. The slices were incubated with orthophosphate-phosphorus-32 for approximately 2 hr. before the addition of acetylcholine (10^{-4} *M*) and eserine (10^{-4} *M*). The radioactivity is expressed as in Table 1. The experiment was carried out three times; in each experiment slices from the same animal were incubated in triplicate for each point. Each value given is therefore the average of nine separate estimations on slices from three animals. ○, samples to which acetylcholine was added; ●, control samples incubated over a comparable time without the addition of acetylcholine

Since the renewal of phosphate in phosphatidic acid in response to acetylcholine is taking place throughout this time of exposure to acetylcholine, the maintenance of a constant level of radioactivity in the presence of a given concentration of acetylcholine must mean that a fraction of the phosphatidic acid of the cell has reached equilibrium with the precursor ATP and that this fraction is turning over without any mixing with the other phosphatidic acid of the slice. The step-wise activation in the presence of increasing amounts of acetylcholine presumably indicates the presence in the membrane of receptor sites associated with the turnover of phosphatidic acid which have different threshold values for activation by acetylcholine.

When all the activated phosphatidic acid is in equilibration with its precursor (that is, the specific activity of the activated phosphatidic acid fraction is the same as that of the ATP from which its phosphate is derived), it is not possible to calculate the rate of renewal of phosphate in the relevant phosphatidic acid fraction. This could be very great. Theoretically, the rate of renewal might be obtained from the time taken for the active fraction to reach equilibration with the ATP. However, in order for this to be related to the amount of Na^+ transported during this time, the measurement must be made under conditions in which there is instantaneous and simultaneous activation of all the sites which respond to a given concentration of acetylcholine. Approximate calculations indicate that, under such ideal conditions, equilibration would be expected to take place within a few seconds of activation. Experimentally, however, these conditions cannot be achieved in salt gland slices. It takes an appreciable time for the added acetylcholine to completely permeate the slice and to activate all the possible sites. Some lag in response can therefore be anticipated in this type of experiment due to non-synchronous activation of all the responsive sites. In view of this, the fact that an activated fraction of the phosphatidic acid reaches the specific activity of its precursor within 2 min. after the addition of acetylcholine to the medium is encouraging. It indicates that the rate of renewal of phosphate in the relevant phosphatidic acid could be sufficiently great to account for Na^+ transport by the mechanism which we have proposed.

This work was aided by grants from the United Cerebral Palsy Research and Educational Foundation, the National Institute of Neurological Diseases and Blindness, and the Wisconsin Alumni Research Foundation. It will be published in detail elsewhere.

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Impairment of Erythropoiesis by Irradiation

THERE is much evidence that erythropoiesis is regulated by at least one humoral factor, erythropoietin[1], the chemical composition of which is considered to be that of a glycoprotein[2]. Investigations indicate that this hormone induces erythrocyte production by stimulating differentiation of primitive cells of the reticulum[3-5].

The ideal nature of the transfusion-induced polycythæmic mouse for *in vivo* studies of erythropoiesis was first demonstrated by Jacobson *et al.*[3]. Recently we have described the orderly wave of erythropoiesis which sweeps through the reticulum of such animals in response to a single injection of erythropoietin[6]. This communication records preliminary work on the impairment of this response produced by total-body X-irradiation of plethoric mice.

Virgin female *CF* No. 1 mice, 7–8 weeks of age, were made polycythæmic by single intraperitoneal injections of 1 ml. of packed red cells from retired breeder mice of the same strain given on two consecutive days. Five days after the second transfusion, hæmatocrits averaged approximately 75 per cent, and the absence of reticulocytes from the peripheral blood, and of morphologically identifiable red cell precursors from bone marrow and spleen, indicated that erythropoiesis was practically eliminated. These mice now received varying doses of total-body X-irradiation (250 kV., 30 m.amp., target distance 79 cm., filter 1/4 mm. copper plus 1 mm. aluminium). Within 30 min. after completion of irradiation, animals were given a single subcutaneous injection of 6 units[7] of erythropoietin dissolved in 0·25 ml. saline, a dose which we have previously demonstrated will evoke maximal erythropoietic response[6]. Two days after this injection (when concentration of normoblasts in the marrow and spleen is rising, and the first reticulocytes are appearing in the peripheral blood of unirradiated controls)[6], each animal received 1 mc. of iron-59 citrate through the tail vein. Four days after this radioiron injection, blood was withdrawn by cardiac puncture, and the percentage iron-59 incorporated in newly formed peripheral red cells was determined[1].

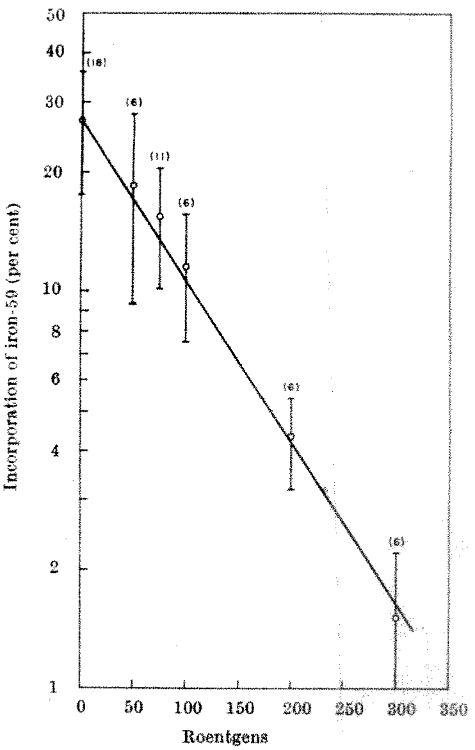

Fig. 1. Impaired response to erythropoietin in the irradiated polycythæmic mouse. Number of mice in brackets

Fig. 1 shows the percentage radioiron incorporation in the red cells of control and irradiated animals plotted on a vertical logarithmic scale. Results are expressed as mean values ± 1 standard deviation, and the number of animals in each group is indicated in parenthesis. Although variation within any group is great, particularly at the lower doses of radiation, mean values indicate that the response of a given group can be predicted, and the suppressive effect of as little as 50 r. is clearly demonstrated. (In a group of mice receiving 400 r., incorporation of radioiron was only 0·6 per cent, indicating almost complete suppression of erythropoiesis.)

While the suppressive effect of radiation on erythropoiesis has been clearly described by previous authors[8], these preliminary results seem worth reporting because they describe a simple quantitative method of evaluating it. In addition, they suggest a way of investigating the sensitivity to irradiation of the stem cells *in vivo*. Since erythropoiesis is virtually suspended in the hypertransfused mouse at the time of irradiation, it seems reasonable to attribute its suppression, as measured by red cell iron-59 incorporation, to the direct action of radiation on the undifferentiated red cell precursors, although an indirect mechanism may also be contributory. Decreased iron-59 incorporation by newly formed red cells is probably not primarily a reflexion of changes in the plasma iron-level following irradiation, since we have recently demonstrated a high level of plasma iron in hypertransfused rats, which, unlike the plasma iron in normal animals, is not further elevated following radiation (Gurney, C. W., and Taylor, K. T., unpublished results). It must be stressed that only effective erythropoiesis is being measured, and future investigation must also include a study of the blood-forming organs to assess the degree to which stem cells damaged by irradiation undergo differentiation and then succumb while still erythrocyte precursors.

In addition to affording a means for the simple quantitative determination of irradiation damage and recovery of a population of cells, we believe this model offers possibilities for investigation into the nature and kinetics of the stem-cell compartment, and it is for these reasons that these preliminary observations are reported.

We are indebted to L. O. Jacobson for discussion of the problem and for constructive criticism of the manuscript.

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Development of the Islets of Langerhans in Man

VERY little has been published about the differentiation of the α and β cells in the development of the pancreatic islets in man. The only work since the introduction of the modern granule stains is that of Ferner and Stoeckenius[1] (based on twelve fœtal pancreases), and Schultze Jena[2] (based on six fœtal pancreases).

The material for the present work was 36 pancreases from human fœtuses with weights ranging from 34–3,000 gm. (10 wk. gestation to full term), stained by Gomori's chrome-alum hæmatoxylin phloxin method[3] and/or by chrome-alum hæmatoxylin with Gomori's one-stage trichrome counterstain. Eleven pancreases were from fœtuses between 10–20 wk. gestation. It is the islets in these early months of gestation, in particular, which have not been adequately described before.

Four stages are observed in the development of the islets. These overlap so that any one pancreas will show islets in more than one stage :

(1) State of budding islets. In the third gestational month, clusters of islet-cells bud off from the ducts. Differentiated α and β cells are already present. The β cells form a central cluster, around which ungranulated islet-cells and occasional α cells are ranged.

(2) Bipolar stage. As the islets grow, the β cells become grouped at the tip of the islet farthest from the duct, while the α cells form a calyx at the base of the islet. The proportion of ungranulated cells diminishes. Islets in this bipolar phase are observed from the fifth to the eighth gestational month.

(3) 'Mantle-islet' stage. The α cells at the base of the islets proliferate and grow right round the β cells. The islet now has a kernel of β cells and a shell of α cells. Some ungranulated cells are still present among the α cells.

There is a good deal of overlapping between the bipolar and mantle-islet stages. Mantle-islets are seen as early as the fifth gestational month, and dominate the picture from about the sixth gestational month.

(4) Stage of mature islets. From the eighth gestational month onward, a few islets can be found where the α and β cells have the more-or-less haphazard distribution characteristic of the mature adult islet, although the majority are 'mantle-islets' right up to term and beyond.

A detailed account of this work, with photomicrographs, will be reported elsewhere.

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PATHOLOGY

Serological Tests for Toxoplasmosis

INTEREST in various serological tests for toxoplasmosis has increased considerably in recent years. Siim and Lind[1], in a preliminary communication, have recently described a flocculation test using toxoplasma antigen adsorbed on to acrylic particles. They stated that preliminary results of absorption experiments showed the existence of more than one toxoplasma antigen. The following experiments carried out in this Laboratory may be of interest.

A high-titre human antitoxoplasma serum was absorbed with toxoplasma-infected mouse peritoneal exudate at room temperature (20° C.). This removed almost all the hæmagglutinating, complement-fixing and dye-test antibodies. Absorption of the same serum overnight with human red cells coated with toxoplasma antigen (as used in the hæmagglutination test of Jacobs and Lunde[2]) removed the hæmagglutinating antibody but the dye test and complement-fixation antibodies were unaffected.

Control tests were set up using a normal human serum, absorbed with toxoplasma suspension, and a normal human serum absorbed with red cells coated with toxoplasma antigen. This experiment excluded effects due to elution of soluble toxoplasma antigen. Absorption of the antitoxoplasma serum with human unsensitized cells did not alter the titres of the dye, hæmagglutinating or complement-fixing antibodies. The results are summarized in Table 1.

The results of this experiment were substantiated by using a HeLa cell tissue culture toxoplasma antigen to absorb a hyperimmune serum from another patient. The effect of using a tissue culture antigen

Table 1. ABSORPTION OF ANTITOXOPLASMA SERUM

	Dye test	Hæmagglutination test	Complement-fixation test
Antitoxoplasma serum unabsorbed	Positive at 1/1,280	Positive at 1/1,280	Positive at 1/640
Antitoxoplasma serum absorbed with toxoplasma suspension	Positive at 1/40	Negative at 1/20	Negative at 1/20
Antitoxoplasma serum absorbed with sensitized human red cells	Positive at 1/1,280	Negative at 1/20	Positive at 1/1,280
Antitoxoplasma serum absorbed with unsensitized human red cells	Positive at 1/1,280	Positive at 1/1,280	Positive at 1/1,280
Normal human serum control absorbed with toxoplasma suspension and then mixed with antitoxoplasma serum	Positive at 1/640	Positive at 1/320	Positive at 1/640
Normal human serum control absorbed with sensitized human red cells and then mixed with antitoxoplasma serum	Positive at 1/640	Positive at 1/640	Positive at 1/640

as an absorbent was essentially the same as that with the mouse exudate antigen.

These experiments demonstrate that the antibody measured in the hæmagglutination test differs from that in either the dye or complement-fixation tests. Therefore the antigen or antigens responsible for the hæmagglutination test differ from those responsible for the dye and complement-fixation tests.

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Effect of a Magnetic Field on Sarcoma 37 Ascites Tumour Cells

THIS communication deals with work on the effect of magnetic field on living ascites tumour cells *in vitro*. The hanging-drop culture was prepared by diluting a 7-day-old transplanted ascites mouse tumour with suitable aliquots of Tyrode solution. The culture slide to be exposed to the field was then placed between the poles of an Alnico permanent magnet in a horizontal position at a constant temperature of 37° C. The field strength used during the experiment was about 4,000 gauss and was determined independently by the susceptibility technique. The control slides not exposed to the field were treated identically. The microscopic examination of the slides was carried out periodically.

The control tumour cells showed normal growth and active differentiation, even after 18 hr., whereas most tumour cells exposed to the field showed complete degeneration at the end of this period. These experiments were repeated several times with reproducible results. The slides made after 18 hr. were stained with acetocarmine and photomicrographs were made.

Although it is not possible to explain these results clearly at the present time, the following postulates and assumptions are considered in working out a hypothesis for this purpose. (1) The 'chemical components' in the cells have varying magnitudes of magnetic susceptibility representing the diamagnetic and the permanent and any transient paramagnetic contributions. Hence, the permanent and any transient components will experience varying forces under the influence of the magnetic field. (2) Although these forces are small from a macroscopic point of view, they may be enough to upset the delicate balance (chemical and other equilibria) inside and outside the cell. (3) Such changes are assumed, at least in part, to produce the degeneration of the cells.

We wish to thank Dr. G. S. Sperti for his encouragement and Messrs. Albert Gerth and Robert Neagele for their valuable assistance. Further work on the effect of a magnetic field on living cells will be published elsewhere.

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Association of Detectable Antibodies produced by *Haemonchus* spp. Infections in Cattle, with Serum Globulin Fractions

EARLIER investigations have shown that cattle which exhibit a marked degree of natural resistance to infection with *Haemonchus* spp. also develop higher complement-fixation titres than susceptible cattle, and that rises in the complement-fixation antibody titres often occurred at the same time as rises in the gamma one fraction of the serum globulin. This resistance has been shown to be heritable[1,2]. These observations were made under field conditions where extraneous stimuli were likely to produce changes in the globulin fractions, so that the correlation between complement-fixation titres and the γ-1-globulin fraction was not always consistent and definite conclusions were not possible. To clarify this situation, cattle sera known to be positive by the complement-fixation test were fractionated and the fractions identified and tested for antibody content. The following is a brief account of the procedure and results obtained.

The β- and γ-globulins were first separated from the serum by precipitation with 16 per cent sodium sulphate at 37° C. The globulin was further fractionated on a diethylaminoethyl cellulose chromatographic column with gradient potassium acid phosphate buffer solutions ranging from pH 7·0, 0·005 M to pH 5·8, 0·1 M, after the technique in refs. 3 and 4. Three antibody positive fractions were isolated in this manner. The protein content of these fractions was estimated by the biuret colorimetric method[2,5]. The relative concentration of the individual globulins in these fractions was ascertained by adding them to a known standard serum and observing the alteration in the globulin fraction content of the serum by electrophoresis in an Antweiler microelectrophoresis apparatus. Complement-fixation test techniques were carried out as described in ref. 1, and hæmagglutination tests following the method in refs. 6 and 7.

Considerable variation occurred in the relative amount of total protein in the samples, and antibody contents have therefore been expressed as antibody potency per gm. of protein. Complement-fixation test results have been expressed as 'index ratios' (refs. 2 and 8), which is the ratio of the amount of complement fixed by the serum in the presence of antigen to the amount fixed in the absence of antigen, after corrections, if necessary, have been made for anti-complementary effects of the antigen. Details of the results are shown in Table 1.

Table 1. ANALYSIS OF ORIGINAL SERUM AND THREE COLUMN FRACTIONS

Serum specimen	Electrophoresis analysis. Per cent content of globulins				Protein content gm./100 ml.	Complement-fixation index ratio per gm. of protein	Hæmagglutination titre per gm. of protein
	Beta 1	Beta 2	Gamma 1	Gamma 2			
Original whole serum	12	28	28	32	1·36*	12·5	1/24
Column fraction 1	0	25	25	50	0·07	21	Neg.
Column fraction 2	0	70	30	0	0·09	33	1/22
Column fraction 3	52	48	0	0	0·06	Neg.	1/33

* Beta and gamma only.

The results support the original suggestion that the complement-fixation antibody is located principally in the γ-1-globulin, and also show that the hæmagglutination antibody is present in the β-globulin fraction, principally in the β-2-globulin. It has been demonstrated by other workers that the complement-fixation and hæmagglutination antibodies are independent in animals infected with *Haemonchus* spp. and the above findings confirm this.

There are at present insufficient data available to form definite conclusions as to the role of the globulin fraction in resistance to *Haemonchus* spp. infection in cattle, but there would, however, appear to be a possible association between the complement-fixation antibody, rises in the γ-1-fraction of the γ-globulins and the occurrence of a natural immunity. Present investigations into the host–parasite relationship in hæmonchosis in this laboratory suggested that rises in the γ-2-fraction of the γ-globulin may be associated with an acquired resistance to the disease.

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HÆMATOLOGY

Significance of the Presence of Lewis Substances in Serum during Infancy

IT has been found that infants who develop into adults with the following phenotype: red cells Le (a − b +), ABH secretor, Les (a + b +)*, go through a phase in which the red-cell phenotype is Le (a + b +), and the secretions, as in the adult, contain ABH, Le^a and Le^b. We have been able to establish that during the phase in which Le^a substance is detectable on the red cells of these infants, it is also detectable in their serum. In the adult with Le (a − b +) red cells, although Le^b substance is present in serum, Le^a substance is not detectable although it is found in the water-soluble secretions. Our observations are summarized in Table 1.

* Les = Lewis substances in saliva.

Table 1

	Red cells		Serum		Saliva	
	Le^a	Le^b	Le^a	Le^b	Le^a	Le^b
Infant	+	+	+	+	+	+
Adult	−	+	−	+	+	+
Infant Adult	+	−	+	−	+	−
Infant Adult	−	−	−	−	−	−

The explanation of the infant Le (a + b +) red-cell phenotype appears to be that the Lewis substances he or she is capable of producing are present throughout the body. We can assume that the disappearance of detectable Le^a substance from the circulation is related to the interactions of Lewis, ABO and secretor systems. There is already evidence for this in the case of some A$_1$, Le (a − b +) individuals in whom the Le^b substance is present in low concentration on cells and in serum, and the power to detect it depends on the potency of the antisera. In our tests for Lewis substances in serum we diluted the sera 2/3 with saline, and added one volume of this to each tube of a titration of the Lewis antisera 1/1–1/16. We were fortunate in having antisera with titres respectable enough to perform the tests and for these we are indebted to Dr. I. Dunsford.

Roman type has been used for the symbols which represent phenotypes, in order to observe a convention in genetics that phenotypes are shown in Roman type and genes in italics.

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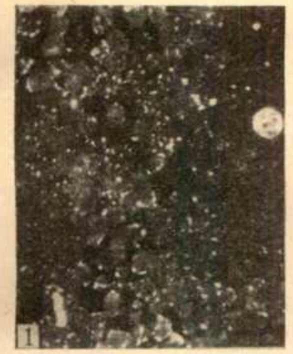
Detection of *C*-Reactive Protein by Fluorescent Antibody Techniques

THE presence of *C*-reactive protein in human serum is a highly sensitive index of inflammation or of tissue destruction[1]. It appears in the blood serum as early as 14 hr. after the injury occurs. Present knowledge points towards its being a β-globulin[2], but its origin and function are yet unknown. The methods available for the detection of *C*-reactive protein are based on precipitation[3,4], complement-fixation[5], hæmagglutination[6] and gel-diffusion[7] techniques, methods which are unsuitable for the precise localization of the substance in cells. For this purpose the fluorescent antibody technique seemed to be promising. Results of staining blood smears from people who are positive and negative for *C*-reactive protein with the fluorescent antibody techniques are described here.

Smears were made on glass slides either directly from blood taken from a finger prick or from clotted venous blood. The smears were air-dried and fixed for 10 min. in 95 per cent ethanol. The presence of *C*-reactive protein in the serum was determined in each case by the usual capillary precipitation method[4] with *C*-reactive protein anti-serum prepared in rabbits. The antiserum, purchased from Schieffelin, of New York, was also used for labelling with fluorescein *iso*thiocyanate. Labelling was carried out by the method of Marshall *et al.*[8]. The fluorescent solution was absorbed twice, once with monkey liver powder and once with guinea pig liver powder[9], and passed



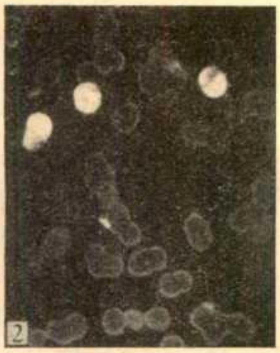


Fig. 1. Fluorescent antibody staining of *C*-reactive protein in blood smear taken from finger. Fluorescent *C*-reactive protein antibody in buffered saline. Leucocyte shows non-specific staining. (× 320)

Fig. 2. Inhibition of fluorescent antibody staining of *C*-reactive protein in blood smear taken from finger. Fluorescent *C*-reactive protein antiserum diluted in concentrated *C*-reactive protein. Staining of the latter was completely inhibited, but leucocytes continue to show non-specific staining. (× 320)

through a column of 'Dowex 2' (ref. 10). This labelled *C*-reactive protein antiserum was used diluted 1 : 8.

Both direct and indirect staining methods were used. The direct method consisted of covering the blood smears with the labelled *C*-reactive protein antiserum and allowing them to stand for 30 min. in a moist atmosphere. The smears were then rinsed for 10 min. in buffered saline, mounted under a cover-slip in glycerol saline at *p*H 7·6 and examined. The indirect method was carried out as follows. Smears were covered with unlabelled *C*-reactive protein antiserum and allowed to stand for 30 min. in a wet chamber. The smears were then washed for 10 min. in buffered saline and covered with labelled anti-rabbit globulin. For the rest the procedure was as described above. Smears were examined from persons who were negative to the *C*-reactive protein by the capillary precipitation method and from persons showing various amounts of *C*-reactive protein in their serum.

Particles with intense green fluorescence, ranging in size from the barely visible to approximately $0 \cdot 3 \mu$, were present in 20 out of 25 people giving a positive response for *C*-reactive protein (Fig. 1). There was rough correspondence between the degree of the capillary precipitation and the quantity of fluorescing particles. Such fluorescing particles were not found in smears of people giving a negative response for *C*-reactive protein. Leucocytes of people giving a positive and those giving a negative response for *C*-reactive protein, as well as those of various laboratory animals, showed slight to intense fluorescent staining. This non-specific staining could be obviated by the addition of normal rabbit, sheep or guinea pig serum to the conjugated *C*-reactive protein antiserum. Thrombocytes from *C*-reactive protein-negative and -positive people, collected separately and stained as above, were negative.

A series of tests was carried out to demonstrate the specificity of the staining. Conjugated normal rabbit serum, when used instead of rabbit *C*-reactive protein antiserum, failed to demonstrate fluorescent staining. The conjugated *C*-reactive protein antiserum was diluted 1 : 8 in saline, and normal rabbit serum, normal sheep serum, normal human serum, concentrated *C*-reactive protein solution prepared from ascitic fluid[2] and in unlabelled *C*-reactive protein antiserum. The normal rabbit and sheep serum did not interfere with the appearance of the stained particles described above, but inhibited the non-specific leucocyte staining. In the presence of unlabelled *C*-reactive protein antiserum, no staining was observed, either of the particles or of leucocytes ; in the presence of concentrated *C*-reactive protein, the staining of the particles only was inhibited (Fig. 2). Surprisingly, labelled *C*-reactive protein antiserum when diluted in some normal human sera also failed to show staining. In the indirect staining procedure, the same results were observed in the staining as well as in the inhibition tests. Here also some normal human sera inhibited staining of the fluorescent particles.

Similar findings were obtained with impression smears and frozen sections from spleen, lymph nodes and liver taken *post mortem* from a patient whose serum gave a positive response to *C*-reactive protein. Fluorescent particles were observed as in the blood smears, but did not seem to be associated with any particular cell.

One of the puzzling features of this work is the fact that some specimens of serum taken from apparently healthy laboratory workers interfered with the fluorescent staining. This interference was observed both in the direct and indirect staining procedures. Seligman *et al.*[11] found traces of *C*-reactive protein in many normal human sera. Assuming that the inhibition of staining is more sensitive than the capillary precipitation reaction, one might speculate that the apparently normal sera which inhibited staining contained traces of *C*-reactive protein. The failure to detect the latter in leucocytes by the fluorescent technique is in agreement with the findings of Seligman *et al.*[11], who were unable to detect it in extracts of normal and leukæmic cells.

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HISTOCHEMISTRY

Nature of the Sinus Lining Cells of the Spleen

VARIOUS opinions have been expressed on the nature of the cells lining the venous sinusoids of the spleen[1]. They have been described as forming a type of internal epithelium, as muscle cells and as separated, longitudinal endothelial ridges. Klemperer

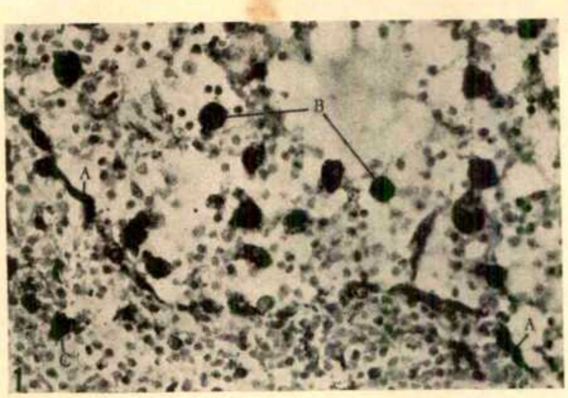

Fig. 1. Frozen section of a lymph node demonstrating non-specific esterase activity in histiocytes, (*A*) lining sinuses (littoral cells), (*B*) lying free in dilated sinus, (*C*) in medulla. α-Naphthyl acetate method. (× *c*. 240)

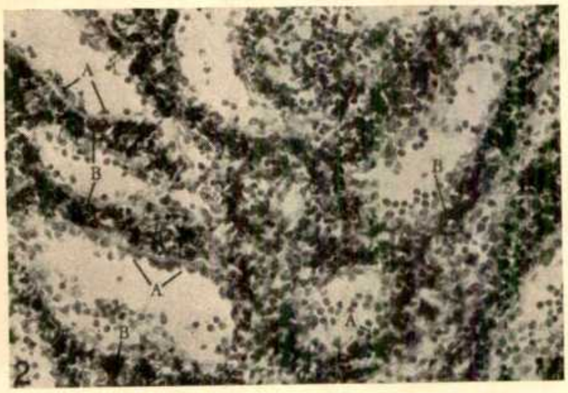

Fig. 2. Frozen section of spleen demonstrating non-specific esterase activity in histiocytes, (*B*) in cords of Billroth, but not in sinus lining cells (*A*). α-Naphthyl acetate method. (× *c*. 150)

accepted Mollier's evidence[2] that the wall of a splenic sinus is formed by a cytoplasmic reticulum with reinforcing fibres, essentially identical to the general cytoplasmic and fibrillar reticulum of the pulp. As recently as 1957 Weiss[3] stated that splenic cords are collapsed splenic sinuses, and the preponderant cell type in the cords is the same as that lining sinuses.

It is evident that interpretations of histological appearances, whether under the light or electron microscope, are influenced by artefacts. Other methods of study may therefore be more fruitful.

Braunstein *et al.*[4], from enzyme histochemical studies, regarded the splenic littoral cells as histiocytes, that is, phagocytic cells like those of lymph nodes, Kupffer cells, reticuloendothelial cells of bone-marrow and monocytes of blood. They stated further that all histiocytes manifest strong non-specific esterase and acid phosphatase activity. They also demonstrated strong alkaline phosphatase activity in capillary and lymphatic endothelium. From this study it would appear that splenic sinusoids are comparable with those of liver in that they are lined by phagocytes.

Using the histochemical techniques described by Pearse[5] for acid and alkaline phosphatase and non-specific esterase, on formalin-fixed frozen sections, I have confirmed the results of Braunstein *et al.*, in regard to the histiocytes of lymph nodes. I have not, however, been able to confirm their statements concerning the splenic sinus lining cells.

The spleens studied were obtained from monkeys, guinea pigs and human patients. The last-named group comprised two patients with lacerated spleens, two with thalassæmia, three with hereditary spherocytosis and one with thrombocytopenic purpura. In all these, non-specific esterase and acid phosphatase were demonstrable in stellate, branching histiocytes in the cords of Billroth and in Malpighian corpuscles. The sinus lining cells, however, were consistently negative for all three enzymes.

Capillary endothelium possessed the characteristic strong alkaline phosphatase activity.

It is of interest to note that Marshall's 'metalophil' technique[6] impregnates those histiocytes in lymph nodes and spleen which show non-specific esterase and acid phosphatase activity. He states, however, that the impregnation of the cells lining the splenic sinuses by metallic methods is often difficult.

These enzymatic reactions provide strong evidence that the splenic sinus lining cells are not identical with histiocytes (reticulum cells) in the pulp and in fact are not true histiocytes.

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MICROBIOLOGY

A Superior Fat-synthesizing Single-spore Strain of *Lipomyces*

CONSIDERABLE attention has been devoted to the investigation of fat-producing micro-organisms as a potential industrial process. Woodbine[1] has recently reviewed this field and showed that, whereas many strains had been examined for their fat-producing abilities, no rationalized investigation for improving the fat-producing ability of an individual strain by selection had been undertaken. Such a strain could be isolated originally as either a single cell or a spore which could then be subcultured to give a genetically homogeneous strain.

Roberts[2] had isolated some single-spore cultures from strains of the fat-producing yeast genus *Lipomyces* and an investigation was carried out on ten of these strains: *B* to *H* were isolated from a parent culture of *L. lipofer*, and *I* to *L* from a culture of *L. starkeyi*.

Lactose was used as the carbon substrate by all strains (Wickerham's medium). Although *L. lipofer* was found to utilize lactose immediately, *L. starkeyi* required an adaptive period of fifteen or more days. This contradicts the evidence of Connell *et al.*[3], who claimed that none of the strains they examined would utilize lactose, and of Lodder *et al.*[4], who found that only strains of *L. lipofer* would utilize lactose and indeed used this as a diagnostic character. Van der Walt[5] and Roberts[2] found that both species would utilize lactose. One reason for the confusion would appear to be due

Table 1. RESULTS FOR THE LOW C : N RATIO IN TERMS OF TOTAL YIELD, SUGAR UTILIZED AND TOTAL FAT IN GM./L. USING *L. starkeyi*

Strain designation		Total yield	Sugar utilized	Economic coefficient	Fat per cent	Total fat	Fat coefficient
Parent strain *P*	Mean	11·2	29·1	38·8	20·1	2·3	8·1
	S.E.	±0·16	±1·38	±0·69	±5·5	±0·61	±2·07
	S.S.D.	*I,K,L*	*I,K,L*	*J*		*I,L*	
Strain *I*	Mean	21·9	64·6	34·0	31·4	7·0	10·7
	S.E.	±0·84	±1·12	±1·11	±2·45	±0·8	±1·14
	S.S.D.	*P,J*	*P,J*	*J*		*P,J,K*	
Strain *J*	Mean	13·2	28·6	50·1	15·6	3·4	11·8
	S.E.	±2·06	±1·29	±6·32	±4·70	±1·13	±3·85
	S.S.D.	*I,K,L*	*I,K,L*	*P,I,K,L*		*I,L*	
Strain *K*	Mean	20·1	66·2	30·4	18·9	3·8	5·8
	S.E.	±1·5	±2·32	±0·6	±2·64	±0·45	±0·95
	S.S.D.	*J,P*	*J,P*	*J*		*I,L*	
Strain *L*	Mean	22·9	62·1	36·9	26·8	7·2	11·7
	S.E.	±0·7	±2·1	±0·84	±0·94	±1·09	±1·76
	S.S.D.	*J,P*	*J,P*	*J*		*P,J,K*	

The 'economic coefficient' is dry $\frac{\text{cell weight}}{\text{sugar utilized}} \times 100$, and the fat coefficient is $\frac{\text{fat produced}}{\text{sugar utilized}} \times 100$.

S.S.D. indicates the strains showing a significant difference ($P < 0.05$) by 'difference between means' analysis.

to the long adaptive period required by *L. starkeyi* before it can break down the lactose. This, indeed, might be used as a diagnostic character in the species differentiation of the genus *Lipomyces*.

Examination for variations in the fat-synthesizing abilities of the strains was done on two media, one with a carbon to nitrogen ratio of twenty and the other with a ratio of sixty.

With the low carbon-to-nitrogen ratio, Wickerham's[6] yeast–nitrogen base ('Difco' brand) medium was employed, containing 5 per cent lactose. Five replicates of 50 ml. of medium in 250 ml. Erlenmeyer flasks were used for each strain. The inoculum was subcultured on the glucose agar used by Starkey[8], and 0·5 ml. of a standardized cell suspension used as inoculum. The flasks were agitated at 120 r.p.m. (3 in. thrust) at 26 ± 1° C., and gathered after 33 days. Dry weight, sugar utilization and the fat percentages were assessed by the methods of Wix and Woodbine[7]. *L. lipofer* strains showed no variation, but considerable variation occurred among strains of *L. starkeyi* (Table 1). Although no variation was found with regard to the fat-synthesizing mechanism, all the single spore strains examined were found to have adapted to lactose faster than the parent strain, which had utilized only half the available sugar. Diauxie occurred, so that the 'parent' strain was adapting from the glucose to the galactose (or vice versa) fraction of the lactose when samples were harvested.

Strains *I*, *K*, and *L* had completely utilized the lactose whereas strain *J* was still utilizing the second sugar in the lactose and consequently had a significantly higher economic coefficient. This indicates that single-spore strain selection might produce strains with shorter adaptation periods toward sugars than the original strain.

In the medium with high carbon-nitrogen ratio fat production was encouraged. Six replicates for each strain were grown in 6-oz. medical flat bottles containing 35 ml. of the medium recommended by Starkey[8] but containing 3 per cent lactose. The bottles were laid on their sides to increase aeration and incubated at 26 ± 1° C. No agitation was applied. Inoculation and gathering were carried out in the same manner as in the previous experiment. No deviation occurred among the strains of *L. lipofer*. The total yield, sugar utilization, and economic coefficient trends were similar to those found in the first experiment. The differences may be summarized by division of the strains of *L. starkeyi* into three groups. Strain *P* gave the lowest yield, strains *I*, *J*, and *K* produced greater yields, whereas strain *L* produced the highest. In no case was all the lactose broken down; this is the result of the high C : N ratio of the medium and the relatively poor aeration retarding growth. Sugar utilization by the strains does not show the same pattern as is evident for the total yield, strain *L* having utilized only the same amount of sugar as strain *P*. The higher total yield of strain *L* is reflected in the higher economic coefficient which is significant at a 1 per cent level. Much of this increase has occurred due to the higher rate of fat synthesis in strain *L*. This may be shown by a comparison of the synthesis of non-fatty components for these two strains. For strain *P*, this amounts to 0·98 gm./l., whereas for strain *L* it is 1·10 gm./l., which is an increase of 11·2 per cent. If the fat-synthesizing mechanisms of both strains were similar, then strain *L* would show 11·2 per cent increase in fat produced over strain *P*, since no significant difference in sugar utilization has occurred between the strains. From Table 2, it can be calculated that the increase in fat produced by strain *L* over strain *P* is in fact 163·6 per cent. This exceptional increase in fat could be due to a more efficient synthesizing mechanism being possessed by strain *L* than by strain *P* under the high carbon/nitrogen ratio conditions used in this experiment. Strain *I* closely resembles strain *P* in all but total yield, which is significantly higher. This may be due to a greater synthesis of the non-fatty components from the same amount of sugar used. Strains *J* and *K* are very similar to each other; both have used more sugar than the other strains. Most of the increase in total yield appears to be the result of the increased synthesis of non-fatty cell components.

The general conclusion drawn from this work is that by investigation of single-spore isolates from *Lipomyces starkeyi*, strains are found with superior power to assimilate sugar and/or more efficient fat-synthesizing ability to those of the parent strain. The results indicate that improvement in such characteristics might be possible by investigating single spores isolated from a parent strain.

Table 2. RESULTS FOR THE HIGH C : N RATIO IN TERMS OF TOTAL YIELD, SUGAR UTILIZED AND TOTAL FAT IN GM./L. USING *L. starkeyi*

Strain designation		Total yield	Sugar utilized	Economic coefficient	Fat per cent	Total fat	Fat coefficient
Parent strain *P*	Mean	1·2	7·6	18·3	7·7	0·22	2·16
	S.E.	±0·2	±1·39	±3·32	±4·98	±0·038	±1·52
	S.S.D.	*I,J,K,L*	*J,K*		*L*	*L*	*L*
Strain *I*	Mean	1·56	7·24	21·9	13·0	0·216	2·96
	S.E.	±0·036	±0·42	±0·85	±2·35	±0·0038	±0·66
	S.S.D.	*P,L*	*J,K*		*L*	*L*	*L*
Strain *J*	Mean	1·54	11·4	14·7	10·9	0·22	1·69
	S.E.	±0·19	±0·87	±1·93	±3·04	±0·022	±0·92
	S.S.D.	*P,L*	*P,I,L*		*L*	*L*	*L*
Strain *K*	Mean	1·56	12·8	12·8	11·9	0·33	1·58
	S.E.	±0·18	±3·07	±1·53	±3·09	±0·03	±0·65
	S.S.D.	*P,L*	*P,I,L*		*L*		*L*
Strain *L*	Mean	1·68	8·0	31·7	30·6	0·58	10·04
	S.E.	±0·19	±3·56	±10·06	±8·06	±0·023	±1·93
	S.S.D.	*P,I,J,K*	*J,K*		*P,I,J,K*	*P,I,J*	*P,I,J,K*

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The Cytochrome of *Pasteurella tularensis*

THE examination of cytochrome in bacterial cells has been reported earlier by W. F. Riedmüller[1], A. F. Brodie *et al.*[2], and W. S. Silver[3]. Recently, T. Yamanaka *et al.*[4] have separated and purified cytochrome from *Pseudomonas aeruginosa.* The present communication describes cytochrome components which have been detected in the highly virulent (Idaho, Nevada) strains (LD_{50} 10^{-7}) and avirulent (*P*38) strain (LD_{50} 32 mgm.) of *P. tularensis.*

Bacterial cells cultured on Hiwatashi's medium for 24 hr. at 37° C. were used. 4 gm. of cells were washed three times with distilled water in a homogenizer and centrifuged for 10 min. at 11,000 r.p.m. Cell-free extracts were obtained by treating the washed cells in a sonic oscillator (9 kc./s.) for 6 min., followed by centrifugation at 11,000 r.p.m. for 10 min. The resulting supernatant was used as the cell-free extract. Freeze-dried cells and frozen cells were prepared by the usual methods.

The optical density was measured with a Shimadzu photo-electric spectrophotometer, type *QB*–50. The absorption spectrum of the cell suspension or cell-free extract was measured using an opal glass, using Calvin and Shibata's method[5]. Cell concentrations were checked by finding the dry weight and by micro-estimation of nitrogen (indophenol method).

Using a hand spectroscope, when excess sodium dithionite was added, the native cells showed absorption bands essentially the same as the virulent and avirulent strains, though an absorption band at around 560 mμ was found to be stronger in the virulent strains (Fig. 1). Similar results were obtained with cells grown for 48 hr. on a blood-free medium.

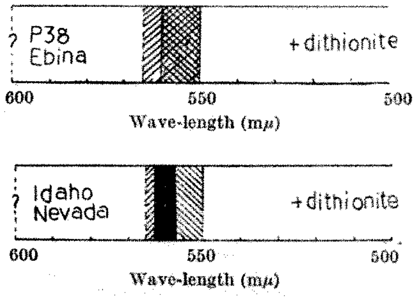

Fig. 1. Cytochrome components of *P. tularensis*. After reduction with sodium dithionate, the samples were examined for their cytochrome components by means of a Zeiss hand-spectroscope at room temperature and during immersion in liquid air

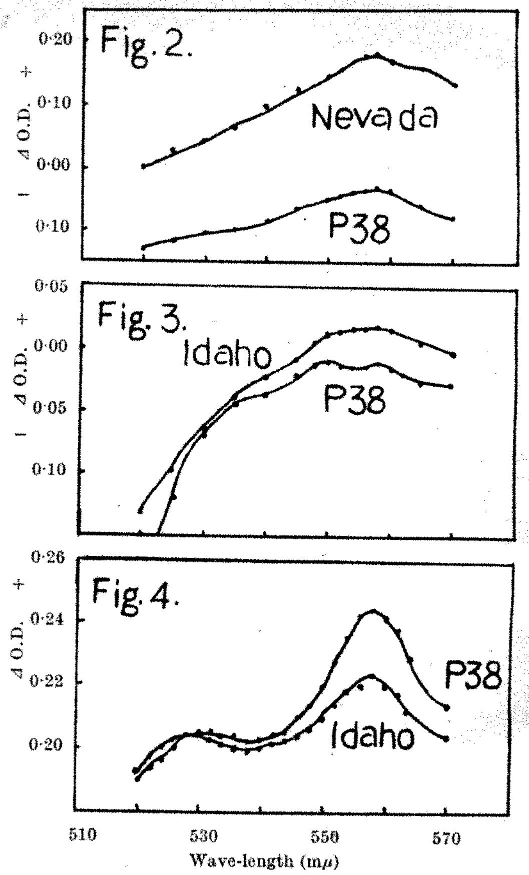

Fig. 2. Freeze-dried cells. Fig. 3. Native cells. Fig. 4. Particles. Optical density (O.D.) represents the difference between the oxidized and reduced forms

(1) Lyophilized cell preparations: 100 mgm. of dry cells were thoroughly dispersed in 10 ml. of 0·2 *M* phosphate buffer (*p*H 7·0) in a homogenizer, and the differential spectra (optical densities) were measured by the difference between cell suspensions previously reduced by excess sodium dithionite and by excess potassium ferricyanide. In Fig. 2, $\Delta E(=E_{\text{max.}} - E_{510})$ is plotted against wave-length for the two strains, and $E_{\text{max.}}$ is seen to be at 558 mμ:

Virulent strain: $\Delta E = 0\cdot17 - 0\cdot090 = 0\cdot080$
Avirulent strain: $\Delta E = -0\cdot040 - (-0\cdot100) = -0\cdot060$

As the dry weight is the same, cytochrome as measured by ($E_{558-540}$) from the virulent strain is greater by $\Delta E = 0\cdot02$ than the avirulent strain.

(2) Resting native cells: 2 gm. of wet cells per 10 ml. of 0·2 *M* phosphate buffer (*p*H 7·0) were suspended by homogenization, and the differential spectra were measured as before. The results are shown in Fig. 3 and Table 1, giving the value of ΔE corrected for the respective protein contents. The latter was remarkably large in the virulent strain, but $E_{\text{max.}}$ is still at about 558 mμ.

Table 1. CYTOCHROME ($E_{558-540}$) CONTENTS OF *P. tularensis*

Specimens	Native cells		Particles	
Strain	*P* 38	Idaho	*P* 38	Idaho
ΔE (O.D.)	0·013	0·035	0·042	0·023
N (γ/ml.)	7,680	6,440	5,040	870
ΔE/gm. protein	0·27	0·87	1·33	4·24
Differences		< 0·60		< 2·91

(3) Cell-free extracts: The cell-free particles extracted from the cells as described were examined spectroscopically. When absorption at about 560 mμ due to contamination had been removed, there was a strong alpha-absorption peak at 558 mμ like the so-called cytochrome b_1 (Fig. 4, Table 1).

These particles can be repeatedly oxidized and reduced like other cytochromes, and showed higher succinoxidase activity by the respirometry or reduction of trimethyl tetrazolium chloride than the other fractions[6]. Hence the absorption band at about 558 mμ may well be that of a cytochrome b_1 type pigment.

Thus it seems that *P. tularensis* contains cytochrome b_1, the amount of which in the whole cell and in the cell-free extract is greater for the virulent strain than for the avirulent strain.

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Pseudomonadaceae as a Source of Bacterial Contamination in Tissue Culture

The pleuro-pneumonia-like organisms cause considerable difficulty in tissue culture work, as many lines of cell cultures are contaminated with these organisms[1]. I have found that members of the Pseudomonadaceae could play a part as contaminants of tissue culture. It is well known that these bacteria are frequently a source of bacterial contamination in stored blood and blood derivatives[2].

I have isolated from apparently healthy tissue-cultures of human origin (human embryonic and baby kidneys and *KB* cells) a Gram-negative bacillus identified according to *Bergey's Manual of Determinative Bacteriology* (7th edit., 1957) as *Pseudomonas fragi*.

The main characteristics of the organism isolated were as follows:

P. fragi is a Gram-negative rod with a polar flagellum (0·5–1·0 × 0·75–4·0μ). It grows well on agar slants as white, glistening and butyrous colonies. In broth cultures a thin pellicle and a sediment are formed. The saccharolytic and proteolytic activities are poor; the organism produces acid from glucose, arabinose and galactose. Litmus milk is coagulated in 2–3 weeks, and it produces a characteristic 'strawberry' odour. On potato, *P. fragi* grows in raised, white colonies, which later become brown. It does not produce pigments.

The important observations for the tissue-culture work are: (1) *P. fragi* does not grow at 37° C., but remains viable at this temperature in the human embryonic and baby kidney tissue-culture for at least 30 days, and it can persist in *KB* cells through 65 passages made at three-day intervals without causing any visible damage to the cells; it grows at temperatures of 10°–30° C., the optimum for growth being 20° C. (2) It is a minute organism and it can pass through sintered glass filters (*G*5) commonly used for the preparation of tissue-culture media.

Because of these two characteristics it can remain undetected in contaminated tissue cultures if the sterility controls are not tested at room temperature (18°–20° C.). The organism is widespread in Nature, and can be encountered in air, soil and water. In my experience tissue-cultures were contaminated from tap water, and the organisms reached the tissue-culture media primarily through contaminated glassware and sintered-glass filters. It was detected by chance.

P. fragi can easily be detected in control tests for sterility. Cultures can be set up on ordinary bacteriological media incubated after plating at room temperature. The incubation period must be sufficient to permit growth of the organism. Direct growth from tissue cultures may be slower on account of the antibiotics added to the media. *P. fragi* grows in visible colonies from a tissue-culture medium containing 100 i.u. of penicillin and 100 μgm. of streptomycin in 48 hr.

A great number of saprophytes and plant parasites belong to the Pseudomonadaceae. Many members of this family have been isolated from water and I believe that, like *P. fragi* in my experiments, they could contaminate tissue cultures and remain there as latent contaminants.

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BIOLOGY

Sun Navigation in Birds ?

C. J. Pennycuick[1] has advanced a new hypothesis of Sun navigation in birds. The bird is supposed to measure the Sun's altitude and the rate of change of altitude and to compare these quantities with the respective memorized conditions at home.

The experimental evidence available does not agree with this theory in at least two major respects:

(1) The Sun's altitude: data have been presented[2] in which pigeons, following a phase-shift of their chronometers of 6 hr. clockwise, were released during the common light period of the artificially shifted and the natural day (which appears to be afternoon to the bird, but actually is forenoon). Only at one particular time of day (9.00 local time) do the altitudes of the anticipated and realized Sun with which the bird is confronted agree. All birds released before that time should, according to the hypothesis presented by Mr. Pennycuick, take their headings towards east to south-east, all birds released after that time towards north-west to north. This clearly was not the case. There was a deviation to the left, with reference to the headings of the controls, no matter what time and from which direction or distance the birds were released.

(2) In determining the rate of change of altitude either: (*a*) the bird has to be able to discriminate between a Sun going up and a Sun going down; or if not, (*b*) there would be an ambiguity during at least some portions of the day. Mr. Pennycuick does

not mention this point in his paper. An additional point of evidence against Sun navigation is implied by the data referred to under (1) if case (2*a*) should obtain for the rate of change of altitude. The birds should expect a decrease in altitude but will observe an increase in altitude. However, instead of eastward headings, as would be predicted by the hypothesis, there was a deviation to the left.

The first evidence of the orientational reaction of pigeons to a phase-shift of their chronometers[2] has, meanwhile, been confirmed by the results of experiments involving other degrees of phase-shift[3-5]. These results, however, were not yet generally available when Mr. Pennycuick's manuscript was submitted for publication.

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(1) In the paper referred to, I followed Matthews[1] in assuming that the pigeon, on release, compares the Sun's altitude with the remembered altitude seen at the same time of day at home. In Schmidt-Koenig's 'clock-shifting' experiments the birds were accustomed to a light–dark rhythm advanced by 6 hr. in a light-proof room. Since they could not see the Sun during the training period, they clearly could not remember its altitude for subsequent comparison with the altitude observed on release. Hence these experiments shed no light on the possible use of altitudes for navigation in the way postulated, and neither the predictions stated nor any others based on altitude measurements would follow from my hypothesis.

(2) Schmidt-Koenig's experiments might have been interpreted in at least two ways by the pigeons.

(*a*) They may (as Schmidt-Koenig seems to assume) accept the 'shifted day', forgetting that the shift had been applied and assuming that they were still at home. On release in normal daylight they should then respond as though they had been taken a quarter of the way round the world, to the west. This is a very large displacement compared with any with which pigeons normally have to contend, and the release distances from home (5·5–100 km.) were insignificant by comparison. They would thus head east if a two-dimensional navigation system were being used.

If rate of change of altitude alone were being used (altitude being ruled out) they might respond to the gross difference which would occur—the Sun going up when, according to the time of day, it should be going down (Schmidt-Koenig gives no reason for his doubts as to whether the birds could distinguish opposite directions of movement). On the simple method of interpreting this, which is postulated in my paper, the birds would then orient themselves at right angles to the Sun's direction, with the Sun on their right. That they did not do so might be evidence against Sun navigation, but more likely it indicates that Schmidt-Koenig's interpretation of his experiment is not the correct one. The fact that behavioural rhythms became synchronized to the shifted day is no proof that the shift had been forgotten: the experiments in which pigeons were trained to take food in a particular direction referred to the Sun, also purporting to show that the clock had indeed been shifted, are also inconclusive, since there is no reason why the birds should associate this task with homing orientation—indeed, it was shown that most of them would not even allow for the Sun's motion during the day unless they were specifically trained to do so.

(*b*) The birds could alternatively have supposed that they had been taken for a few days to a place where the light–dark rhythm was advanced by 6 hr., and then brought back and released in the general neighbourhood of home (as had in fact happened). It is well known that pigeons will return to their old home after confinement in a distant place for much longer than the short training periods (4–10 days) used by Schmidt-Koenig, which amounts to the same thing. The experiment then becomes a test of ability to orient homeward after being denied sight of the Sun for a few days, during which the time-sense of the birds was subjected to severe stress. No specific effects would be predicted from this, but one might expect some general disorientation, giving increased scatter of direction of departure and reduced success in homing. These were observed.

It is true that the differences observed in directions of departure between experimentals and controls would not be predicted on my hypothesis, but Schmidt-Koenig has not explained these either. Probably the design of these experiments was too ambiguous to lead to any definite predictions.

Schmidt-Koenig's results are of great interest in that they confirm that pigeons have a time-sense, and that certain manifestations of it can be interfered with by applying shifted light–dark rhythms; but he is not justified in assuming that an experiment in which the bird is not allowed to see either the real Sun, or an artificial sun moving in a calculated path, will lead to results predictable from a theory of Sun navigation. In any event the time-shifts used (6–12 hr.) were unreasonably large: shifts of the order of 5–30 min. should produce effects comparable to those of the homing distances which have been used in previous experiments on pigeons.

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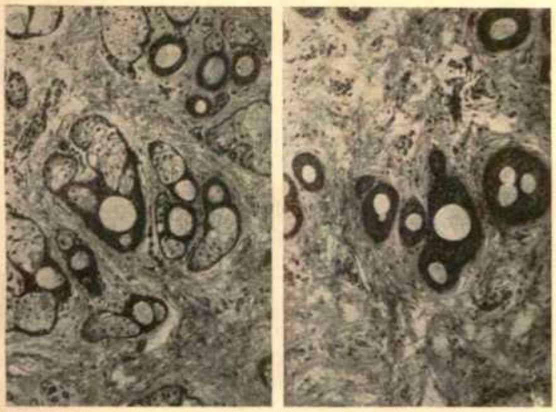
Bundles of Follicles in Adult Romney Ewes

Bundles of secondary follicles have been shown in the Karakul[1], Herdwick[2], Cheviot[2], Merino[3] and several other breeds[4] of sheep, and the formation of these bundles by the branching of follicles during their development has been demonstrated in the Karakul[1] and the Merino[3]. Branched primary (*P*) follicles have been described in the Merino and the Corriedale by Lyne[5], who also discussed his findings in relation to the fact that secondary/primary (*S*/*P*) ratios are normally determined from sections cut at the level of the sebaceous glands. At this level, branching follicles



(*a*) (*b*)

Fig. 1. A primary follicle bundle and a secondary follicle bundle ; *a*, at the level of the sebaceous glands ; *b*, above the level of the sebaceous glands, showing the coalescence of the root sheaths of the follicles. (× 100)

will not always be apparent so that some branching P follicles will be classified as secondary (S) follicles.

In recent work at Massey Agricultural College, skin samples were taken from the face (0·5 cm. trephine) and the mid-side (1 cm. trephine) of a flock of 111 2-year-old Romney ewes. Transverse skin sections were cut about the level of the sebaceous glands and stained with hæmatoxylin and eosin. During the determination of S/P ratios and follicle densities, branching P and S follicles were found in both mid-side and face samples.

It should be pointed out that the purpose of the work was not to determine whether branching follicles existed in the Romney breed ; therefore, these observations are not based on complete serial sections of the follicles. However, in the light of Hardy and Lyne's[3] and Lyne's[5] work, it is reasonable to assume that if the root-sheaths of two follicles coalesce at a level above that of the sebaceous glands, then, nearer the skin surface, the lumens of the two follicles will do so as well (Fig. 1). Furthermore, as the branching follicles were observed mainly in those sections or part of sections cut higher than the level of the sebaceous glands, it is clear that the proportion of sheep showing branched follicles will depend on whether such sheep had sections cut at this level.

Branching S follicles were found in the mid-side samples of twenty-one of the 111 sheep (Fig. 1). Even within the limitations of the sections there appeared to be variations between sheep in the number of bundles of S follicles in the area sampled as the number of bundles varied from 1 to more than $80/\pi$ cm.^{2}. Again, within the limitations of the sections, the number of fibres/bundle varied from 2–5.

The occurrence of six branched P follicles in the mid-side sample of one sheep, which also showed marked S follicle branching, and the appearance of several more in the face samples of five sheep, was noted, again in those sections cut above the level of the sebaceous glands (Fig. 1).

In view of the work by Hardy and Lyne[3] and Lyne[5], it is unlikely that the branching follicles found in this study are anomalies as suggested by Auber and Ryder[2], although until the factors leading to the formation of these follicles have been determined, this must remain a possibility.

These findings suggest, therefore, that an investigation into the method and time of development and extent of branching of follicles in the Romney breed is needed. Such work, making use of Lyne's[5] suggested definitions of follicle types, could establish the importance of branching follicles to the type of fleece in this breed. Furthermore, the occurrence of bundles of both P and S follicles in the Romney and Merino breeds suggests the necessity for investigating the follicle populations of breeds of other wool types, as branching follicles may be a more common unit of the fleece than was previously thought.

I am indebted to Mr. P. G. Schinckel for confirming the identification of branching P and S follicles, Mr. G. A. Wickham and Mr. B. Thatcher for preparing and examining the sections and Dr. F. Cockrem for the use of his material and for helpful criticism.

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Independence of Temperature of the Rate of Ageing in *Drosophila subobscura*

THE fact that in poikilotherms an increase in temperature reduces the life-span has naturally been taken as showing that the rates of ageing processes, like those of chemical reactions, are dependent on temperature. If so, an animal which is kept for an appreciable part of its life-span at a high temperature and is then transferred to a low temperature would be expected to die at a chronologically younger age than a similar animal kept continuously at the lower temperature. It has already been reported[1,2] that this expectation is not realized in the case of *D. subobscura*. Flies kept when young at 30° C. for periods equal to about half their expectation of life at that temperature and then transferred to 20° C. died at the same chronological age as did flies kept continuously at 20° C.

These results were interpreted, wrongly as we now think, as evidence that the causes of ageing at the two temperatures are different. But they can be explained in another way. Ageing processes may be defined as those processes occurring in individuals which render them more likely to die as they grow older, either from internal or external causes. It is convenient to describe the result of such processes as a decline in the 'vitality' of individuals as they grow older. It is supposed that the ageing process is not only the same at 20° C. and at 30° C., but also that its rate is the same at the two temperatures. Death occurs when the vitality of the individual falls below some threshold value, the level of which depends on the temperature at which the individual is living at the time, and which corresponds to the level of vitality necessary for survival at that temperature. Differences in longevity at different temperatures are then due, not to differences in the rate of the ageing process itself, but to different threshold-levels below which the vitality must not fall if life is to be maintained.

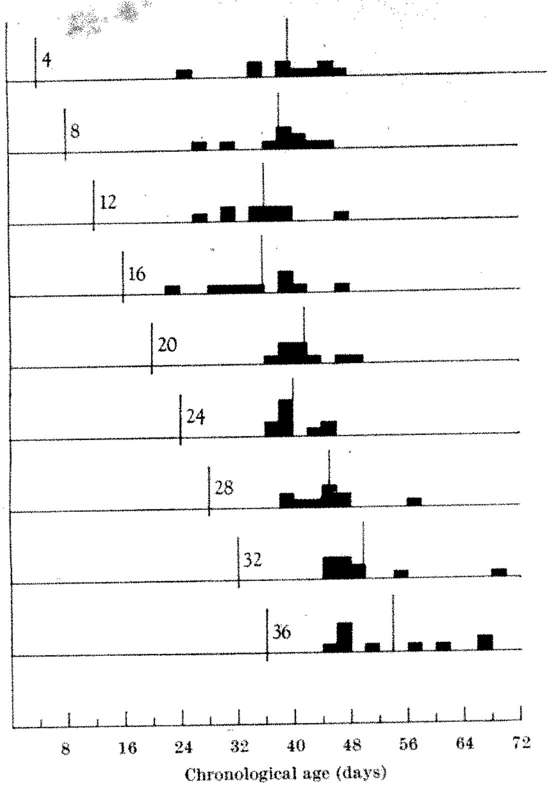

Fig. 1. Chronological ages at death of males kept for periods from 4 to 36 days at 20° C., and afterwards at 26° C.

This hypothesis would explain the earlier findings, since, provided that flies are transferred from the high to the low temperature before their vitality has fallen to the threshold-level corresponding to the higher temperature, their physiological state would be the same as that of flies of the same chronological age kept at the lower temperature. But there is another prediction from it which can easily be tested. Animals kept at a low temperature for a period equal to or greater than their expectation of life at some higher temperature should die immediately if transferred to that higher temperature. The experiment now to be described fully confirms this prediction, provided that the word "die" in the previous sentence is replaced by the phrase "start dying".

F_1 hybrid males between the *B* and *K* inbred lines were raised at the usual laboratory temperature of 20° C. A control group kept continuously at 20° C. had a mean longevity of 83·4 days. Groups of 10 males each were kept at 20° C. for periods varying from 4 to 36 days after emergence, and were then transferred to 26° C. and kept at that temperature until they died. The results are shown in Fig. 1. The first 6 groups to be transferred all died at the same chronological age of approximately 40 days, although the first group had a mean survival-time at 26° C. of 35·4 days, and the 6th group of only 16·2 days. This is exactly the result to be expected if the rate of ageing were independent of temperature. But the last four groups all had approximately the same survival time at 26° C. of about 16 days. This suggests that once the vitality has fallen below the threshold value appropriate to the temperature at which the flies are living, a second process is set in motion, a process we shall refer to as 'dying'. The threshold appropriate to 26° C. is reached at a chronological age of about 24 days, regardless of whether the flies have been living at 20° or at 26° C. If flies, aged 24 or more days, are transferred to 26° C. they start dying, a process which, at that temperature, is completed in about 16 days.

Similar results have been obtained in a number of experiments on both males and females, at temperatures ranging from 15° to 30° C. It has also been found that the 'dying' process is reversible. Males aged 12 or more days at 20° C. die after 3 or 4 days at 30° C. But males kept alternately for one day at 30° C. and for three days at 20° C. died at chronological ages varying from 53 to 85 days, after periods of from 8 to 17 days in all at 30° C. This shows that the changes occurring during the dying period at 30° C. can be repaired at 20° C.

The earlier experiments, together with those described here, lead to the following conclusions. The processes which ultimately result in the death of old flies can be divided into two stages, here called 'ageing' and 'dying'; these may correspond to the processes of induction and development suggested by Neary[3] from a study of mice. Ageing is an irreversible process the rate of which is roughly independent o temperature, at least from 15° to 30° C., and which leads to a continuous decline in the 'vitality' of the individual; by vitality is meant merely the capacity of the individual to withstand the various factors which may cause death. When the vitality falls below a certain threshold, a second process, that of dying is set in motion. The level of this threshold is dependent on temperature, being lower at lower temperatures; it is differences in the threshold-level, and no in the rate of ageing, which are mainly responsible fo differences in longevity at different temperatures. The dying process occurs at a rate which is dependent o temperature, and is terminated by death, but it ca be reversed in individuals transferred to lower temperatures.

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Further Evidence of Polar Movement of Nutrients in Plants

In two earlier papers I showed that there was goo evidence for postulating polar rather than transvers movement of materials in plants[1a,b]. This conclusio was supported by later work on the movement o fluorescein in the xylem system[1c], of labelled carbon and of labelled phosphorus[3]. The two last paper describe experiments which show that carbon-14 o phosphorus-32 when introduced on one side into th leaves or the roots respectively of plants pass freel and rapidly up to the tissues on that side, but do no move transversely across the plant.

The present communication describes some exper ments which confirm the previous results, but whic have the advantage that the tissues concerned wer not mutilated by defoliation nor affected by the use c staining materials which are, to a greater or les extent, toxic to the living cells.

The technique is simple and can be carried ou without the necessity for any precautions which ma attend the use of radioactive materials. Further, th

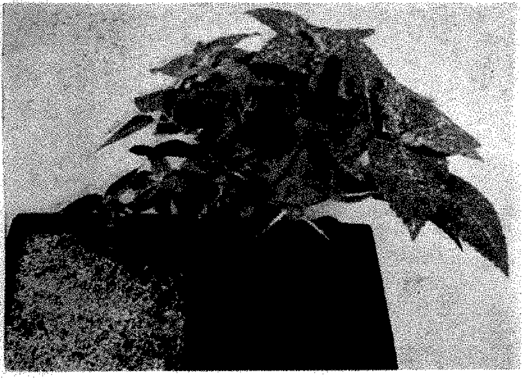

Fig. 1. Photograph of *Coleus* plant showing marked growth on the side of the plant with roots in soil

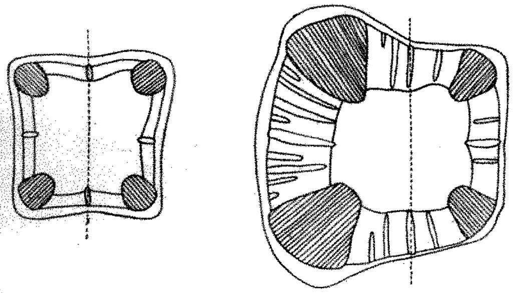

Fig. 2. Camera lucida drawing of section of *Coleus* stem (*a*) before experiment, (*b*) after three months. The dotted lines indicate the approximate position of the glass plate dividing the two media in which the cutting was grown

experiments were of comparatively long duration, showing that during a period of even three months the major portion of the metabolites move up and remain within the side on which they were originally absorbed or elaborated.

The experiments were set up as follows: young shoots of *Coleus* plants with 3–4 pairs of leaves were taken as cuttings and rooted in coarse sand without any added nutrients. After a couple of weeks or so, the end had developed a callus and adventitious rootlets had emerged. The end of the stem was then carefully slit with a razor by an incision up the middle at right angles to a pair of sides. It will be recalled that *Coleus* stems are square with pairs of opposite leaves inserted on the flat sides. In these stems there are four main vascular bundles, one at each corner of the stem. In the treated cuttings, therefore, there were two bundles on each side of the incision and the stems were symmetrical for all practical purposes.

The cuttings were then so placed on a sheet of glass dividing a wooden box into two compartments that each side of the stem and its adventitious roots was on one side of the sheet of glass. One compartment was filled with a rich loam, the other with a coarse sand without any added nutrients. Within a few days it was noted that the side of the plant above the soil was growing much more rapidly than that above the sand. The leaves on one side were much larger than the opposite members of the pairs, the axillary buds were developing into branches and the stem was much better grown on the soil side than on that over the sand. Clearly, the nutrients being absorbed from the soil were passing into and being retained by the tissues on that side of the plant.

At the end of three months a plant was photographed (Fig. 1), and all the leaves and axillary branches on the side above the soil and on that above the sand were removed. Though the alternate leaves on the other two sides also showed marked distortion —the side above the soil had grown much more than that above the sand, and similarly the axillary branches were correspondingly distorted—they were not taken into account for this measurement. Three sets of leaves and axillary branches were taken and dried at 100° C. The dry weights are given in Table 1.

Table 1

	Above soil	Above sand
Plant No. 1 (after 1 month)	1·07	0·54
Plant No. 2 (after 2 months)	1·85	0·41
Plant No. 3 (after 3 months)	7·54	1·57

Sections were made of the stem at an internode about the middle of the plant and Fig. 2 indicates the differences in the vascular tissues.

It will be noticed that the main vascular bundles on the corners of the stem above the soil had increased by about six times in area, while those above the sand had only doubled in cross-section after three months.

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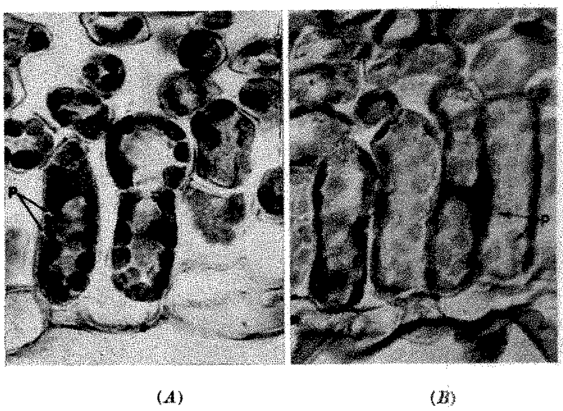
Staining Plastids in Chlorophyll-deficient Mutant Plants

Childers and McLennan[1] described collapsed, distorted palisade cells in a *viridis*-type chlorophyll mutant in alfalfa. A modification of Johansen's[2] quadruple stain was used for differentiating the plastids.

The technique is described here. Leaves were pre-fixed in Carnoy's fluid 6 : 2 : 2 for 30 sec., then fixed in Carpechenko's modification of Navashin's fluid. The tissue was dehydrated in Johansen's[2] tertiary butyl alcohol series. Sections were cut at 6μ and stained in the quadruple stain. The clearest differentiation was achieved by reducing the time in methyl violet to 7 min., while maintaining the time



(*A*) (*B*)

Fig. 1. Cross-sections of alfalfa leaves; (*A*) green leaf showing heavily stained plastids; (*B*) yellow leaf showing lightly stained but fully formed plastids. (× *c*. 575)

in fast green at 15 min. The nuclei were stained reddish-blue and the chloroplasts green. An advantage of this schedule is that de-staining is not critical and is accomplished by brief immersion in the de-staining solutions.

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Nothofagus Pollen in the London Clay

THE existence of southern families and genera with disjunct distributions, such as the Restionaceae and *Nothofagus*, has been used by many authors[1,2] as evidence supporting continental drift. Some[3] have stressed the importance of Antarctica as a Tertiary link between these presently disjunct areas without explicitly invoking the notion of drift. The presence of *Nothofagus* among the pollen and spores from Seymour Island, Antarctica[4], supports such a suggestion.

Bandulska[5] assigned a leaf from the British Tertiary to *Nothofagus*, but this isolated record received no further support. Recently, Erdtman[6] has recorded pollen of the Restionaceae from the British Early Tertiary. If supported by further evidence, these reports of tropical and southern hemisphere disjunct groups in the northern hemisphere temperate region might lead some to suggest that the Eurasian land mass may have served as a 'bridge' between the southern continents. A distinct Australian element exists among the genera represented by the fossil fruits and seeds of the London Clay ; the proportion of Australian genera (40 per cent), although less than that of the genera of the Malay Islands (73 per cent), is nevertheless considerable[7].

In the course of an investigation of the pollen of the (Eocene) London Clay, I have now found, in addition to that of the Restionaceae and Proteaceae, *Nothofagus* pollen. The slides were sent to Prof. G. Erdtman of Stockholm for confirmation. He stated (personal communication) that he found it "impossible, at the present state of our knowledge, to avoid referring the grains to *Nothofagus*"; he regards the grains as more similar to certain recent *Nothofagus* pollen grains than to any other pollen grains so far known. The pollen came from the London Clay of Alum Bay, Isle of Wight, and the grains, which are 5- to 6-aperturate, compare favourably with those of *N. obliqua* of the *menziesii* group (Figs. 1–5). Harris[8] has shown that the aperture number in this group varies between 5–9. Although the exine of *Nothofagus* pollen has generally been considered to be undifferentiated, Prof. Erdtman has shown (personal communication and ref. 9) that the pollen grains in *N. obliqua* have delicate infrategillar bacula. The grains from the London Clay also have very short bacula between the nexine and the spinule-bearing tegillum.

The possibility of mis-identification has been ruled out because the pollen of *Nothofagus* is distinctive and characteristic. Its occurrence could be accounted for either by: (1) the pollen being transported long distances by sea (for example, from Australasia along the Tethys), or (2) its presence here in cooler upland areas. The first possibility is highly unlikely, although pollen has been known to cover considerable distances, while the second would be in accordance with what is implied by Bandulska's discovery.

In addition to members of disjunct groups dealt with above, I have also obtained pollen of temperate families, for example Betulaceae and Ulmaceae, from the London Clay. These families have not hitherto been recorded as megafossils at this horizon. Brett[10,11]

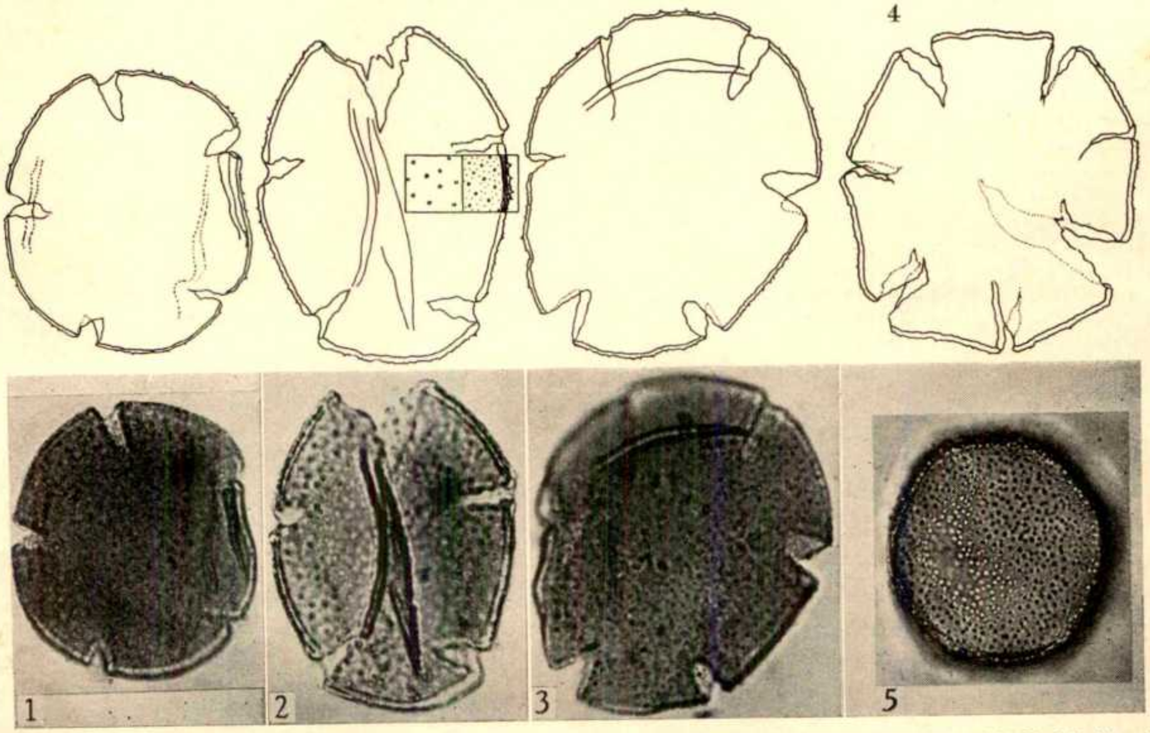

Fig. 1–3. Fossil *Nothofagus* (×1,000). Fig. 4. Recent *N. obliqua* (×1,000). Fig. 5. Recent *N. obliqua* (×750, photograph by Prof. Erdtman)

reported two temperate families (Cercidiphyllaceae, Fagaceae) preserved as petrified wood. These records of pollen and wood thus indicate the existence of a more temperate element (possibly an upland one ?) in the British Eocene flora, which is under-represented in the fruits and seeds of the well-known London Clay flora. Evidently the fossil pollen (and to a lesser extent the fossil woods) represents a broader picture of the entire flora, and it may be that the fruits represent predominantly the swamp association. Comparable results have been reported by Gray[12], who recorded the pollen of a number of temperate genera in the Eocene flora of Alabama, believed, on the evidence of megafossils, to have been tropical.

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Proteolytic Activity of Crude Stem Extracts from Normal and Tumour Tissues of Plants

IN an effort to characterize more fully the tumorous tissue (crown-gall) induced in plants by *Agrobacterium tumefaciens*, the proteolytic content in the neoplastic tissues of several plants was investigated. Although a variety of enzymes have been found associated with crown-gall[1], no reports are available concerning proteolytic activities of such tissue extracts. Recent findings of increased proteolytic activity in mammalian neoplastic tissues[2,3] indicate a need for measuring the relative quantity of proteases in comparable plant tissues.

Greenhouse plants approximately 25 cm. high were inoculated by needle-puncture into stem tissue with 18-hr. agar-grown cultures of virulent or attenuated strains of *A. tumefaciens*. Tumours developing on tobacco (*Nicotiana tabacum* var. Wisconsin) were harvested approximately 5 wk. post-inoculation; on sunflower (*Helianthus annus* var. Mammoth Russian) and tomato (*Lycopersicon esculentum* var. Marglobe), 3 wk. after inoculation; and on beans (*Phaseolus vulgaris* var. Black Valentine) and *Kalanchoe daigremontiana*, approximately 6 wk. post-inoculation. Comparable areas on control stems which had been punctured under sterile conditions or inoculated with the avirulent strain *IIBNV6* were harvested at the same time, and constituted the 'normal' tissue to be compared with 'tumorous' tissue in later tests.

The excised tissue materials were ground in a Lourdes model *MM* tissue-homogenizer for 75 sec. at approximately 16,000 r.p.m., at a temperature of 1° C. A ratio of two parts buffer–potassium chloride solution to one part wet weight of plant tissue was used in the extractions for possible soluble fractions containing active proteolytic material. At *p*H 5·5, the buffer solution consisted of 0·1 *M* acetate–1·6 *M* potassium chloride[4]; at *p*H 7·5, it consisted of 0·1 *M* *tris*(hydroxymethyl)aminomethane - maleate – 1·6 *M* potassium chloride. The homogenized tissue was decanted into 50-ml. stainless steel tubes and centrifuged at –1° C. for 50 min. at approximately 20,000*g* in a Servall Superspeed refrigerated centrifuge. Supernatants were then adjusted to desired *p*H and kept at 1° C. or frozen at –17° C. until used in the digestion-reaction experiments. The Lowry[5] method was employed for all O.D. readings at 750 mμ (Spectronic 20). The Folin–Ciocalteu reagent was used in a dilution of 1-to-1, and a 1-to-5 dilution of supernatant from the TCA (trichloroacetic acid)-precipitated samples was used to eliminate interference in the development of the colorimetric reaction.

A digestion-reaction mixture consisting of 3 ml. buffer, 2 ml. demineralized water, 2 ml. substrate (urea-denatured bovine serum albumin, fraction V), and 1 ml. tissue extract was contained in a glass-stoppered reaction flask, and controlled at 35° C. for 18 hr. A 4-ml. aliquot was removed for the Lowry determination, and the remaining 4 ml. was employed as a final *p*H check on the reaction solution, which had previously been adjusted to the desired *p*H (buffer, substrate, and extract were all at this *p*H before addition to the reaction mixture). 'Zero' time systems containing exactly one-half the quantities of the respective digestion reaction mixtures served as control (blank) comparator systems for all readings at 750 mμ. A 5 per cent TCA solution was added to each 'zero' system immediately before incorporation of the tissue extract. Spontaneous hydrolysis controls for substrate and tissue extracts were tested with the other test systems. Since a low level of spontaneous hydrolysis prevailed, no corrections were made for the final O.D. readings of the digestion reaction.

The results in Table 1 indicate that, with the exceptions of sunflower and Kalanchoe stem tumour tissues, all other plants showed a much higher level of proteolytic activity in tumorous tissue than in their normal counterparts. The O.D. readings for tumorous tissues consistently reflected, in part, the proteolytic levels in normal tissues. For example, when normal tobacco tissue extracts yielded O.D. readings of 0·080 and 0·023, their respective tumour tissue extracts resulted in O.D. readings of 0·345 and 0·152. The total and protein nitrogen results appear

Table 1. COMPARISON OF NITROGEN CONTENT AND PROTEOLYTIC ACTIVITY OF CRUDE STEM EXTRACTS FROM VARIOUS PLANTS AT *p*H 5·5

Plant	Normal tissue*			Tumour tissue†		
	O.D.‡ 750 mμ	Total nitrogen§ mgm./ml.	Protein nitrogen‖ mgm./ml.	O.D. 750 mμ	Total nitrogen mgm./ml.	Protein nitrogen mgm./ml.
Tobacco	0·073¶	0·111	0·009	0·335¶	0·742	0·182
Tomato	0·070	0·142	0·023	0·210	0·415	0·052
Bean	0·085	0·349	0·051	0·222	1·383	0·289
Sunflower	0·035	—**	—	0·038	—	—
Kalanchoe	0·022	—	—	0·022	—	—
Kalanchoe††	0·028	0·047	0·013	0·111	0·214	0·030

* A mixture of sterilely punctured tissues and tissues inoculated with attenuated strain *IIBNV6* of *A. tumefaciens*.

† A composite of extracts from tissues inoculated with *A. tumefaciens* strains *B6*, *A6K1* and 11156 (*ATCC*).

‡ Optical density readings based on aliquots from an 18-hr. digestion at 35° C.

§ Micro-Kjeldahl.

‖ Method of Markham (ref. 8).

¶ Extracts heated to 75° C. for 10 min. inactivated the proteolytic activity.

** Not done.

†† Leaf tissue.

Bacteriological examinations of the reaction mixtures at termination of digestion period indicated no gross contamination.

to correspond to the relative proteolytic activities of normal and tumour tissue extracts from a given plant. However, it should be noted that no absolute quantitative relationship exists between O.D. readings and nitrogen contents. Until such time as one can evaluate the relative ratios between non-enzymatic nitrogen (nucleic acid, nucleo-protein, etc.) and proteolytic-enzyme-nitrogen, the relationship can only serve as an indicator of higher or lower proteolytic activity within a single set of stem extracts.

Reports of protease activity in leaf tissue of tobacco[6] and more pronounced proteolytic activity in leaf than in stem or root tissue in soybean[7] prompted a study of the relative proteolytic activity in leaf-tumour and normal tissues. Kalanchoe (Table 1) was selected because of its low level of activity in stem-tissue extracts. The results indicate a definitely higher level of activity in leaf tumour than in the stem tumour, although no apparent difference seems to exist in the respective normal tissues. Experiments are now in progress which are intended to evaluate the proteolytic relationship of leaf to stem tumour extracts of all the plants used in this work.

In conjunction with the results in Table 1, there are three additional observations: although the recorded results are based on freshly extracted material, extracts of tobacco frozen for 64 days showed no diminution in proteolytic potency. Secondly, activators such as BAL (2,3 dimercaptopropanol) and cysteine-hydrochloride actually decreased the proteolytic response of both normal and tumour tissue extracts when incorporated into the digestion-reaction mixture. Weil *et al.*[9] reported a comparable finding for proteases of certain aerobic bacteria with cysteine. Lastly, preliminary studies with stem extracts from tobacco and tomato at *p*H ranges from 2·5–8·5 indicate that the best range for proteolytic activity of the crude extracts lies at approximately 4·5. Additional work with frozen extracts, extended *p*H ranges for the best activities, and the effect of activators is now in progress.

The majority of our initial stem work was carried out at *p*H 5·5. Later experiments at alkaline *p*H ranges indicated a low level of activity. It was thought advisable to find what relationship exists between the extracting *p*H and the digestion *p*H. The results in Table 2 indicate clearly that, although a tissue may be extracted at *p*H 7·5 (low activity), it still possesses an active proteolytic principle, which can be demonstrated by adjusting this extract to *p*H 5·5 (high activity) and carrying out the digestion at this *p*H. Conversely, if an active extract at *p*H 5·5 is adjusted to *p*H 7·5 and the digestion reaction conducted at this latter *p*H, there will be a very low level of proteolytic activity.

In conclusion, it can be noted that crude extracts from tumorous stem tissue of tobacco, tomato and bean possess greater proteolytic activity than their respective normal tissues; that no apparent difference in proteolytic activity between tumour and normal stem extracts from sunflower and Kalanchoe can be demonstrated; and that with an acid *p*H (5·5) of digestion there is greater proteolytic activity than with alkaline conditions.

Table 2. EFFECT OF RECIPROCAL ADJUSTMENT OF *p*H ON PROTEOLYTIC ACTIVITY OF EXTRACTS FROM NORMAL AND TUMOROUS TOBACCO STEM TISSUE

*p*H of extraction	*p*H of digestion reaction †	O.D.* — 750 mμ	
		Normal tissue	Tumour tissue
5·5	5·5	0·088	0·390
5·5	7·5	0·005	0·010
7·5	7·5	0·010	0·026
7·5	5·5	0·045	0·270

* Digestion at 35° C., for 18 hr.

† *p*H of extracts adjusted before addition to the digestion-reaction system.

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Fungal Colonization of a Developing Salt Marsh

DURING work on fungal colonization of a developing salt marsh at Gibraltar Point, Lincolnshire, National Grid reference *TF*555576 (a preliminary account of which has been given[1]), the rhizosphere populations were used as indicators of changing fungal populations in the different areas of colonization by phanerogams. The number of fungal isolations from mud uncolonized by higher plants and from the rhizospheres of the common salt marsh plants are given in Table 1. Five Petri dishes per sampling site were inoculated with approximately 0·5 gm. fresh mud each month that samples were collected. This mud was covered with mud extract agar[2] (Warcup soil plate technique[3]) using larger inocula than recommended in the original technique to compensate for the paucity of fungi in the muds as compared with normal soils.

The percentage differences from bare mud have been calculated to illustrate the increased fungal coloniza-

Table 1. NUMBERS OF FUNGAL ISOLATIONS FROM DIFFERENT SAMPLING SITES

	Sampling site	No. of isolations	No. of samples	Average isolations per sample	Percentage difference from bare mud †
	Bare mud	148	12	12·3	—
A	Low *Salicornia**	162	12	13·5	+9·8
	Lower *Spartina*	102	11	9·3	−24·4
B	Middle *Salicornia*	111	8	13·9	+13·0
	Upper *Spartina*	97	10	9·7	−21·1
C	High *Salicornia*	104	8	13·0	+5·7
	Halimione	90	6	15·0	+22·0
	Suaeda	113	7	16·1	+30·9
	Puccinellia	100	6	16·6	+35·0

* Plants grouped under *A* were sampled at the same horizontal level on the marsh: this applies equally to *B* and *C*.

† Percentage of difference = difference between number of fungi isolated from rhizospheres and from bare mud, calculated as a percentage of the bare mud isolations.

tion up the shore. These changes may reflect the greater amount of organic matter present as a result of increasing cover by higher plants, and may also reflect the reduced periods of inundation by the sea.

The exceptions to the general increase call for comment. The high *Salicornia* zone shows a smaller difference from bare mud than the lower *Salicornia* zone, and this is the more striking when compared with the results obtained from *Halimione*, *Suaeda* and *Puccinellia* at the same level. This may be a reflexion of the stunted growth of the high *Salicornia* plants.

The two *Spartina* sites show a marked decrease in numbers of isolations compared with bare mud, and again this is emphasized by the increases obtained from the rhizospheres of nearby *Salicornia* plants. Depression in fungal numbers under *Spartina* may be a concomitant of the growth habit of these plants: many, particularly those in the lower zone, grow in water-filled depressions where conditions frequently lead to the formation of black muds by the activities of sulphate-reducing and possibly other sulphide-producing bacteria. Similar black muds are also found in the upper *Spartina* zone, where there may be interpolated sandy horizons. A preliminary comparison of non-reduced and reduced *Spartina* rhizosphere muds indicates that it is the condition of the reduced muds (possibly the low Eh, or the toxic effect of accumulated sulphides) and not any inhibitory effect of the *Spartina* which causes the depression in fungal numbers.

Physiological aspects of this work are now being studied: ecological aspects are being prepared for publication.

Thanks are due to Prof. C. G. C. Chesters for his help in the preparation of this communication, and to Mrs. M. Turner and Mr. T. R. G. Gray for many fruitful discussions.

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Molybdenum and the Fixation of Nitrogen in *Myrica* Root Nodules

We have shown elsewhere[1] that nodulated plants of *Casuarina* and *Alnus*, grown under conditions which entail dependence on the fixation of elemental nitrogen, develop symptoms of severe nitrogen deficiency when deprived of molybdenum; this and other evidence obtained is wholly consistent with the view that, as in other biological nitrogen-fixing systems, molybdenum fulfils an essential role in the fixation proceeding in the root nodules of these non-legumes.

Work has now been extended to a third genus, *Myrica*. Young plants of *M. gale*, from seed sown in peat (experiment I) or purified sand (experiment II), were transplanted at the two-leaf stage into water-culture in 'Pyrex' beakers, the culture solution being prepared from reagents purified from molybdenum at the Long Ashton Research Station. All elements known to be essential were provided, except that no combined nitrogen was supplied (apart from an initial addition of 0·4 mgm. ammonium–nitrogen per plant in experiment I and 2·0 mgm. per plant in experiment II, to sustain the plants over the period of nodule formation), while molybdenum was omitted from certain cultures. Nodulation was secured by application to the root systems of an inoculum prepared by crushing nodules from field plants of *M. gale* in water. The plants were grown by daylight in the greenhouses of the Botany Department, University of Glasgow; other details of procedure were as described elsewhere[1].

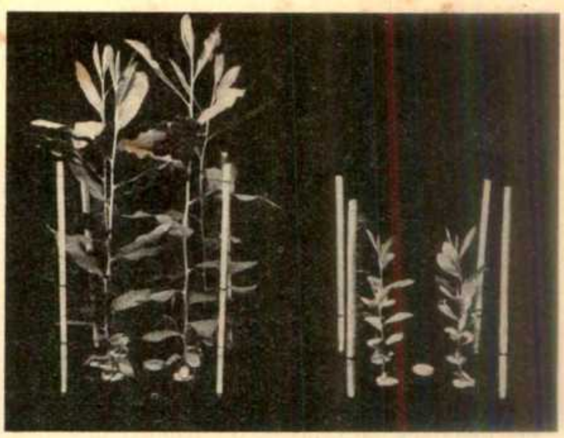

Fig. 1. Nodulated plants of *Myrica gale* after 12 weeks growth in water culture with (left) and without molybdenum supplied. (× 3/16). Photograph by Mr. R. Cowper

Nodules appeared some 2 wk. after inoculation, followed, after a further interval, by the commencement of vigorous growth in the plants supplied with molybdenum, indicative of the onset of fixation of nitrogen in the nodules. The plants not supplied with molybdenum lagged behind in their growth and developed symptoms of nitrogen deficiency; they were much reduced in stature and had small, pale-green leaves. Fig. 1 illustrates the disparity between the two types of plant at harvest after twelve weeks growth in water culture, while results based on the examination of some twenty plants in each treatment are provided in Table 1.

The results confirm the poor growth of the plants deprived of molybdenum and their deficiency in nitrogen. Fixation of nitrogen per plant, calculated by deducting from the total nitrogen per plant (Table 1) the mean nitrogen content of non-nodulated control plants, amounted to 15·0 mgm. (+Mo) and 1·5 mgm. (−Mo) in experiment I, and 13·7 mgm. (+Mo) and

Table 1

	Treatment	Mean dry weight per plant, mgm.*	Mean per cent nitrogen in plant dry matter	Mean total nitrogen per plant, mgm.	Molybdenum, p.p.m. in dry matter		
					Nodules	Shoot	Roots
Experiment I	+Mo	557	2·76	15·3	78·61	31·10	5·83
	−Mo	97	1·59	1·5	1·58	0·16	2·71
Experiment II	+Mo	510	2·92	14·9	273·70	52·34	33·94
	−Mo	150	1·95	2·9	0·93	0·18	0·97

* Statistical analysis of the dry weight data showed the differences between treatments to be significant at $P = 0·01$.

1·8 mgm. (−Mo) in experiment II. Relative to plant size, nodulation was actually better in the plants deprived of molybdenum, since nodule dry weight amounted to 14·4 per cent of whole plant dry weight in their case, but only to 7·1 per cent where molybdenum was supplied (mean values for the two experiments). Where molybdenum was provided, 383 mgm. of nitrogen was fixed per gm. of nodule dry matter formed, whereas the corresponding value for the molybdenum-deficient plants was only 83 mgm.; thus with molybdenum available, the nodule tissues were nearly five times more effective in fixation. Certain plants, deprived initially of molybdenum, were supplied with the element at an intermediate stage and showed obvious benefit within a few days.

The results in Table 1 also indicate the high accumulative capacity of the nodules for molybdenum. The higher molybdenum content, particularly in nodules and roots, shown in the plus-molybdenum plants of experiment II is possibly related to the circumstance that this experiment was conducted during the most favourable period climatically (May–August). In both experiments 0·05 p.p.m. of molybdenum was supplied in the culture solution where appropriate, some 17 per cent of the total supplied being absorbed by the plants in experiment I and 34 per cent in experiment II. Of the total molybdenum present in the plants at harvest, 24 per cent was in the nodules when molybdenum was supplied and 32 per cent when it was not.

The above results closely resemble those obtained in experiments with *Casuarina* and *Alnus*[1], and point to a special role for molybdenum in fixation in *Myrica* nodules also. A further finding of possible significance is that the two samples of field nodules of *M. gale* examined in this work proved to have molybdenum contents of 3·04 and 1·40 p.p.m. in the dry matter respectively; the latter value is of the same order as those for the nodules of plants cultured without molybdenum which fixed little nitrogen.

Technical assistance was provided by Miss K. McCormick and Mr. D. James.

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Gibberellin Action on Growth of Seedlings in the Absence of Photosynthesis

A MAJOR effect of gibberellins on growing seedlings is often an increased dry weight, especially in the shoots growing in the light[1]. These and other observations have frequently led to suggestions that this particular gibberellin effect may be related to an increased photosynthetic carbon-fixation. We have found, however, that in light-grown seedlings that are prevented (by darkness) from performing photosynthesis, gibberellic acid can increase their dry weight over water-control seedlings. The effect of gibberellic acid in these experiments thus cannot be attributed to either direct or indirect actions on photosynthetic fixation.

Wheat, *Triticum vulgare*, var. Thorne, was germinated on 'Lucite' bars wrapped with moist paper towels in covered germinating dishes at 23 ± 1° C. during a 16-hr. day (∼500 ft. candles white light) and 17·5 ± 1° C. during the night. Four days after sowing the seedlings were transferred to darkness permanently with or without gibberellic acid. Plants, the heights of which differed by less than 2 mm., were paired so that within each experiment the population receiving treatment with gibberellic acid was comparable with the corresponding water controls. Seedlings were sown on two pieces of Whatman No. 1 filter paper moistened with 25 ml. of solutions, all at about *p*H 5·7, in 9-cm. Petri dishes. The dishes were kept in light-proof wooden boxes in the same growth-room. The plants remained in complete darkness except for a few moments of low-intensity green light while watering. After 4 days in darkness the shoots and roots were detached from the scutella, washed, and dried in an oven at 60° C. at least overnight. All plant material from a given experiment was dried for 3 days and ground in batches to a fine powder in a Wiley Mill using a 40-mesh screen. Within each batch the ground material thus was mixed so that portions removed for analysis were homogeneous and representative of the entire group of shoots or roots after each treatment (treated with gibberellic acid or control). Total carbon was determined by a modified Pregl method[2]. Soluble carbohydrate was extracted in boiling water and analysed by the anthrone method[3]. Total nitrogen was determined by a micro-Dumas method[2]. Protein was determined by the method of Lowry *et al.*[4]. In all these analyses duplicate samples never differed by more than a few per cent.

The dry weights of 8-day-old seedlings which had been transferred to darkness with or without gibberellic acid when 4 days old are listed in Table 1. In all experiments the plants weighed approximately two to three times more at 8 days than they did at 4 days. The treated seedlings, compared to control seedlings, showed typical gibberellin effects: greater dry weight of the shoot, less dry weight of the root system, and in most cases a greater dry weight of the whole plant. Uptake of the gibberellic acid itself could not have been sufficient to cause these differences in dry weight. Treatment with sucrose during the 4 days in darkness did not significantly alter the results.

Chemical analyses of dried shoots and roots from one experiment are given in Table 2. The increases in total carbon, soluble carbohydrate, total nitrogen, and protein in the shoots treated with gibberellic acid

Table 1. DRY WEIGHT OF WHEAT SEEDLINGS TREATED WITH GIBBERELLIC ACID IN DARKNESS. SEEDLINGS WERE 4 DAYS OLD WHEN TREATED, 8 DAYS OLD WHEN DRIED

Exp.	Sucrose concentration (per cent)	Concentration of acid (*M*)	No. of plants	Dry weight per plant (mgm.)		
				Shoot	Roots	Total
1	0	0 3×10^{-4}	44 44	8·76 9·67	4·87 4·44	13·63 14·11
2*A*	0	0 3×10^{-4}	48 48	7·76 9·07	4·92 4·69	12·68 13·76
2*B*	1	0 3×10^{-4}	48 48	8·25 8·95	5·10 4·70	13·35 13·65
2*C*	2	0 3×10^{-4}	48 48	7·25 8·51	4·73 4·71	11·98 13·22
3	0	0 3×10^{-4}	80 80	7·17 7·81	4·95 4·24	12·12 12·05

Table 2. CHEMICAL ANALYSIS OF SEEDLINGS TREATED WITH GIBBERELLIC ACID IN DARKNESS*

Analysis	Content and concentration	Shoot		Root system		Total	
		Water controls	Treated	Water controls	Treated	Water controls	Treated
Total carbon	mgm./plant	3·34	3·80	2·17	2·01	5·51	5·81
	percentage of dry weight	43·1	41·9	44·1	42·9	43·5	42·2
Soluble (boiling water) carbohydrate	mgm./plant	1·88	2.02	1·03	0·94	2·91	2·96
	percentage of dry weight	24·3	22·3	21·0	20·0	22·9	21·5
Total nitrogen	mgm./plant	0·307	0·377	0·142	0·127	0·449	0·504
	percentage of dry weight	3·96	4·16	2·88	2·71	3·54	3·66
Protein	mgm./plant	0·98	1·09	0·62	0·61	1·60	1·70
	percentage of dry weight	12·6	12·0	12·6	13·0	12·6	12·4

* Analyses were performed on finely ground dried material from plants in exp. 2*A* in Table 1

were approximately proportional to the dry weight increase.

Since the plants were not exposed to light during the time they were in contact with gibberellic acid, there could be no photosynthesis while the acid was increasing the dry weights. The gibberellic acid effects therefore could not be related in any way to photosynthetic fixation. The endosperm reserves plus photosynthesis that occurred before treatment with gibberellic acid were sufficient to produce the dry weight increases, since the addition of sucrose did not seem to greatly alter the results. The proportional increases in total carbon, soluble carbohydrate, total nitrogen, and protein suggest a general acceleration of growth under conditions in which neither carbon nor nitrogen is deficient. This effect of gibberellic acid treatment in darkness is on seedling growth proper, and has no relation to any action of the acid in breaking dormancy, because the plants were not treated with the acid until 4 days after sowing, when they were several cm. high.

These results are consistent with the finding that gibberellic acid does not increase directly either the rate or the pattern of photosynthetic $^{14}CO_2$ fixation in short-time experiments[5]. They also weaken suggestions that dry weight increases from treatment with gibberellic acid must necessarily be attributed to indirect effects on photosynthetic fixation. The fact that leaves treated in the light do not increase in chlorophyll content even though they increase in area[6] is further evidence against the theory that fixation is increased because of a greater photosynthetic area[1,7]. In his argument that fixation is increased by an accelerated mobilization of photosynthate, Alvim[8] apparently has equated photosynthesis with 'net assimilation rate'. Although we do not question that gibberellic acid can increase net assimilation, we do question that such increased net assimilation must necessarily be related to an increased photosynthetic fixation. Photosynthesis is but a part of the complex of many anabolic and catabolic processes which together determine net assimilation. Thus we are inclined to believe that an increase in dry-matter content in plants treated with gibberellic acid in light does not necessarily result from either direct or indirect increases in photosynthetic fixation, but can be attributed to altered utilization of photosynthate and other metabolites, regardless of their origin. Coulombe and Paquin[9] reported transient changes in transpiration as well as both respiratory and photosynthetic net gaseous exchanges of carbon dioxide after treatment of tomato leaves with gibberellic acid. To our knowledge, however, no one has demonstrated that gibberellin treatment can increase photosynthetic fixation, that is, the light-dependent reduction of carbon dioxide in green tissues. Products derived from photosynthesis undoubtedly are involved in the metabolic processes which underlie the response to gibberellic acid of light-grown plants, just as they are involved in other aspects of plant metabolism. But at the present time it seems doubtful whether gibberellic acid generally increases shoot dry weight by increasing photosynthetic fixation.

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GENETICS

Non-disjunction of the Sex-chromosomes in the Male of *Drosophila melanogaster*

THE discovery that certain human abnormalities have a chromosomal basis has stimulated renewed interest in the phenomenon of non-disjunction. Theoretically, non-disjunction may occur in both the male and female germ cells at either the first or second meiotic division and may give rise to a considerable variety of non-disjunctional types in the progeny. In practice, only non-disjunction occurring at the first meiotic division in oocytes has so far been studied in *Drosophila*. The present communication reports the occurrence of spontaneous non-disjunction of the sex-chromosomes in *Drosophila* males.

The scheme used involves *brown-variegated*, bw^{VA}, an inversion originally obtained by H. J. Muller in the second chromosome which, in combination with the sex-linked gene *vermilion*, *v*, produces a characteristic yellow eye colour. This position effect of bw^{VA} is suppressed by the presence of an extra *Y*-chromosome, so that males of the genotype *XYY* and females of the genotype *XXY* exhibit the bright red eye colour normally associated with *v*. With the aid of this scheme, a search was made for the presence of *XXY* and *XYY* individuals in the progeny of normal *XY* males and *XX* females. Markers on the paternal and maternal *X*-chromosomes were used to decide whether non-disjunction had occurred in the father or the mother. The presence of an additional *Y* in the exceptional individuals was confirmed by progeny tests.

In addition to the already known types of non-disjunction, the following new ones occurred:

(1) *XXY* females with one paternal and one maternal *X*, which thus must have arisen through fertilization of a disjunctional egg by a non-disjunctional *XY* spermatozoon.

(2) *XYY* males with a maternal *X*. These probably arose through non-disjunction at the second meiotic division in the male. An alternative interpretation cannot be excluded, however. Lamy[1] found a small proportion of patroclinous *XXY* daughters with detached *X*'s among the progeny of attached-*X* females and attributed their occurrence to equational doubling of the *X*-chromosome in the sperm. She did not consider the possibility of non-disjunction at the second meiotic division. The frequency of *XXY* females in her experiments was considerably higher than that of *XYY* males in those reported here. If Lima-de-Faria's[2] observation that, in grasshopper chromosomes, euchromatin divides more rapidly than heterochromatin, should apply also to the chromosomes of *Drosophila*, equational doubling of the *Y* may take place very rarely or never.

The results obtained in this preliminary observation do not yet permit accurate quantitative estimates of the frequencies with which the various types of non-disjunction take place. The approximate frequencies, however, appear to be of the order of 0·01 per cent for type (1) and 0·004 per cent for type (2). The accurate frequencies as well as a possible influence of parental age will be determined in a more comprehensive investigation.

P. J. Kelsall

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[1] Lamy, R., *Drosophila Information Service*, **23**, 91 (1948).

[2] Lima-de-Faria, A., *Science*, **130**, 503 (1959).

Determining Mating Type in *Neurospora* without Crossing Tests

Strains of *Neurospora crassa* belonging to either *A* or *a* mating type are morphologically indistinguishable. Mating type determinations are usually made by observing whether perithecia occur after crossing strains of unknown mating type to *A* and *a* tester strains. The tests are usually run either in two series of culture tubes or in pairs of Petri dishes. The use of tester strains on a large scale, however, may become laborious, ambiguous tests have to be re-run, and infertility sometimes confuses the results.

An alternative method which has been found useful for sex-testing numerous progeny from crosses employs a strain unable to grow on complete medium at elevated temperatures due to a mutant allele very closely linked to the mating-type locus. This strain, *unknown* (55701) (*un*), grows slowly on minimal medium at 25° C., but is stimulated by addition of acetate. At 35° C., however, no growth occurs on any medium yet tried[1]. *un* has shown very close linkage with mating type in tetrad analysis, no recombination between these two loci having as yet been obtained (Table 1). Since no recombination has been obtained, all the derived strains of *un* at present known are of the same mating type, *a*, as the one in which this mutation was originally induced[2].

Table 1. Crosses showing Linkage of *un*(55701) to *sex*

Cross	No. of asci	Recombination between *un* and *sex**
1*A* × *un a*	38	None (4)
vis A × *un a*	16	None (4)
pyr(68902) *A* × *un a*	22	None (4)
74*A* × *un ad*-5 *a*	94	None
ad-5 *A* × *un a*	21	None
frost A × *un a*	40	None
frost leu-3 *A* × *un ad*-5 *a*	56	None
leu-3 *A* × *un a*	18	None
leu-3 *A* × *un ad*-5 *a*	30	None
Total	335	

* Fiducial upper limit of recombination frequency between *un* and *sex* at the 5 per cent level is 0·011.

In practice, the *un* allele is incorporated into one of two parental stocks prior to crossing these two stocks with each other. Segregants isolated from the cross either by dissection or by sorbose plating[3] are placed in culture tubes of complete medium and incubated at 25° C. until initial growth verifies isolate viability. Complete media should include 333 mgm. sodium acetate per litre to stimulate growth of *un* at 25° C. As soon as initial growth occurs the tubes are transferred to 35° C. for 24–48 hr. Tubes should be placed at 35° C. only after initial growth is observed, because germinating *un* ascopores or incipient *un* mycelial transfers die if held excessively at 35° C.

The tubes which show no further growth at 35° C. contain the *un* allele and are, with high probability, of the same mating type as the *un* parent used in the cross. The tubes showing completed growth at 35° C. contain the *un*$^+$ allele and are, with high probability, of opposite mating type. All tubes are then returned to 25° C. where the *un* isolates will complete growth. No crossing tests and no subculturing are necessary.

Alternatively, scoring may be done exclusively at 25° C. by omitting acetate from the medium. In this case the *un* allele is readily distinguishable by markedly slow growth, provided no other segregating markers give sub-wild growth-rates.

The *un* segregation method is especially convenient when large numbers of segregants have to be scored for mating type. Investigations in which chromosomal aberrations such as translocations are being followed or where the mating type locus itself, for example, may require the sex-testing of numerous progeny. In such cases it seems more efficient to incorporate *un* into a parental stock and follow *un* segregation by means of temperature variation or slow growth-rate than to cross each isolate to two tester strains.

The accuracy of the method depends on the strength of the linkage between the *un* and *sex* loci. So long as this linkage holds, segregant mating types may be identified by the *un* phenotype. The method may be used with both visible and nutritional mutants provided none possesses the *un* type of temperature sensitivity.

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University of Georgia,
Athens, Georgia.

[1] Houlahan, M. B., and Mitchell, H. K., *Arch. Biochem.*, **19**, 257 (1948).

[2] Beadle, G. W., and Tatum, E. L., *Amer. J. Bot.*, **32**, 678 (1945).

[3] Newmeyer, D., *Genetics*, **39**, 604 (1954).

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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, June 12

Institute of Cancer Research: Royal Cancer Hospital (in the Conference Room of the Chester Beatty Research Institute, Fulham Road, London, S.W.3), at 3 p.m.—Prof. Antoine Lacassagne: First Sir Ernest Kennaway Memorial Lecture.

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. M. Munitz: "The Logic of Cosmology".

Tuesday, June 13

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

Wednesday, June 14

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. Honor B. Fell: "Tissue Culture in Experimental Pathology".*

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. N. T. Gridgeman: "The Sensory Appraisal of Food".

Women's Engineering Society (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Annual General Meeting.

Thursday, June 15

Institution of Mining and Metallurgy (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 4 p.m.—Annual General Meeting. 5.15 p.m.—Mr. A. R. O. Williams: Presidential Address.

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. J. A. Shannon: "The National Institutes of Health—Programmes and Problems".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. V. C. A. Ferraro: "Magnetohydrodynamics".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Demonstrator in Organic Chemistry—The Registrar, University College of North Staffordshire, Keele, Staffs (June 14).

Assistant Lecturer in the Geology Department to teach Mineralogy—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 16).

Assistant Lecturer or a Lecturer in Applied Mathematics—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (June 17).

Assistant Lecturer (with an honours degree or its equivalent, and a special knowledge of crop and grassland production) in the Department of Agriculture—The Registrar, University College of North Wales, Bangor, North Wales (June 17).

Lecturer (well qualified academically, with teaching, and preferably some industrial experience) in Organic Chemistry at Hendon Technical College—Clerk to the Governing Body, Town Hall, Hendon, London, N.W.4 (June 17).

Lecturer (with a good honours degree in mathematics and interested in mathematical statistics or in economics with statistics) to teach Mathematics and Mathematical Statistics to Institute of Statistics and economics degree finals standard—The Director of Education, The Polytechnic, 309 Regent Street, London, W.1 (June 17).

Research Demonstrator (graduate in geology), to engage in research; and a Research Assistant (graduate in geology or chemistry, or graduating this year), to assist the Professor of Geology in a study of the structure and composition of fossil and recent shells—The Registrar, University College of Swansea, Singleton Park, Swansea (June 17).

Research Students (with the degree of Bachelor or Master of a British University other than Manchester) in Science—The Registrar, The University, Manchester 13 (June 17).

Senior Lecturer/Lecturer (qualified in applied mathematics) in Mathematics—The Registrar, University College of North Staffordshire, Keele, Staffs (June 17).

Lecturer in the Department of Physiology—The Secretary, The University, Aberdeen (June 18).

Lecturer (Grade II) in Theoretical Physics—The Registrar, The University, Bristol 8 (June 19).

Senior Lecturer in Mathematics or Applied Mathematics at the University of Natal, Durban, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, June 20).

Graduate in Mechanical Engineering or Applied Mathematics, to conduct research on an aspect of vibration concerned with transport—The Secretary, University College, Gower Street, London, W.C.1 (June 24).

Lecturer in Crop Production—The Secretary, Wye College (University of London), near Ashford, Kent (June 24).

Lecturer in Metallurgy—The Registrar, The Manchester College of Science and Technology, Manchester 1 (June 24).

Assistant Lecturer (preferably with qualifications in sedimentology and an interest in recent sediments) in the Department of Geology—The Registrar, University College of Wales, Aberystwyth (June 25).

Assistant Lecturer (preferably applied mathematician) in the Department of Mathematics—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (June 30).

Lecturer in Philosophy at Monash University, Clayton, Victoria, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

Lecturer (with a science degree with zoology as a major subject, followed by postgraduate experience leading to a higher degree and/or to published work) in Zoology at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

Research Assistant (with special interests in climatology and meteorology, and ecology or physical geography) in Geography—The Secretary, The University, 38 North Bailey, Durham (June 30).

Research Fellow (honours graduate in chemistry, biochemistry or agricultural chemistry) in the Department of Soil Science, to study chemical changes in forest litter—The Secretary, The University, Aberdeen (June 30).

Biochemist (with experience in tracer techniques) in the Division of Experimental Biology and Virology—The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2 (July 1).

Chair of Philosophy in the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 3).

Lecturer (with a good honours degree) in Philosophy at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 5).

Chief Officer (with a recognized degree or equivalent qualification in botany, agricultural botany or agriculture, and preferably experience in laboratory organization and staff management) of the Official Seed Testing Station—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (July 7).

Lecturer in Psychology at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, July 7).

Senior Lecturer in Psychology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 15).

Senior Lecturer (with experience in concrete technology and structural design) in Civil Engineering at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 25).

Chair of Organic Chemistry; and Chair of Chemistry in the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 31).

Reader in Mathematics at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 31).

Senior Lecturer-in-Charge of the Department of Pharmacy, University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 31).

Lecturer (with a degree) in Agricultural Chemistry (Animal Nutrition); and a Lecturer (with a degree or diploma) in Agriculture (Economics)—The Principal, Harper Adams Agricultural College, Newport, Shropshire.

Postdoctoral Fellow in Organophosphorus Chemistry—The Secretary, Department of Chemistry, King's College (University of London), Strand, London, W.C.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Northampton College of Advanced Technology, London. Report of the Governing Body for 1959–60. Pp. 19. Examination Successes and Prizes, Session 1959–60. Pp. 27. (London: Northampton College of Advanced Technology, 1961.) [203

Ministry of Education: Central Advisory Council for Education (Wales). Technical Education in Wales. Pp. ix+146. (In English and Welsh). (London: H.M. Stationery Office, 1961.) 7*s*. net. [203

Imperial College of Science and Technology. Postgraduate Courses, 1961–62. Pp. xii+114. (London: Imperial College of Science and Technology, 1961.) [213

Why a Census: Why do we Need It? How is it Carried Out? What Questions will be Asked? Pp. 31. (London: H.M. Stationery Office, 1961.) 1*s*. net. [223

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Institute of Petroleum. A Glossary of Petroleum Terms. Edited by George Sell. Third edition, revised and enlarged. Pp. vii+39. (London: Institute of Petroleum, 1961.) 5*s*. [233

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Anthropology and History. By Prof. E. E. Evans-Pritchard. (A Lecture delivered in the University of Manchester with the support of the Simon Fund for the Social Sciences.) Pp. ii+22. (Manchester: The University Press, 1961.) 3*s.* 6*d.* net. [273

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Bulletin of the British Museum (Natural History). Geology. Vol. 5, No. 3: Notes on Eocene Tarsioids and a Revision of some Necrolemurinae. By Elwyn L. Simons. Pp. 43–69+plates 12–14. 12*s.* Vol. 5, No. 4: Fractured Chert Specimens from the Lower Pleistocene Bethlehem Beds, Israel. By J. Desmond Clark. Pp. 71–90+plates 15–23. 17*s.* 6*d.* (London: British Museum (Natural History), 1961.) [273

Department of Scientific and Industrial Research: Geological Survey and Museum. British Regional Geology. The Hampshire Basin and Adjoining Areas. By C. P. Chatwin. Third edition. Pp. iv+99+8 plates. (London: H.M. Stationery Office, 1960.) 5*s.* net. [273

Report of the Rugby School Natural History Society for the year 1960. Pp. 16. (94th issue.) (Rugby: George Over, Ltd., 1961.) [273

Sir John Cass College. Report, Session 1959–1960. Pp. 25. (London: Sir John Cass College, 1961.) [273

Privy Council. Medical Research Council Report Series, No. 298: Physiological Responses to Hot Environments. Compiled by R. K. Macpherson. (An account of work done in Singapore, 1948–1953, at the Royal Naval Tropical Research Unit, with an appendix on preliminary work done at the National Hospital for Nervous Diseases, London.) Pp. xv+323. (London: H.M. Stationery Office, 1960.) 35*s.* net. [273

Planning, Vol. 27, No. 451 (27 March, 1961): Four Approaches to the European Problem. By Miriam Camps. Pp. 81–108. (London: Political and Economic Planning, 1961.) 3*s.* 6*d.* [283

Other Countries

Institut National pour l'Étude Agronomique du Congo. Carte des ols et de la Vegetation du Congo et du Ruanda-Urundi. Le Tableau Général Classification des Séries de Sols, ci-joint, est à annexer à la Farde 15—Kasai. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1961.) [203

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Connecticut Agricultural Experiment Station, New Haven. Bulletin No. 624: Chemical Control of Weeds and Brush Along Roadsides. By John F. Ahrens. Pp. 32. Bulletin No. 625: Commercial Feeding Stuffs, 1958—Report on Inspection. By H. J. Fisher. Pp. 139. Bulletin No. 626: The Climate of Shade: a Tobacco Tent and a Forest Stand Compared to Open Fields. By Paul E. Waggoner, A. Boyd Pack, and William E. Reifsnyder. Pp. 39. Bulletin No. 627: The Storage of Moisture in Connecticut Soils. By David E. Hill. Pp. 30. Bulletin No. 628: Commercial Fertilizers—Report for 1959. By H. J. Fisher. Pp. 80. Bulletin No. 629: The 62nd Report on Food Products; and the 50th Report on Drug Products, 1957. By H. J. Fisher. Pp. 84. Bulletin No. 630: The Pedigree of Connecticut 49 Shade Tobacco. By Seaward A. Sand. Pp. 12. Bulletin No. 631: Laboratory Studies on House Fly Populations. 3: The Influence of Insecticides on Population Trends. By R. L. Beard. Pp. 22. Bulletin No. 632: Mosquitoes in Connecticut. By Robert C. Wallis. Pp. 30. Bulletin No. 633: Commercial Feeding Stuffs, 1959—Report on Inspection. By H. J. Fisher. Pp. 133. Bulletin No. 634: Plastic Mulching: Principles and Benefits. By Paul E. Waggoner, Patrick M. Miller and Henry C. De Roo. Pp. 44. (New Haven, Conn.: Connecticut Agricultural Experiment Station, 1960.) [213

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World Health Organization. Specifications for Pesticides: Insecticides, Rodenticides, Molluscicides, Herbicides, Auxiliary Chemicals, Spraying and Dusting Apparatus. Second edition. Pp. 523. 30 Swiss francs; 50*s.*; 10 dollars. Technical Report Series. No. 205: Expert Committee on Malaria—Eighth Report. Pp. 50. 2 Swiss francs; 3*s.* 6*d.*; 0.60 dollars. No. 211: Expert Committee on Addiction-Producing Drugs—Eleventh Report. Pp. 16. 1 Swiss franc; 1*s.* 9*d.*; 0.30 dollars. [233

Institut Royal des Sciences Naturelles de Belgique. Mémoires. No. 146: Further Conifers of the Pinaceae from the Wealden Formation of Belgium. By Kenneth Leonard Alvin. Pp. 39+10 plates. Deuxième Série. Fasc. 63: Révision Systématique et Biogéographique des Tabanidae (Diptera) Paléarctiques. Vol. 1: Pangoniinae et Chrysopinae. Par Marcel Leclercq. Pp. 77+10 planches. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1960.) [233

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TECHNICAL ASSISTANCE TO OVERSEAS COUNTRIES

THE Department of Technical Co-operation Bill, which received its second reading in the House of Commons on April 25, gives effect to the Government's decision, announced by the Prime Minister on March 11, to create a separate Department, under the charge of a Minister, to co-ordinate responsibilities and functions in regard to technical assistance to overseas countries which are at present divided between the Foreign Office, the Commonwealth Relations Office and the Colonial Office. The Minister will be known as the Secretary for Technical Co-operation, and his Department will be concerned with technical assistance, defined in the White Paper issued in March as "training in the United Kingdom and overseas, the provision of experts, administrators and other professional men and women, the provision of advisory technical and consultant services and expert missions, and the supply of equipment for training, demonstration, pilot schemes or surveys". It will not, as Sir Edward Boyle, Financial Secretary to the Treasury, emphasized at the outset of the debate, be responsible for the provision of capital, which will remain with the overseas Departments.

Sir Edward Boyle, who was moving the second reading of the Bill, said that at the beginning of 1960, about 47,500 overseas students were receiving training of some kind or other in Britain, of whom about 2,000 held some form of United Kingdom scholarship, and about 2,500 trainees have come to Britain under the technical assistance arrangements of the Colombo Plan, while 380 British experts have been employed under the Plan in south and south-east Asia. Altogether, some 6,000 professionally qualified officers were recruited in Britain to serve overseas Governments during the years 1955–60, although the table given in the White Paper* shows that the annual figure has declined from 1,467 in 1956 to 816 in 1960. Some of this educational assistance is at present handled by the Ministry of Education, the British Council, the Commonwealth Scholarship Commission and other bodies, and Sir Edward said that there is no intention of disturbing such arrangements where they were working well, though some marginal adjustment of responsibility may be necessary. In particular, he quoted the recruitment of specialists, such as biologists, as being handled more easily and efficiently if centralized in one department, irrespective of where the specialist was to serve. Sir Edward, in reply to a question about social services, to which the Bill refers, said that provision of medical services would certainly be included, as well as provision of experts to advise on starting a new industry or the provision of skilled administrators in every field.

An important responsibility of the new Department would be the administration of the scheme described in the White Paper last October for the continued employment of overseas officers. This scheme is expected to cost about £12 million a year. The responsibility for the transfer, promotion and discipline of the overseas service in dependent territories, however, would remain with the Colonial Secretary. The new Department will be concerned with the technical assistance which the United Kingdom provides through the United Nations, in particular the United Nations Expanded Programme of the Technical Assistance and Special Fund, and will take over the present work of the Technical Assistance Recruitment Unit of the Ministry of Labour, which recruits in Britain for service with the United Nations. It will also deal with technical assistance under the Colombo Plan and under the newly formed Special Commonwealth African Assistance Plan. It will be responsible, on behalf of the United Kingdom, for any technical assistance discussions in the Development Assistance Group and in the new Organization for Economic Co-operation and Development, and for the provision of technical assistance to a number of Middle East countries under the Central Treaty Organization arrangements and through the Middle East Division. The work of the British Council in education will retain its full importance, and the Ministry of Education will continue to be responsible for Britain's contribution to the United Nations Educational, Scientific and Cultural Organization.

Sir Edward anticipated that the staff of the new Department would number about 1,000, initially by transfer of posts and staff from existing overseas Departments and including about 550 from the directorates of the Overseas Services and the Overseas Geological Surveys, and its vote was estimated to be about £30 million, including certain expenditure now financed out of Colonial Development and Welfare Funds, but not involving any new expenditure. In replying on the debate, Sir Edward Boyle pointed out that British expenditure on technical aid had increased from £75 million in 1957–58 to about £100 million in 1958–59 and about £150 million in 1959–60, with a further £100 million of private investment in less-developed countries. He emphasized that there is no intention of changing the Government's policy in this respect, and that British commitments to the Colonies and under-developed countries are likely to increase. The Colonial Secretary is still responsible for more than thirty Colonial territories, and Sir Edward agreed that one of the dangers to be watched in operating the new Department is that of excessive consultation with the existing overseas departments, hindering prompt decisions. He also agreed as to the importance of an adequate supply of managers and engineers to enable the power stations and factories, etc., provided by technical aid, to be operated, that the relief of poverty in under-developed lands is one of the greatest moral challenges of our times, and as to the importance of bringing more people to Britain for training in skills which would be valuable in their own country when they returned ; but he pointed out also that we should not think entirely of engineering and technical skills, and that it is important to make greater use of expert knowledge and skill.

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* Technical Assistance from the United Kingdom for Overseas Development. Pp. 43. (Cmnd. 1308.) (London: H.M.S.O. 1961.) 2s. net.

The scale of the technical assistance already provided from the United Kingdom for overseas development is more fully displayed in the White Paper, which was freely quoted in the debate. This document includes among its annexes an impressive list of advisory committees and councils under the Colonial Office, and also of technical and other institutions in the United Kingdom which provide services overseas. Much of this service, like the contribution of private enterprise, clearly could not be included in the estimate of £150 million (£125 million bilaterally and £25 million multilaterally) given in the White Paper. The work of many of these bodies, like that of various advisory committees on research or the Colonial Development Corporation, is already known to many readers of *Nature*, though the White Paper gives some figures conveniently for reference. Thus, by the end of June 1960, the United Kingdom spent or formally committed for technical assistance to the countries of south and south-east Asia some £6,800,000, and the Government has declared its willingness to spend up to £9,000,000 for technical assistance under the Colombo Plan in the period April 1956–March 1963. Of this, about £1,585,000 was spent on the provision of some 380 experts and about £1,800,000 on equipment for training and research. About £6 million was contributed in 1960 to the various United Nations Technical Assistance activities—about double the amount contributed in 1959.

On the contribution of private bodies in the United Kingdom, the White Paper gives no information; but it refers with appreciation to the formation in 1958 of Voluntary Service Overseas, which will enable young men and women from the United Kingdom to serve as teaching and social welfare auxiliaries in the Commonwealth. This organization was welcomed in the debate in the House of Commons, though Mr. R. E. Prentice suggested that the new Ministry should initiate something on the lines of the Peace Corps started more recently by the President of the United States. Much more stress, however, had been laid on this aspect in a debate in the House of Lords on February 22, which dealt moreover with the fundamental purpose of technical aid—that of enabling the under-developed countries to raise their economies to a point where they could survive on their own. It is a combination of capital and technical skill that is required, and while these under-developed countries are at present receiving something like 4,000 million dollars a year, more than half of which is found by the United States, the United Nations Special Fund has only been able to support some 70 of the 250 projects for which it has received applications.

Lord Grantchester, after a tribute to the part Britain has played in establishing universities in its dependent or emerging territories, suggested that there is room for more co-ordination between the universities in dealing with overseas students. He also urged in particular the need for more individual advice for the students and the Governments of the countries from which they come. His suggestions for the establishment of training centres in London, where a limited number of students could obtain satisfactory training prior to entrance to a university, and for an alternative to the university course for overseas students, with particular emphasis on administration, seem worthy of consideration. Moreover, they are of the type where the initiative may well lie more fittingly with the Department of Technical Co-operation than with the Ministry of Education.

The Marquess of Lansdowne, who spoke for the Government, pointed out that if the effectiveness and volume of aid are to be increased, donor countries must encourage their experts—technicians, teachers and medical men—to serve where they are so vitally needed in the less-developed countries, concentrating on the projects of real value to the recipients and providing training facilities in their own countries. It follows from this that a Department of Technical Co-operation should be closely concerned with the establishment of the conditions which make it as easy and attractive as possible for professional men and women, as well as technicians, to serve overseas. This aspect was also urged strongly by the Bishop of Chichester. Suggesting first that it might be worth while examining our methods of rendering technical assistance in the light of ten years experience, he stressed the need especially to consider technical assistance in the context of educational assistance. The economic resources of a country cannot be developed fully without a continuing supply, from within that country, of technicians, administrators, and leaders of all sorts. He emphasized the need for public opinion to recognize and support the overriding need for teachers and educational assistance generally. He hoped that authorities in schools and universities will urge this opportunity on members of staff and students, and that technicians and scientists from industry may also contribute to such teaching for a few years.

Obviously, this is the point which the Duke of Edinburgh pressed in his Graham Clark Lecture to the Institutions of Civil, Mechanical and Electrical Engineers on April 13, and in the House of Lords debate Baroness Summerskill suggested that the universities should be assisted to establish supernumerary appointments so as to enable staff to go overseas. The Earl of Perth, who replied on the debate, referred particularly to the work of the Inter-University Council for Higher Education Overseas, and said that some £17 million from Colonial Development and Welfare Funds have been used for research and higher education; but the 30,000 students from the Commonwealth in British universities and technical colleges represent a further hidden contribution of some millions of pounds, including a very considerable effort in medical training. Scientists and technologists are already playing an important part in this effort; indeed, one theme of the Duke of Edinburgh's Graham Clark Lecture was that engineers have a responsibility to help in improving the material welfare of the poorer countries. It should also be remembered that a number of leading scientists have for many years been urging this responsibility and its implications on their colleagues.

At the Committee stage of the Bill on May 10, Sir Edward Boyle said that the Overseas Research Council will not itself be responsible for expenditure from Exchequer funds, but the new Department will, in general, take over research work in the United Kingdom which is now financed by the three overseas Departments, and in particular it will take over certain research work now financed from Colonial Development and Welfare Funds. Sir Edward also agreed as to the importance of the new Department having ample sources of information and that it is desirable that the new Department, which will have a small but important information branch, should give some publicity to its technical assistance work. Sir Edward's remarks did not, however, make the exact position of the Overseas Research Council very clear, and it is essential that the position should be carefully watched.

The debate on the Department of Technical Co-operation Bill scarcely touched on the aspect of capital investment, but some speakers were not entirely satisfied that capital aid is not within the Department's scope. Nevertheless, the debate showed that the interrelation of capital aid and technical aid, in the sense of personal service, is fully recognized, more so, perhaps, than in Dr. V. K. R. V. Rao's eighteenth Montague Burton Lecture on International Relations at Leeds last November*. In this lecture, on "International Aid for Economic Development: Possibilities and Limitations", which has recently been published, Dr. Rao, after reviewing the various attempts which have been made to accelerate the economic development of the backward territories with international and bilateral aid, suggests that all such aid should be internationalized and channelled through a modified International Development Association, for which he outlines a revised plan. He believes that such a scheme would deal automatically with the limitations at present placed on international aid—by resources, capacity for repayment or for absorption, or by the risk of demoralizing the recipient countries so that dependence replaces self-reliance.

The fundamental condition stressed by Dr. Rao, however, is a change in psychology, the recognition on the part of nations that they are all parts of the world, and that their citizens are all members of the human race. The establishment of a Department of Technical Co-operation certainly recognizes that need and should help to co-ordinate the British contribution both to British dependent territories and elsewhere. What is also required, however, is the clear understanding and firm support of those who will provide the expert knowledge, and for that reason alone the Department when it is established should receive careful attention by scientific and professional workers of all kinds, both individually and through their professional associations. There is nothing in the White Paper to suggest that weaknesses already apparent in regard to overseas research are recognized, and while the magnitude of the overseas research effort of Britain is not apparent either, that effort is sufficiently large in terms both of man-power and of expenditure for it to be important that resources should be wisely and efficiently used. Apart from that, however, the formation of the new Department should be a further stimulus to the constructive thinking as well as the personal service that are essential to enable technical assistance to match the opportunities becoming available, either in magnitude or in quality.

INSECT BIOCHEMISTRY

The Biochemistry of Insects

By Darcy Gilmour. Pp. xii+343. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961) 64*s*.

In recent years an increasing number of biochemists have come to use insects as the source of their material for study; and entomologists studying the toxicology of insecticides have found the need for more knowledge of insect biochemistry. The time was therefore ripe for the appearance of this first text dealing with the subject. A difficult problem in such a book is how much introductory matter to include. The author assumes a general knowledge of chemistry and biochemistry; but whenever metabolic cycles or sequences are discussed the reader is tactfully reminded of the main outline of what is known of these processes in general biochemistry. Emphasis throughout is on what has come to be called 'dynamic biochemistry': the description of firmly established stepwise transformations and of the enzymes by which they are catalysed. The author recognizes the need for a sound basis of descriptive chemistry; but where well-controlled biochemical studies are lacking—as in the hardening of the insect cuticle—he makes the gap apparent to his reader.

There are large areas of the subject where the insect biochemist is mainly confirming the existence of processes already established elsewhere. But there are interesting variants, such as the predominance of glycerophosphate over lactate as the end-product of the glycolytic sequence in the flight muscles of some insects. Not all insect biochemistry, however, is of this derivative sort. In some quarters the insects lead the field. The cytochromes, which were soon recognized as the universal chain for electron transfer to oxygen, were discovered and characterized in the insect. Even the recent addition to the series, the microsomal cytochrome b_5, was discovered in an insect. The pteridines, which were first identified as inert pigments in the wings of butterflies—what

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* International Aid for Economic Development: Possibilities and Limitations. By Dr. V. K. R. V. Rao. (Eighteenth Montague Burton Lecture on International Relations.) Pp. 29. (Leeds: The University, 1960.) 6*s*.

Gowland Hopkins called 'waste products that function in ornament'—seem ever to be increasing in metabolic importance as growth factors, photolabile receptors, co-factors for the transfer of 'active formate' and so on; and some of the most suggestive work is still being done on insects. Although best known in insects, the ommochromes and their relation to tryptophan metabolism are of general interest for the light they have thrown on gene action. The sclerotin of insect cuticle, formed by the quinone tanning of protein, constitutes a new type of natural polymer which still awaits biochemical analysis: and insects provide the ideal material for studying the biochemistry of morphogenesis.

The author recognizes physiology as a reputable discipline, but he does not allow himself to be diverted into physiological discussions, beyond the minimum necessary to link up his biochemical arguments. He always draws a clear distinction between what is surmised and what has been demonstrated. All this makes a book that is distinctive and stimulating in the sense that the reader feels a strong urge to go and fill the gaps in our knowledge. Almost every point made is documented by references to original work. V. B. WIGGLESWORTH

EVOLUTION AND PHILOSOPHY

The Ascent of Life

A Philosophical Study of the Theory of Evolution. By Prof. T. A. Goudge. Pp. 236. (London: George Allen and Unwin, Ltd., 1961.) 30*s.* net.

THIS book may be warmly recommended precisely because it gives a sustained philosophical discussion of modern evolutionary theory. It is eminently readable and its moderate tone should ensure for it a place on the bookshelf of every professional evolutionist. This is not to say, however, that the arguments in the book are wholly convincing or without blemish. The philosophical approach is chiefly that of the neo-positivist or empiricist, and this approach has clearly limited the scope and value of the book. Prof. Goudge seems to consider that a philosophical explanation, while going beyond the empirical facts and assumptions, is somehow immanent in them but cannot be transcendent to them unless capable of empirical application. This point of view would again seem to narrow the value of the interpretations given. I am sure many people consider that the value of a philosophical explanation of a biological theory is precisely because it may go far beyond the theory into a wider realm of meaning which the theory may have for us in both our sentient and intellectual lives.

After an introduction and a second chapter, which is described as historical but does not give much actual history, Prof. Goudge begins the main chapters of the book. The evolutionary theory mainly considered is that of modern selection, and even here this explanation is practically limited to evolution in the light of population genetics. A certain courage is here manifested by the author because population genetics is new, highly abstracted, speculative, and its models are greatly simplified versions of the real picture. It may be fashionable to consider that evolution occurs through 'populations', but it is a serious mistake to give a philosophical account of evolution which implies that evolution occurs only by this method. There just is no evolutionary or genetical reason why a single individual (or a single pair) of organisms should not start, or have started, a new evolutionary line. This is a point which is important in considering the evolution of man, and it is missed completely by Prof. Goudge.

Other legitimate biological theories which are often misunderstood and have never been philosophically refuted, such as vitalism, finality or neo-Lamarckism are, indeed, mentioned but are unfortunately given scant treatment. On the question of finality alone the fundamental point, that without the assumption of finality many scientific statements could scarcely even be made, is ignored. It is a pity also that the basic assumptions of the theory of evolution are not given any philosophical treatment. Nevertheless, there is a wealth of ideas and suggestions in this book which should enlarge the vista of the evolutionary biologist. P. G. FOTHERGILL

HUMAN GENETICS

Recent Advances in Human Genetics

Edited by Prof. L. S. Penrose, with the assistance of Helen Lang Brown. Pp. vii+194. (London: J. and A. Churchill, Ltd., 1961.) 27*s.* 6*d.* net.

HUMAN genetics is now a branch of biology with its own methods. This is due largely to Penrose on the genetical side, and Tjio and Levan on the cytological. This small book is not a text, but consists of eight chapters by Penrose and six colleagues. In the first chapter Penrose summarizes recent work on human mutation-rates and their dependence on age. He is sceptical of the high rates (median 28 mutations per chromosome per generation) estimated for autosomal recessive defects, as compared with a median value of 6 for dominants. He believes that they often are or have been advantageous to heterozygotes. The data on ages of parents of mutants are equally interesting, and make it clear that mutation at different loci must be due to wholly different processes. Harnden and Miller deal with chromosomal abnormalities, and some sexual abnormalities in chromosomally normal people. The most striking item in Penrose's chapter on fœtal genetics is perhaps the map on p. 71 showing the distribution of anencephaly in Europe, with one peak in Ireland and western Britain, and another around Naples.

Gerald's article on the abnormal hæmoglobins is a useful snapshot of a very rapidly developing subject, and contains some 'stop-press news' which very much interested me. The subject will probably remain a little confused until a new technique is devised which will be as quick as electrophoresis, and which can distinguish between some chemically different variants that are not electrically separable. Holt deals with the ridge patterns of fingers and palms, which are among the most accessible of human characters, and, in view of the correlations exceeding 0·95 between monozygotic twins, must be mainly determined genetically. The last two chapters are largely statistical. Renwick describes the search for human linkage, and its estimation, while Smith deals mainly with other statistical problems, including his own remarkably efficient iterative method.

To a reader who had been cut off from reading for as short a time as two years, the chapters on chromosomal abnormalities and sex determination would be the most striking. Two years hence we should know a great deal more on these matters. But the whole book is very good. It is not free from errors, for example, the statement on p. 87 that "individuals heterozygous for the gene determining *Hb S* invariably possess both *Hb A* and *Hb C*". This is corrected later in the same paragraph. However, I have detected no serious mistake. It can be read with great benefit by students of other branches of genetics, and of medicine. Some knowledge of both genetics and pathology is assumed. The alternative would have been to double the volume and price of the book. Helen Lang Brown, who helped in editing, and was almost surely responsible for the very full bibliography with 526 references, deserves special thanks.

J. B. S. HALDANE

TECHNICAL APPLICATIONS OF FRESHWATER BIOLOGY

Handbuch der Frischwasser- und Abwasser-Biologie.

Von Prof. Hans Liebmann. Band 2. Lieferung 1: Pp. xi+1–160. Lieferung 2: Pp. 161–320. Lieferung 3: Pp. 321–480. Lieferung 4: Pp. 481–640. Lieferung 5: Pp. 641–800. Lieferung 6: Pp. 801–960. Lieferung 7: Pp. 961–1149. (München: R. Oldenbourg, 1960.) 20 D.M. each number.

THIS volume is the second of a pair. The first, published in 1951, dealt with freshwater biological methods and the most important chemical techniques which are needed to supplement them. It contained also an exhaustive treatment of the indicator organisms which can be used in the study of pollution, and a new exposition of the Kolkwitz and Marsson *Saprobiensystem*, which is widely used in Europe for assessing organic pollution.

The two volumes are intended to be used together, and the second contains many references to the first. In both, the emphasis is on the 'application' of biological knowledge to water technology, and although much basic freshwater biology is included it is clear that the author intends this only as a background. This approach to a subject which is often regarded as primarily academic should commend itself to readers in many disciplines concerned with the uses of water in their widest sense.

The second volume begins with a discussion of the various processes which result in natural self-purification of organically polluted waters, and this leads to a brief survey of the biology of waters in general, including artificial lakes and reservoirs. It then considers drinking water, with special reference to its origin, since the organisms which occur, or develop, in it vary according to whether it comes from underground or surface sources. Here also are described the various filtration and other methods which are normally employed, and the biological problems which may arise in their use, as well as the difficulties caused by water blooms and their control. A similar, shorter, chapter deals with swimming pools and spas.

A further section covers freshwater fisheries of all kinds, from natural rivers and streams to fish farms, and here again the emphasis is on practical management.

The author then discusses the methods of sewage purification, with particular reference to the biological processes involved. This section is a mine of information on the various processes in use on the Continent, including some, such as fish ponds, sewage ponds and irrigation, which are not used in Britain, but which might well be considered for introduction. Many readers of biology could learn much about civil engineering from these chapters, as could engineers about how their steel and concrete structures enable micro-organisms to purify effluents. Of particular interest are the accounts of the use of sewage in Munich for rearing carp, and of the biology of trickling filters and activated sludge.

The final section, written in collaboration with Dr. H. A. Stammer, gathers together the scattered literature on the toxicity of inorganic and organic chemicals to freshwater organisms, and the effects of suspended solids and high temperature on fish. It will be most useful for reference.

The book is well illustrated with photographs, and contains diagrams from many original papers. It ends with a hundred closely printed pages of references and a good index. It is, in short, an excellent book to have on one's shelf, or in the nearest library, and Dr. Liebmann is to be congratulated on having performed such a Herculean task.

I have, however, some criticisms. First, 140 D.M. is a very high price for any book, and for this one it seems unnecessarily high. Many points are discussed several times, and liberal application of a blue pencil could have made the book much shorter with no loss of content. Moreover, many figures are reproduced two or three times. There is also a very large number of misprints, only some of which are corrected in three pages of errata. Many of these involve the names of invertebrates, and Dr. Liebmann would have been well advised to submit the proofs to a zoologist. This would also have saved him from calling *Suberites* a freshwater sponge, mislabelling at least three of his photographs and making several other unnecessary mistakes. More careful editing could have removed these blemishes and lowered the price.

H. B. N. HYNES

PROTON RESONANCE

Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry

By L. M. Jackman. (International Series of Monographs on Organic Chemistry, Vol. 5.) Pp. xii + 134. (London and New York: Pergamon Press, 1959.) 35*s*. net.

FROM birth to maturity, the application of nuclear magnetic resonance to problems in organic chemistry spans a period of only five years. Dr. Jackman's book is therefore timely, for which both author and publisher are to be congratulated.

The questions that nuclear magnetic resonance is asked to solve in organic chemistry are: (1) What types and number of protons can be distinguished by their chemical shift? (2) What information about the environment can be obtained when the spin-coupling components are resolved? Both topics are treated in the book although, appropriately (1) is dealt with more thoroughly. While many important spectra are described and analysed, the theoretical content is strongly qualitative, and it is possible to doubt if this severely practical outlook really conveys the elements of the subject to the uninitiated. This is perhaps not the purpose of the book, but it fails to meet the condition that the full deployment of nuclear magnetic resonance in problems of structure involves a better appreciation of its principles than, for example, ultra-violet or infra-red spectroscopy.

An account of the general theoretical and experimental background is followed by two central chapters dealing with the relationship between proton shifts and chemical structure. The information summarized here will be of immense value to structural chemists, and it is likely that the carefully assembled tables will form part of the intellectual furniture of organic laboratories for a considerable time: in fact, the exposition and illustration of factors governing the chemical shift form the strongest part of the book. Spin-spin interactions are described in a subsequent chapter wherein the position is a less happy one. The discussion ranges from examples of AB coupling which are readily apprehended even in large molecules to complex cases, for example A_2B_3, where complete resolution can only be accomplished with great difficulty. The tendency to quote, without derivation, formulæ for the frequencies and intensities of lines in a spin multiplet is unfortunate if spin-coupling constants are to be fully exploited as instruments for structure determination. It would seem that an account of electron-coupled interactions in terms that will commend themselves to organic chemists has yet to be achieved.

The book is well produced and illustrated, though misprints are fairly common. There is no doubt it will occupy a place in the bookshelves of most, if not all, organic chemistry laboratories.

J. C. D. Brand

WAVE MOTION OF OCEANS AND ATMOSPHERES

Hydrodynamics of Oceans and Atmospheres

By Carl Eckart. Pp. xi+290. (London and New York: Pergamon Press, 1960.) 63*s*. net.

This book has a misleading title in the sense that it is neither a balanced introduction nor a treatise on the hydrodynamics of oceans and atmospheres. The dust cover and preface hint at a narrower topic; the list of contents makes it clear. Most of modern dynamic oceanography and meteorlogy—general circulation, weather prediction, current and wind structure, interaction of wind and sea, turbulence, diffusion, internal friction—are scantily treated, if at all. In fact this book is a monograph, suitable for research workers in geophysical fluid dynamics, on wave motion in an inviscid fluid. Its approach is akin to that taken by the Norwegian school of meteorologists in their book *Physikalische Hydrodynamik* of 1933. Prof. Eckart considers the effects of compressibility, vertical variation of density, gravity, and Coriolis force, but not instability due to shear in the unperturbed state of equilibrium. To this limited topic he applies new methods, drawn from his great experience of mathematical physics.

The perturbation equations for various basic states are set up in the opening Chapters 1–5. At the same time, the time-mean motion of the Earth's atmosphere and sea is discussed perfunctorily, and some simple stationary models for the basic state are described in Cartesian and Mercator co-ordinates. The remaining chapters, 6–17, contain an original and powerful treatment of the perturbation equations. Ray theory of geometric optics, the Wentzel–Kramers–Brillouin approximation, and Sturm–Liouville theory are used for a variety of cases depending on the basic variation of density, and on the neglect or retention of the Earth's rotation and of the curvature of the geopotential surfaces. This treatment embraces and unifies much classical work on sound, internal gravity waves, Lamb waves, tides and Rossby waves.

Throughout the text are remarks claiming the importance of linear wave-motion. The arguments are broadly of three kinds: those that maintain the physical relevance of wave motion, those that deny the adequacy of other theories, and those that promote wave theory as a first step toward understanding more complicated phenomena such as turbulence. Lack of space has prevented the author from properly discussing rival theories or observations of the sea and atmosphere, so I have yet to be convinced that wave-motion is more than one of many topics important in geophysical fluid dynamics. However, to this one topic the author has made an extensive and original contribution.

P. G. Drazin

MAN AND HIS ANCESTRY

Man and His Ancestry

By Alan Houghton Brodrick. Pp. 256+16 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1960.) 35*s*. net.

The recent spate of semi-popular books on man and his ancestry has given the reader a wide choice. This one is useful for reference purposes since it covers a wide field and quotes many of the sources. It includes a good many new discoveries which are not widely known outside specialist journals (for example, the post-war hominid remains from China and the Neanderthal footprints cave at Toirano) and the author has some stimulating ideas about familiar facts. There are chapters on "What is Man?", "The Genealogy of the Apes", "The Discovery of Man's Antiquity" and others on the Australopithecines, the Pithecanthropoids, the Neanderthaloids and *Homo sapiens* fossils. Each chapter is divided by numerous sub-headings which help to lessen the confusion caused by rapid leaps from one subject to another.

Inevitably, Mr. Brodrick's book will be compared with *Mankind in the Making* by Prof. W. W. Howells; both have appeared within the past few months and cover essentially the same ground, both are easy to read and sprinkled with anecdotes. But while Prof. Howells is a trained anthropologist who can be relied on for accuracy, the general reader (for whom *Man and his Ancestry* is presumably intended) will be badly misled if he accepts all Mr. Brodrick's statements.

The Australopithecines are said to have lived more than a million years ago; and the beginning of the Pleistocene is set at "about two or possibly three million years ago". The Ternifine fauna is stated to be post-Villafranchian "as the presence of *Equus* indicates" (*Equus*, of course, first appeared during the Villafranchian, as the author himself points out earlier in the book). Apart from a good many minor inaccuracies of this kind, there are more serious misinterpretations. The principles of natural selection, for example, seem to have been overlooked: "Apes do not have long arms because they live in trees, they live in trees because they have long arms".

If most of this review has been taken up with criticisms, it is in the hope that the faults will be corrected in subsequent editions. If this were done, the book could be thoroughly recommended as a lively introduction to the subject of man and his ancestry. SONIA COLE

PHYSIOLOGY OF EFFECTOR ORGANS

Vergleichende Physiologie

Von Prof. W. von Buddenbrock. Band 5: Physiologie der Erfolgsorgane. (Lehrbücher und Monographien aus dem Gebiete der Exakten Wissenschaften. Reihe der Experimentellen Biologie, Band 10.) Pp. 390. (Basel und Stuttgart: Birkhauser Verlag, 1961.) 54 D.M.

WHEN, in 1936, W. von Buddenbrock published the second edition of the *Grundriss der vergleichenden Physiologie*, the rapid development of comparative physiology had made necessary a subdivision of the work. The first volume was devoted to the sense organs and the nervous system. The comparative physiology of the effector organs was the subject of Volume 2 of the new edition. After the War von Buddenbrock undertook the Herculean task of revising the whole work and incorporating it in the enormous advances in the subject that had been made, especially in the English-speaking world, immediately before and during the War. The result was the publication of a work in which Volumes 1 and 2 were devoted to sensory physiology and nervous physiology respectively. Nutrition, water and mineral economy were dealt with in Volume 3 and the comparative physiology of hormonal control in Volume 4. The present volume, the fifth in the series, is devoted to the comparative physiology of effector organs. These include muscles, cilia, flagella, chromatophores, luminous and electric organs, but there is no account of glandular effectors. A sixth volume dealing with blood, heart and respiration is in preparation.

Volume 5 is a consistent and equal partner to the preceding volumes of the work. It shares with them both their merits and their faults. On the merit side it can be said that the treatment of the subject benefits from the author's wide-ranging insight into the fundamental biological problems as well as his astonishingly up-to-date information on modern trends in research and their theoretical implications. Especially the chapters on muscle, colour-change, luminous and electric organs well bear out his claim of having incorporated the literature up to and, in some cases, including 1959. This has enabled him at least to sketch out an account of the results of modern experimental work against a background of older observational data. The faults may be considered inherent in an attempt to present comparative physiology in its entirety in a work of 6 volumes written by one author, at a time when a comparative monograph on muscles alone has appeared filling 3 volumes written by a galaxy of experts. As a reference work, then, and a progress report, the book is bound to fall short of the research worker's needs. Its usefulness as a text-book, quite apart from its bulk and price, is also doubtful. The author, having introduced a wealth of frequently conflicting research data, is justifiably reluctant to arrive at the sort of generalizations on which a student depends on to find his way into, and about, a new subject. More often than not, the student is left with a shrug of eminent shoulders where he has been looking for guidance, if only in guessing.

Thus it may well be that the book's justification and importance lie in its use by the academic teacher, especially in German-speaking countries. To him it can be an invaluable guide to the literature not least by means of the excellently arranged lists of references divided into various categories, namely review articles, older original papers and recent publications.

The volume, like the others, is well illustrated and contains many tables of qualitative and quantitative research data. The style is as lucid as ever, and the presentation bears witness to the unflinching enthusiasm, scientific honesty and wisdom of a man who has, perhaps, contributed more than any other towards the creation, moulding and consolidation of comparative physiology as an important branch of biological science. O. LOWENSTEIN

REPRODUCTION IN ANIMALS

Marshall's Physiology of Reproduction

Vol. 1, Part 2. Edited by Prof. A. S. Parkes. Third edition. Pp. xx+877. (London: Longmans Green and Co., Ltd., 1960.) 240*s*. net.

THIS is the third volume of the third edition of Marshall's *Physiology of Reproduction* to appear since 1952. The fourth and final volume is due to be published within the next twelve months.

The major part of the present volume is devoted to reproduction in the male (spermatogenesis, structure and metabolism of spermatozoa, natural and artificial insemination) and fertilization. These topics have been studied so extensively since the 1922 edition of Marshall's book that some 400 pages are necessary to summarize present-day knowledge (as against 80 pages devoted to the topics in the previous edition). It is a tribute to the editor, Prof. A. S. Parkes, and his team of distinguished authors (including the late Dr. Arthur Walton, Dr. C. R. Austin, Dr. M. W. H. Bishop, and the editor himself), that the present volume maintains the high standard of workmanship. elegant style and accuracy that we have been led to expect from the preceding two volumes.

The chapter on gonadal and gonadotrophic hormones in lower vertebrates, contributed by Prof. J. M. Dodd, provides the best comprehensive review published hitherto of this new and rapidly expanding subject. The chapter is amply and beautifully illustrated, and will be invaluable to zoologists and physiologists alike. The penultimate chapter, by Prof. A. S. Parkes and Prof. A. J. Marshall, deals with reproductive hormones in birds. The last chapter, on external factors in sexual periodicity, was drafted by F. H. A. Marshall before his death, and has been revised and brought up to date by Prof. E. C. Amoroso. It deals with many controversial topics, which are stated with both clarity and impartiality, and will prove of immense value to the many workers currently engaged in research in this field.

It is unfortunate that the volume lacks a table of contents of the preceding two volumes, and it is to be hoped that the final volume will carry a cumulative list of contents for the whole series.

Although the price of this volume is high, there can be few serious students of reproductive physiology who can afford to be without it. ANITA M. MANDL

Resonance Absorption in Nuclear Reactors

By Lawrence Dresner. (International Series of Monographs on Nuclear Energy. Division 10: Reactor Design Physics, Vol. 4.) Pp. x+131. (London and New York: Pergamon Press, 1960.) 40*s*. net.

AN important problem in the physics of nuclear reactor design is to predict the proportion of neutrons lost to the chain reaction by absorption in the nuclear resonances of uranium-238 and other elements. During recent years the accumulation of basic experimental data on resonances has enabled reactor physicists to attempt to calculate the absorption directly from neutron transport theory, as an alternative to the customary use of empirical methods.

The present monograph gives a timely account of modern theoretical work in the field, as well as a critical comparison of theory with experiment. It is primarily intended for those well versed in reactor physics, and contains little on the more descriptive type of theoretical treatment developed by the early workers.

There are two introductory chapters giving a brief historical survey and an account of the general transport theory of neutron slowing-down in absorbing media. The greater part of the book is devoted to the development of approximate methods to deal specifically with resonance absorption in homogeneous and heterogeneous systems. Various special topics are discussed, for example, the interaction between neighbouring absorbers, and the Doppler broadening of resonances due to rise in temperature. The only significant omission noted is an explanation of the empirical formulæ used for representing the experimental results. A critical discussion of these formulæ in the light of the preceding theoretical chapters would have been desirable.

A valuable feature of the book is the orderly presentation of key results from many published papers and official reports. It can be warmly recommended.

J. CODD

Plant Physiology

A Treatise. Edited by F. C. Steward. Vol. IB: Photosynthesis and Chemosynthesis. Pp. xvii+348. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 12 dollars.

THIS volume contains two articles, a longer one extending to 242 pages of text by H. Gaffron on the mechanism of photosynthesis, and a shorter one of 37 pages by M. Gibbs and J. A. Schiff on the energy relations of chemo-autotrophic bacteria. Both articles are followed by an adequate list of references.

The work of Van Niel in 1931 on photosynthetic bacteria, the discovery of the chloroplast or Hill reaction some eight years later, and subsequent work with radioactive isotopes, aimed at discovering the fate of the carbon in the carbon dioxide absorbed in photosynthesis, led to views on the mechanism of photosynthesis which are now generally, though not universally, supposed to involve the photolysis of water and the incorporation of the absorbed carbon into a phosphorylated pentose, giving a substance from each molecule of which two molecules of a phosphorylated triose, phosphoglyceric acid, are produced. In the present volume Gaffron gives a detailed account of all the processes and reactions involved in photosynthesis as suggested by recent researches and of the findings on which present-day views are based. The mechanism of photosynthesis is obviously a very complex one, and the botanist who comes to read this account without some acquaintance with the present position of the subject may find some of it, particularly the part dealing with photochemical considerations, rather difficult reading. The arguments for the views adopted are put forward very fairly.

The small article on the energy relations of chemo-autotrophic organisms brings together what is known concerning chemosynthesis in nitrifying, nitrating and non-photosynthetic sulphur bacteria, and will be found useful by those requiring information on this topic.

W. STILES

Birds of the West Indies

By James Bond. Pp. 256 + 8 plates. (London: William Collins, Sons and Co., Ltd., 1960.) 35*s*. net.

THIS is a field guide to 429 species of which 266 are illustrated, 80 being in colour. There are short descriptions of the main features of each family, which will be a help to the novice in the absence of keys. The text of each species consists of the usual descriptive material, including local names, a useful addition in a region where several languages are in use. Mercifully there are no sub-species.

Colour figures are mainly good; the line drawings have rather too much detail for the size reduction and are inclined to be fuzzy, though this may be partly the fault of the paper used, for the letters of the text also are sometimes lacking in sharpness.

James Bond has been associated with ornithology in the West Indies for half a lifetime, and his knowledge of the birds of that region is unequalled. This illustrated guide will be a great boon to professional and amateur, even traveller and visitor with the most casual interest in birds.

Servomechanisms

An Introduction to the Practice and Theory of Closed-Loop Position-Control Systems, with an Account of Methods of Data Transmission and Computation. By P. L. Taylor. (Electrical Engineering Series.) Pp. xiv+418. (London: Longmans Green and Co., Ltd., 1960.) 42*s*. net.

MR. P. L. TAYLOR, who has had considerable experience in teaching the theory of control systems to students of aeronautical and other branches of engineering, has written this introductory text-book for use in technical colleges and universities. It has refreshing elements of novelty in substance and arrangement. Within its intentionally limited scope, it is well adapted to the needs of both students and lectures.

The analysis is developed in a simple manner using, in the first half of the book, no more mathematics than simple linear differential equations. An account of such physical elements as are involved in measurement, data transmission, operation and analogue computation is given in the second chapter.

The second half of the 400 pages develops the theory of linear continuous systems, proceeding via an excellent summary of the essential properties of the complex variable to the Laplace transform, leaving the response to sinusoidally varying input quantities to be developed by an application of the Laplace transform. This sequence is not the usual one, partly because the teaching of the analysis of linear systems with sinusoidal inputs can usually presume familiarity with the techniques of electrical engineering, and partly because the physical significance of the relationships between sinusoidally varying quantities is more obvious than that between Laplace transforms. It is an interesting feature, but one that will not find favour with all teachers.

The book is confined to linear systems, except for one chapter that describes the effect of certain simple types of non-linearity, but it satisfactorily covers such matters as root loci and phase-gain diagrams. There is no discussion of on-off or sampled data systems, or of any form of discontinuous operation, or of the behaviour with randomly varying inputs. The book is thus essentially an introductory one but, within its scope, it is very good. The emphasis is on physical rather than mathematical explanations. A good selection of problems and exercises is included, with answers and hints for solution. The book is likely to be widely used in connexion with courses of limited scope, where at the commencement of the course the students have only a limited knowledge of the required mathematics.

A. TUSTIN

Abstract Set Theory

By Prof. Abraham Fraenkel. Second, completely revised edition. (Studies in Logic and the Foundations of Mathematics.) Pp. viii + 295. (Amsterdam: Elsevier Publishing Company, 1961.) 60*s*.

THE second edition of this book is in many respects different from the first edition. Its size has diminished from 479 to 295 pages; the first sections have been made more concise, remarks of secondary importance and references to literature of minor significance have been omitted. New material has been added in the text, and exercises, based on publications which have appeared during the decade 1950–1959. The bibliography has been greatly reduced, only those works actually referred to in the book appearing therein.

In the text there are three chapters. Chapter 1 on foundations, the concept of cardinal numbers; Chapter 2 on equivalence and cardinals; Chapter 3 on order and similarity, order types and ordinals. In these chapters there are sections on Cantor's set concept, finite and infinite sets, denumerable sets, the continuum, transfinite cardinals; arrangement of cardinals by magnitude, addition and multiplication of cardinals, exponentiation of cardinals, transfinite numbers and infinitesimals; ordered sets, similarity and order-types, linear sets of points, general theory of well-ordered sets, ordinals and alephs, well-ordering and comparability, the origin and significance of set theory.

The book is very ably written, and will be of great value to undergraduates (and some postgraduates), and to mathematical philosophers.

R. G. COOKE

Name This Insect

By Eric Fitch Daglish. Revised edition. Pp. xxvi + 294 (64 plates). (London: J. M. Dent and Sons, Ltd. 1960.) 21*s*. net.

THERE are said to be some 20,000 species of insects to be found in the British Isles, and possibly a million in the world. The author of a book for their identification in 300 pages of small octavo, fitting easily into the pocket, has clearly performed a feat of selection and compression.

It can be said at once that Mr. Daglish has done this most competently, within the limits that he has set himself. He has chosen some 700 British insects and presented them in the form of a key, using only such characters as can be seen at a glance, and avoiding almost all technical terms. The intention is that anyone on a country walk may carry the book, and use it to identify any insect that is big enough, or brightly coloured enough to attract his attention. The small, the obscure, and the rare insects are ignored.

The keys are clearly set out, but would be easier to use if the details of habitat and distribution were more distinct from those of colour and appearance. There are rather few text-figures; perhaps it would be difficult to have had more without introducing the technical terms that the author has avoided. The 64 plates show the appearance of 360 species, and are generally good pictures poorly printed, especially those in colour. Some are arbitrarily labelled 'all enlarged', though in fact they may be natural size, or even smaller.

Many serious entomologists would condemn such a key for giving a spurious appearance of precision, when scarcely one British insect in thirty is included, but one should not rely on any key for precise identification. The best of keys is no more than a guide, eliminating some possibilities and suggesting others. The fact that this book has been revised and reprinted after eight years suggests that the public for which it is intended has found it helpful.

HAROLD OLDROYD

Mineraly

Spravochnik. [Minerals: a Reference] Book. Vol. 1. (In Seven Volumes.) Pp. 617. (Moscow: Academy of Sciences, 1960.) 3.5 new roubles.

EARLY last year the U.S.S.R. Academy of Sciences announced the forthcoming publication of the first comprehensive compendium of mineralogy to be compiled in the Soviet Union. From the first of the promised seven volumes of this work it is now apparent that the compilation will be of outstanding importance, in many respects comparable to the seventh edition of Dana's *System of Mineralogy* (the two published volumes of which were translated into Russian in 1950 and 1954), but differing from this leading Western authority in paying much more attention to Russian research work. This initial volume sets a high standard and seems to be unusually free from errors, even in references to Western literature. It covers the native elements and intermetallic compounds, carbides, nitrides and phosphides; arsenides, antimonides and bismuthides; sulphides; selenides; and tellurides. The index lists close on 1,500 names of these minerals, printed both in Cyrillic and Roman characters. The second volume will deal with halides and oxides, Volumes 3 and 4 with silicates, Volumes 5 and 6 with other oxygen compounds, and Volume 7 with phase-equilibrium diagrams. For each mineral is given the preferred name, formula, etymology and synonyms, diagnostic characteristics, crystal structure and morphology, chemistry, behaviour on heating, occurrence in Nature, alteration, practical significance, means of distinction from similar minerals, and a selected bibliography. The editors-in-chief are F. V. Chukhrov and E. M. Bonshtedt-Kupletskaya, aided by G. P. Barsanov, N. V. Belov, and the late O. M. Shubnikova. Another 19 authors have collaborated in producing the first volume. This new text will undoubtedly rank with Dana's *System* as a principal reference work in any internationally minded mineralogical laboratory.

C. F. DAVIDSON

THE ORIGIN OF OCEAN BASINS AND CONTINENTS

By DR. J. J. GILVARRY
Allis-Chalmers Manufacturing Company, Milwaukee, Wisconsin

RECENT reviews of the problem of the origin of ocean basins and continents have been given by Howell[1], and by Jacobs, Russell and Wilson[2]. The classical hypotheses in this connexion are the tidal resonance theory of Darwin[3], involving creation of the Pacific Ocean by escape of the Moon, the theory of migrating continents originated by Taylor[4] and Wegener[5], and the various theories based on thermal convection[6]. Most such theories were devised without taking into account a discovery of the last decade that the Mohorovičić discontinuity rises from its depth of about 33 km. under the continents to within about 5 km. from the floors of the oceans. This fact implies a profound difference in the structures of ocean basins and continents[7], which creates grave difficulties for most theories of the origin of these features, in particular for the theory of migration of continents[8].

In a recent article[9], I suggested that the circular lunar maria are simply large meteoritic craters with sedimentary floors, excavated by explosive impact of meteorites on the lunar surface in the presence of a hydrosphere. The purpose of the present article is to propose an exactly analogous mode of formation of ocean basins, as the results of explosive impact of large meteorites at a pristine time when the hydrosphere covered the Earth to a roughly uniform depth. The disparity in the levels of the Mohorovičić discontinuity under continents and oceans enters the theory as an integral part.

My attention has been directed by the Editors of *Nature* to a paper by Harrison[10] which appeared about the time the original manuscript of this article was submitted for publication. Harrison hypothesizes that the Pacific Ocean was created by impact of a planetesimal, a terrestrial satellite, or a satellite of the Earth–Moon system, and conjectures a similar mode of formation for every ocean. Thus, the conclusions presented here, arrived at independently, are identical in principle with the suggestions of Harrison, but the basis of the argument is entirely independent and the proposed mechanism of crater formation differs in an essential respect. The reasoning of the present article is based on a reconstruction of the mensuration of the pristine oceans, and a correlation of their dimensions with those of the lunar maria, lunar craters (of Class V), and meteoritic and explosion craters in water[9]. Thus, the presence of a terrestrial hydrosphere at the time of formation of the ocean basins is an essential element in determining the relative dimensions of the pristine craters. In contrast, Harrison's basic argument is that creation of the Pacific Ocean by the impact of a planetesimal or satellite is energetically possible.

Origin of Ocean Basins

The largest terrestrial ocean, the Pacific, can be divided geologically into two major basins, the North Pacific and the South Pacific, by prolongation to the coast of South America of an east–west line coinciding approximately with the andesite line north of Australia. On the side of the andesite line away from the Pacific, the rocks tend to be continental in type. A similar natural division of the Atlantic into the North Atlantic and the South Atlantic can be made by a line joining South America and Africa at their closest points, roughly between Cape São Roque and Cape Palmas. With these divisions, each of the five oceans, the North Pacific, the South Pacific, the North Atlantic, the South Atlantic, and the Indian, possesses a roughly circular outline.

At the seaward limit of the continental shelf, the demarcation of each oceanic basin is an escarpment as precipitous as that forming the boundary of a lunar mare of the type of Mare Imbrium. Thus, the terrestrial oceans possess at least two features, a roughly circular outline and an encircling scarp, in common with the lunar maria of Imbrian type. A third common feature exists : as is the case with the lunar maria, the floors of the oceans lie below the mean level of the land mass. In view of these similarities, it will be postulated that the terrestrial oceans and the lunar maria of Imbrian type were formed in the same manner, by explosive impact of large meteorites in the presence of a hydrosphere. At the outset, it should be emphasized that the hypothesized meteorite impacts occurred far back in the Pre-Cambrian era (a period of time lasting about four milliard years)[11].

In the case of the lunar maria, my argument on the mode of formation was based on a correlation of their dimensions with those for explosion and meteoritic craters formed in water. For the present terrestrial oceans exclusive of marginal seas, the diameter D and depth d' are shown in Table 1, as determined from data of Kossinna[12]. For simplicity, the line of division between the North Pacific and the South Pacific, and between the North Atlantic and the South Atlantic, has been taken as the Earth's equator. Because of the inherent uncertainties, no effort has been made to correct the entries for the Pacific Oceans to take account of the location of the andesite line. The diameter shown is an equivalent diameter D fixed from the ocean area A by :

$$\tfrac{1}{4}\pi D^2 = A \qquad (1)$$

The depth d' appearing corresponds to the maximum given by Kossinna for which a significant floor area of the ocean exists ; it includes 1 km. corresponding to the mean height (840 m.) of the continents[12].

Table 1. MENSURATION OF OCEAN BASINS

Ocean	D (km.)	d' (isostatic) (km.)	d (anisostatic) (km.)	l (anisostatic) (km.)
S. Pacific	11,000	10·5 ± 0·5	39 ± 4	22 ± 2
Indian	9,700	8·5 ± 0·5	34 ± 3	19 ± 2
N. Pacific	9,500	10·5 ± 0·5	34 ± 3	19 ± 2
S. Atlantic	7,600	8·5 ± 0·5	27 ± 3	15 ± 2
N. Atlantic	6,800	9·5 ± 0·5	24 ± 2	14 ± 1

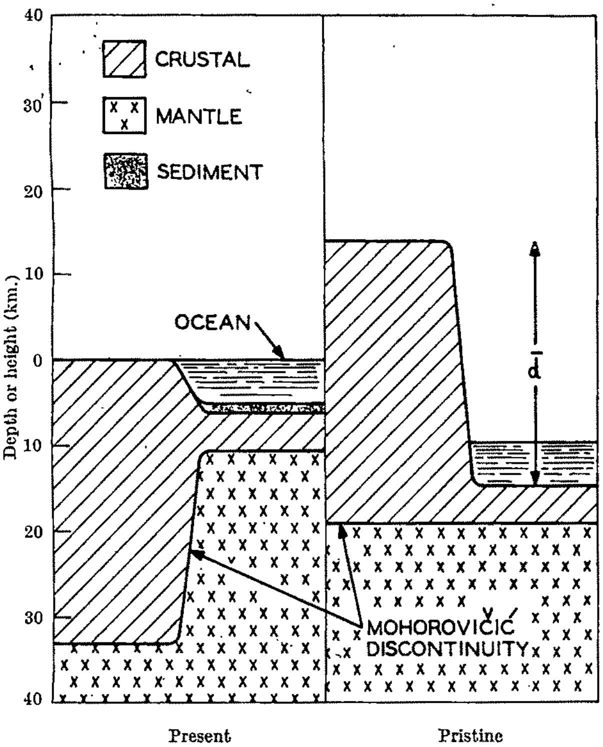

Fig. 1. Standard sections through rocks under continents and oceans at the present time (left), compared with corresponding pristine sections (right) immediately after the impacts of the meteorites forming the oceans

The values of ocean dimensions in question cannot be made the basis of a correlation with the corresponding dimensions of the lunar maria because the terrestrial ocean basins are, and have been, subject to isostatic compensation. On the other hand, the evidence is strong that the Moon is not and never was in hydrostatic equilibrium, in view of its strongly triaxial shape. This shape reflects the strength of the component materials, and is consistent with a cold origin for the Moon[13,14]. However, even if the Earth were formed cold, radioactive heating certainly would be sufficient[15] to render the mantle plastic up to the present depth of isostatic compensation (roughly between 50 and 100 km.). Thus, the ocean depths d' appearing in Table 1 must be corrected for the presence of isostasy.

For precision in terminology, the Earth's crust will be defined as the region above the Mohorovičić discontinuity, and the solid region below will be referred to as the mantle. On the left (labelled 'present') in Fig. 1 are shown standard sections through the Earth's crust and mantle, for continental and ocean columns, as deduced by Worzel and Shurbet[16] by means of relatively accurate seismic data from explosions. The standard land column consists of 33 km. of crustal rock of density 2·84 overlaying mantle rock of density 3·27. The corresponding ocean column consists of 5 km. of sea water (density 1·03), 1 km. of sediments (density 2·30), and 4½ km. of crustal rock (density 2·84) over the mantle for a total depth of 10½ km. of crust. With these densities, the land and ocean columns are in isostatic equilibrium at a depth of 33 km., corresponding to the depth of the Mohorovičić discontinuity under the continents.

At the time of impact of the meteorites forming the ocean basins, the Mohorovičić discontinuity must have been level under the areas now corresponding to continents and oceans, as shown on the right (labelled 'pristine') in Fig. 1. Its depth must have been intermediate between the present depths of this discontinuity below the oceans and continents. Using the data of Kossinna for the total area of the Earth corresponding to continents (including marginal seas) and oceans, it can be computed from the volume balance that the level in question lay 14 km. above the present depth of this discontinuity under the continents, as shown in Fig. 1. The standard land and ocean sections above the Mohorovičić discontinuity appear superposed on this base-level (with the sedimentary layer omitted). The figure represents the proper relative position of the land and ocean columns immediately after meteorite impact, when anisostatic conditions prevailed, provided two conditions have been fulfilled. The first is that isostatic adjustment of oceans and continents, from pristine to present conditions, took place by horizontal flow of mantle rock at depths below the Mohorovičić discontinuity, as occurs in the isostatic balance of the present day. The second is that each standard column (land and ocean) above the Mohorovičić discontinuity has remained approximately the same throughout the Earth's history since the oceans were first formed. This assumption is reasonable in view of the small thickness and mass of oceanic sediments compared with the corresponding quantities for a total column, and implies that isostatic readjustment was fast compared with erosive processes.

On these assumptions, a first approximation to the mean depth $\bar{d}$ of the craters corresponding to the primordial ocean basins before isostatic adjustment is fixed approximately as the difference of the thickness of crustal rock in the standard continental and oceanic columns, with the sediment thickness ignored in the latter, as indicated in Fig. 1. This estimate can be improved in two respects. The first is to include in the estimate an additive correction for the ocean sediments, since primordially these deposits must have existed in the continental column. The present thickness (1 km.) of such sediments yields 2 km. for this correction, corresponding to the fact that the total area of the deep oceans is about twice that of the remainder of the Earth. Secondly, to take account of the fact that the depths of the lunar maria were reckoned from the approximate maximum heights of the rims[9], one can include 1 km. in $\bar{d}$ corresponding to the mean height (840 m.) of the continents. The resulting value of $\bar{d}$ is entered in Table 2 with a possible error of ±10 per cent of its value. The mean height of rim $\bar{l}$ of the meteorite craters forming the primordial ocean basins can be obtained. Prior to the meteorite impact, the crustal rocks must have overlain the Mohorovičić discontinuity to an approximately uniform height L. The value of L can be computed directly from the present thicknesses of crustal rock under the continents (including marginal seas) and oceans, and the corresponding areas[12] on the surface of the Earth, as 15 km. The mean rim height $\bar{l}$ is then simply the difference of the height of the standard continental crustal column and L (the correction associated with the sediment thickness cancels approximately). The value of $\bar{l}$ appears in Table 2 with a possible error of ±10 per cent of its value. The mean diameter $\bar{D}$ of the craters corresponding to the primordial ocean basins, given in Table 2, was fixed from equation (1) with A taken as the mean area of a present ocean. These computations presume that a right circular cylinder represents a reasonable geometrical model for both the present and primordial ocean basins, and that isostatic adjustment leaves the diameter approximately the same.

Table 2. MENSURATION OF AVERAGE PRIMORDIAL OCEAN BASIN (ANISOSTATIC)

$\bar{D}$ (km.)	$\bar{d}$ (km.)	$\bar{l}$ (km.)	$\bar{r}$ (km.)
9,000	32 ± 3	18 ± 2	2,900

To find the depth and height of rim of an individual ocean basin under anisostatic conditions it will be assumed as an approximation that each basin was geometrically similar to the average basin, primordially. Thus, the anisostatic depth d corresponding to the diameter D is given by:

$$d = (\bar{d}/\bar{D})D \qquad (2)$$

and the anisostatic height of rim l is fixed by:

$$l = (\bar{l}/\bar{D})D \qquad (3)$$

These primordial dimensions are shown in Table 1 for each ocean, with a possible error of ±10 per cent of the value.

A sequence of events involving isostatic compensation, similar to that postulated here for the ocean basins, has been invoked by Daly[17] in explaining the Vredefort ring-structure of South Africa as the result of a meteorite impact. Harrison[10] recognizes the need to consider isostatic readjustment of the pristine ocean basins after meteorite impact, but he does not attempt a quantitative treatment, as carried out here. He considers the Pacific Ocean to arise from the fall of a single meteoritic body, whereas the more reasonable assumption that two impacts were responsible is made in the present work.

Correlations

Fig. 2 shows the correlations obtained between the diameter D and depth d for terrestrial explosion and meteoritic craters, on one hand, and lunar craters and circular maria, on the other[9]. The solid curves shown for craters on land represent the quadratic form:

$$D = a_1 d\,[1 + (d/a_2)] \qquad (4)$$

for hard and soft rock. The parameter a_1 was fixed from the dimensions of explosion craters[18] in basalt and sedimentary rock, while the corresponding a_2 was chosen by a least-squares fit to the dimensions of lunar craters[19] of Classes IH and IS, respectively. The craters of Class IH, lying in the highlands, and of Class IS, in the floors of the maria, correspond by inference to hard and soft rock, respectively. A close correlation exists between the data representative of terrestrial meteoritic craters in hard and soft rock, and the corresponding curves constructed from the dimensions of terrestrial explosion craters and the lunar craters in question.

The curves for craters formed in hard and soft rock under water correspond to the form:

$$D = a_1 d\,[1 + (d/a_2)^n] \qquad (5)$$

The coefficient a_1 for hard and soft rock was determined from the dimensions of craters, Glasstone (H) and Glasstone (S), respectively, formed by explosion of nuclear bombs in water[20]. This procedure presupposes that the parameter μ defined in terms of the depth of water δ by:

$$\mu = \delta/d \qquad (6)$$

have a value of the order of unity for a crater[9]. However, the resulting curves are approximately independent of the precise value of μ, so long as it is in the range $1/12 < \mu < 2$, roughly. For both hard and soft rock, the coefficient a_2 and exponent n correspond to a least-squares fit of the data for lunar craters[19] of Class V, which are the nine craters for which the representative points are displaced farthest from the curves for Class I in Fig. 2. The representative points for the lunar maria fall closely on the curve for craters formed in hard rock under water. The final link in the argument that the lunar maria are simply meteoritic craters formed in the presence of a hydrosphere is provided by the terrestrial meteoritic crater of Deep Bay[21]. It can be argued strongly that this crater must have been formed by impact of a meteorite in water, and the corresponding point lies close to the curve expected.

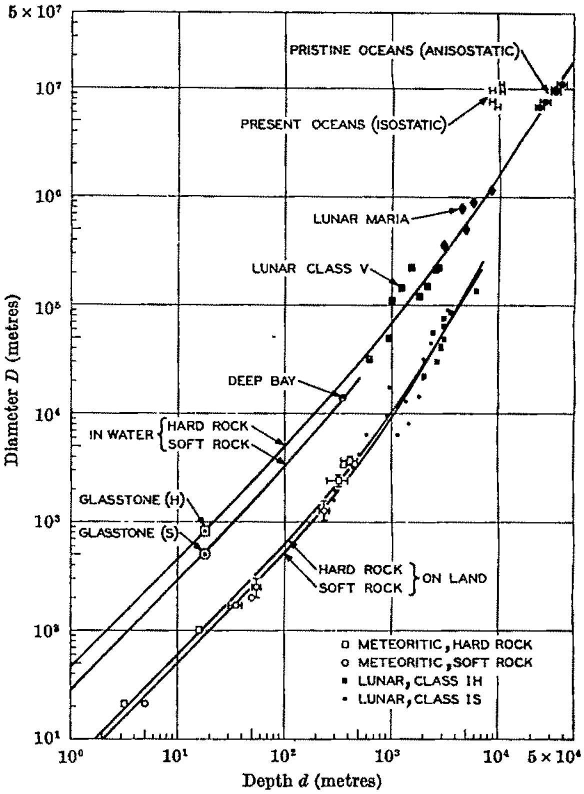

Fig. 2. Correlation of diameter versus depth for the present and pristine oceans, the lunar maria, and various classes of craters. The curves correspond to fitted functions for craters on land and in water, for hard and soft rock

Prior to the impact of the meteorites forming the ocean basins, water must have covered the Earth to a uniform depth $\bar{\delta}$ above the roughly level surface of the crustal rock. On the assumption that the total volume of sea water has not changed greatly since the ocean basins were formed, the value of $\bar{\delta}$ is approximately 3 km. Accordingly, the parameter $\bar{\mu} = \bar{\delta}/\bar{d}$ corresponding to equation (6) had a value of roughly 1/11 for the crater corresponding to the average primordial ocean, and lies within the range for which the curves of Fig. 2 for craters in hard and soft rock under water apply. The diameter D, as a function of the anisostatic depth d for each primordial ocean basin, is plotted in Fig. 2 from the data of Table 1. The correlation of the points for these data with the curve for craters formed in hard rock under water is excellent, agreeing with the fact that the substratum of an ocean basin is basaltic. Furthermore, no data on the dimensions of the lunar maria or terrestrial oceans were used in the construction of the curve in question. On the other hand, the points corresponding to the diameter D and present depth d' of an ocean show agreement with the curve only within an order of magnitude.

One might argue that the excellent correlation of diameter and depth of crater obtained is fortuitous as regards the craters corresponding to the primordial oceans. To rule out this possibility, the correlation of diameter and height of rim will be examined. It will be assumed that the relation between the diameter D and height of rim l of a crater formed in water (for $\mu \approx 1$) can be written as the linear form:

$$D = b_1 l \qquad (7)$$

with the coefficient b_1 fixed in the cases of hard and soft rock by the dimensions of Glasstone (H) and Glasstone (S), respectively. The corresponding straight lines appear in Fig. 3.

For the lunar craters of Class V, only a few uncertain heights of rim are available for comparison with the result of the corresponding line for hard rock[19]. However, the heights of rim of the lunar maria can be approximated as the maximum heights of the encircling mountains less the mean height (2 km.) of the lunar highlands above the lowlands[9]. From Schmidt's results[22], this yields the representative points for the lunar maria shown in Fig. 3. The agreement of the points with the line for craters formed in hard rock under water clearly is good. Also shown are the diameters of the primordial ocean basins as a function of the anisostatic height of rim l, from Table 1. The correlation of the points with the line for craters formed in hard rock under water is excellent. Furthermore, the fitting line was determined completely from the dimensions of Glasstone (H). As a final argument, it may be noted that the representative point[23] for the terrestrial meteoritic crater of Deep Bay (formed in soft rock) lies reasonably well on the appropriate straight line.

Analogous curves for craters in hard and soft rock on land are shown also in Fig. 3. The two curves were determined from dimensions of explosion craters in basalt and sedimentary rock[18], and of lunar craters[19] in Classes IH and IS, respectively. The correlation of the points for terrestrial meteoritic craters in hard and soft rock with the corresponding individual curves is not close, because of the sparsity and inaccuracy of the data. The general similarity of Fig. 2 and Fig. 3 is obvious.

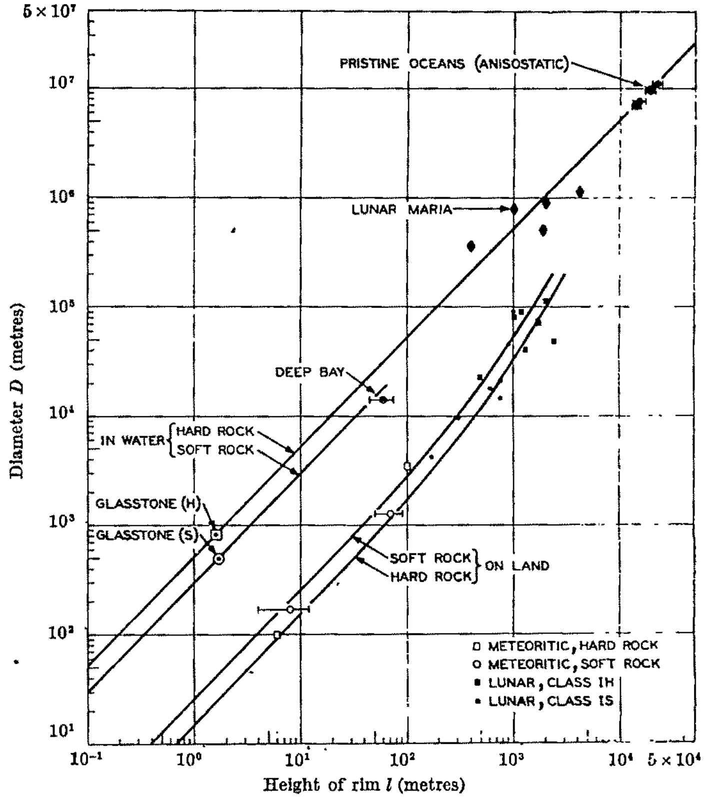

Fig. 3. Correlation of diameter versus height of rim for the pristine oceans, the lunar maria, and various classes of craters. The curves correspond to fitted functions for craters on land and in water, for hard and soft rock

In general, the correlation of the pristine dimensions of the terrestrial oceans with those of the lunar maria is excellent.

Origin of Continents

If the primordial ocean basins were large meteoritic craters, it follows that the primordial continents were simply structures corresponding to the rims of these craters. To see if the model is adequate quantitatively, one can calculate the average width of the rim of a crater corresponding to a primordial ocean. It will be assumed that the cross-section of the rim is a right triangle in the plane of the axis of symmetry of the crater, with the hypotenuse forming the outer slope and one side parallel and one perpendicular to the axis. The altitude of the triangle is the height of rim l, and the length of the base will be designated by r. The average value $\bar{r}$ of the base of the rim can be calculated from the data of Table 2 by invoking Schröter's rule[19,24] for lunar craters, that the volume of the material displaced from the crater equals the volume of the rim. This gives:

$$\bar{r} = (3/4)\bar{D}\,\{[1 + 4(\bar{d} - \bar{l})/3\,\bar{l}]^{1/2} - 1\} \qquad (8)$$

on these assumptions, and the average value of the base of the rim is entered in Table 2. It can be noted that Schröter's rule is a necessary consequence of explosive origin of a crater[25,26].

For the continents regarded as circular in plan, one can determine an average diameter from equation (1) by taking A as one-sixth of the total area of the continents (considering Eurasia as a single continent). The corresponding average diameter is approximately 5,600 km. From the result of Table 2 for $\bar{r}$ it can be seen that two abutting rims from adjacent craters corresponding to primordial oceans yield an average width of 5,800 km. for a continent. Thus, the hypothesis is closely consistent with the mean linear dimension of a continent in plan, as observed.

At the time of formation, the primordial crater rims were mountains of lunar type, and not of the folded type characteristic of present terrestrial topography. Further, these primordial mountains were of about twice the height above the Earth observed to-day for the highest peak (Mount Everest). The eroded remnants of these mountains are the continental shields of crystalline igneous and metamorphic rock of Pre-Cambrian age, forming the nuclei of the continents. The view that the continental shields are simply the eroded roots of ancient mountain systems has been expressed by Collins[27] and Wahl[28], and seems to have gained general acceptance[29].

These ideas yield a direct and natural explanation of Wegener's law[30]. This law states that the frequency curve of elevation on the Earth shows two pronounced maxima, corresponding to the ocean floor and to the continents. On the thesis of the present article, one maximum arises from the floor-level of the craters forming the primordial oceans, and the other corresponds to the rims of the craters. During the time since these rims first reached continental level, the processes of erosion, deposition, and diastrophism have maintained a balance, to yield a roughly constant mean height of the continents. The possibility of explaining the law in question was a major inducement to Wegener in formulating his theory of floating continents.

Discussion and Conclusions

It is clear that the hypothesis proposed yields a direct explanation of the origin of ocean basins and continents. The correlation of the pristine dimensions inferred for the ocean basins with those for lunar features and terrestrial craters formed in water represents a strong argument for the general validity of the ideas. The disparity in the depths of the Mohorovičić discontinuity under continents and oceans appears directly as an essential element in the argument, without the need for *ad hoc* assumptions. Furthermore, the theory is consistent with the observed average diameter of a continent, and Wegener's law follows naturally.

The considerations of this article have an important bearing on the meteoritic impact theory of the formation of lunar craters. In the view of Baldwin[19], Urey[31] and Kuiper[32], the prevalence of craters formed by meteoritic impact on the Moon is not a feature peculiar to that body, but implies a similar bombardment of the Earth by meteorites in the past. However, the lunar craters obviously extend over a wide spectrum of sizes and ages. On the other hand, only 17 craters or clusters of craters were known at the time of Baldwin's work, and all these craters were small (of diameter less than 2 km.) by lunar standards and of recent geological age.

During the past decade, the situation has changed markedly. Discoveries of meteoritic craters in this period have enlarged significantly the total number known[9,33]. The age horizon has been extended to Early Palæozoic (Brent and Holleford Craters) and the size of crater to a diameter of about 14 km. (Deep Bay). Furthermore, the discovery of cœsite in Barringer Crater[34] in Arizona and in the Rieskessel[35] in Austria has reinforced strongly the contention that crypto-explosive features[19,36] are in fact meteoritic in origin. If this view is accepted, the Vredefort structure[17] provides an example of a meteoritic crater which is roughly 130 km. in diameter and is Pre-Carboniferous in age. Finally, two large circular arcs have been noted in Canada, which may be meteoritic in origin[33], and one has a diameter of 400 km.

Since the meteorite impacts forming the ocean basins occurred far back in Pre-Cambrian times, it is implicit in my thesis that the bombardment of the Earth by meteorites has shown the same intensity throughout the ages as in the corresponding case of the Moon. At the present time, the Earth is experiencing merely the terminal phase of the process, marked by relatively sparse and small meteorites. In the past, the smaller craters on land vanished rapidly through erosion, sedimentation and diastrophism. The larger craters are relatively resistant to these processes; but another factor, isostatic readjustment, operates to make identification difficult, as in the cases of ocean basins and crypto-explosive formations. Only for craters formed by impact of large meteorites in the hydrosphere is there hope of preservation over an extended period of time, because of the protection afforded by the water from subaerial erosion. In view of the enhanced rate of sedimentation in the shallow seas close to a land mass, the chance of preservation increases with the depth of the water[37].

The primordial ocean basins were simply the largest meteoritic craters ever formed on the Earth, entirely analogous to the circular lunar maria. They owe their preservation to their size, in spite of the effects of isostatic readjustment, mountain growth, erosion, and sedimentation in masking the characteristic features of a meteoritic crater. Because of their extent, transcending that of any other hydrospheric features on the Earth, it has been possible here to correct their dimensions for isostatic readjustment in a simple manner. This particular method cannot be applied in the case of a marginal sea of the ocean; it is possible that many such seas are meteoritic in origin, but demonstration of the fact may be difficult in an individual case. A possible example is the arm of the Gulf of St. Lawrence, outlined by the coasts of Nova Scotia and New Brunswick[38]; the diameter is 300 km.

Ideas similar to mine and Harrison's[10] have been adumbrated by others. Chenoweth[39] has pointed out the close similarity between the Northern Canary Basin and Mare Crisium, and he has noted analogies in the features of the floor of the North Atlantic with structures in the basins of lunar maria. Gold[37] has speculated that the patterns of circular arcs in the zones of tectonic instability and gravity anomaly, and in regions of earthquakes and volcanoes on the Earth, may reflect the destruction of hydrostatically stratified balance by ancient meteorite impacts. A theory of Howell[1] bears a superficial resemblance to that presented here, but differs in that the meteoritic impacts considered are not explosive in nature. A full account of this work will appear elsewhere.

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GALACTIC EXPLOSIONS AS SOURCES OF RADIO EMISSION

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The Problem of the Energy Sources

IT is generally believed that the major source of non-thermal radio emission, both inside and outside our Galaxy, is high-energy electrons and positrons moving in weak magnetic fields. The strongest non-thermal sources in our own Galaxy, such as the Crab and the Cassiopeia *A* source, are known, or thought, to be supernova remnants, and it is possible that the majority of the non-thermal radio-frequency radiation emitted has arisen directly or indirectly from supernovæ[1]. The strongest extragalactic sources are radiating at power-levels up to 10^7 times that of our Galaxy.

The most difficult problem associated with this mechanism of emission has always been the source of the energy now contained in the high-energy particles and magnetic field. The first idea as to the source of the energy came when Baade and Minkowski[2] suggested that the source Cygnus *A* was two galaxies in collision. This was followed up by the spectroscopic work of Minkowski[3] on the source identified as *NGC* 1275. He showed that there were two large-scale regions in the system with a large relative velocity between them. However, neither this result nor the somewhat more subjective argument that some sources look like galaxies in collision is conclusive, and fundamental objections have been raised to such an explanation. On one hand, Ambartsumian[4] has shown that the frequency of collisions of galaxies in the general field is too small to explain the observed numbers of radio sources. Secondly, the major theoretical argument in favour of the collision hypothesis is that the kinetic energy dissipated in the collision is converted to magnetic and particle energy. However, as has been pointed out by me[5,6] and by Shklovsky[7], this argument requires that, depending on the system being discussed, between 1 and 100 per cent of the energy available in the collision must be transferred into these energy modes. Since the transfer process appears to be very inefficient, such a requirement seems impossible to fulfil.

Other explanations for particular sources have been proposed. In analysing the results for *M* 87, I mentioned the possibility that the annihilation of a comparatively small amount of anti-matter could give the required energy in the form of electrons and positrons without any nucleon component[8]. This argument was applied more generally by Hoyle and me[9]; but, although such an idea still has its attractions, we have been faced by a fundamental difficulty concerning the origin of anti-matter and its separation from matter. I have considered a modified version of the collision hypothesis which would apply to clusters of galaxies[10]. This theory suffers from the objection that, though some radio sources lie in clusters, many new identifications by Bolton[11] and Mills[12] show that the majority do not. Finally, Hoyle[13] has given a new treatment of the problem, again using as a basic hypothesis the idea that, in the cases of Cygnus *A* and *NGC* 1275, the only strong extragalactic sources he discusses, collisions are the source of the energy.

Since this work was done, further evidence of the different types of galaxy which are abnormally strong radio emitters has come from optical identifications using the accurate positions obtained by the observers, in particular Bolton and the California Institute of Technology group[11]. The least predictable of these results, in the light of any of the foregoing hypotheses, is the frequency with which many strong sources can be identified with single or double elliptical galaxies. The radio source which was the first system to be identified in this category, and which may be the prototype of this kind of source, is *M* 87. A number of others are listed by Bolton[11].

In what way do elliptical galaxies differ from other types in their general characteristics? First, it is believed that, on the average, they are more massive than either spiral or irregular galaxies. Secondly, they appear to contain very little gas and dust. Although the presence of the [O II] λ 3727 feature in about 30 per cent of all elliptical galaxies shows that some excited gas is present, detailed investigation in one or two cases[14] shows that there is very little gas—10^{-5}–10^{-8} of the mass—and that it is concentrated at the centre. Thirdly, studies of the luminosity distribution and the apparent sizes, together with mass estimates, suggest that elliptical galaxies are much denser than spiral galaxies.

Unquestionably, for sources such as this, the collision hypothesis can be ruled out, and it appears most probable that the sources of energy must lie in the stars in these galaxies. The only sources of energy which are adequate to explain the phenomenon in single galaxies are anti-matter, which was discussed earlier, rotational energy, and nuclear energy. Many of the elliptical galaxies probably have little rotation (*M* 87 is a spherical system) and even if they do, with very little gas spread throughout the galaxy, it would plainly be a very difficult matter to utilize such energy, so that nuclear energy is by far the most plausible origin. Shklovsky[7], in rejecting the collision hypothesis for other sources, has generalized the argument that supernovæ are the basic sources of energy for objects such as the Cygnus source and *NGC* 5128, both of which contain much gas, and also *M* 87. Without going into a critical appraisal of this latter work I would like to propose that supernovæ are also the sources of energy in all those elliptical galaxies which are strong radio emitters. In developing this hypothesis, we must bear in mind that the scanty spectroscopic data on objects of this type suggest that they do not differ noticeably from the majority of other elliptical galaxies in their stellar content.

The magnitude of the effect that we have to explain can be taken from the energy calculations that have been made for *M* 87 (ref. 8) and *NGC* 6166 (ref. 6), with these treated as typical examples. For *M* 87 it has been found that the minimum total energy for particles and magnetic field is of the order of 10^{58} ergs. In this galaxy the jet is due to visible acceleration or synchrotron radiation with electron and positron energies in the range 1–100 BeV., and magnetic fields in this region having strengths of the order of 10^{-3}–10^{-4} gauss. In all the other sources in this category, however, there is no component of optical radiation so far detected which can be due to accelerated particles. The minimum magnetic field strengths deduced are accordingly, for all these sources, not significantly larger than the fields which I believe to exist normally in the interstellar matter of our own Galaxy.

In a typical elliptical galaxy, in the central region, most of the light is contributed by *K* giants which are probably stars in the range of 1–1·5 $M_\odot$ that have evolved into the giant region of the Hertzsprung–Russell diagram. Evidence for this comes from the spectra[15,16] and the colours[17]. Much of the stellar content is, therefore, probably stars with masses $\lesssim 2\,M_\odot$.

In the normal course of evolution some stars in such a galaxy will eventually become supernovæ. Now supernovæ exploding at a rate of one every few hundred years per galaxy cannot provide the energy required for the strong sources to emit at the observed rates. This is easily shown as follows. The total energy in a radio source such as *M* 87 is $\sim 10^{58}$ ergs or the energy emitted by 10^7–10^8 supernovæ. In the normal course of events, this energy would be released by the nuclear explosions in a period of 10^9–10^{10} years. However, the time-scale for radio emission in such a source, which can be estimated from the energies of the electrons and the assumed strength of the magnetic field, is of the order of 10^7–10^8 years. Thus the reservoir of energy from the supernovæ would build up to only $\sim 10^{-2}$ of that required. For sources stronger than *M* 87, such as *NGC* 6166 or Cygnus *A*, this difficulty becomes even more severe since the normal time-scale would be $\sim 10^{11}$ years. What is needed here is a mechanism that gives rise to a large number of supernovæ explosions in a comparatively short time. The suggestion that I make, therefore, is that one supernova acts as a trigger to set off a chain reaction of stellar explosions. Is such a situation possible ?

Let us review our knowledge of stellar densities in galaxies.

Density of Stars in Galaxies

In the centre of *M* 31, a spiral galaxy, there are some $1{\cdot}3 \times 10^7$ stars within a radius of 7·4 parsecs or a mean density of 1,700 $M_\odot$/parsec^3 (ref. 18). In elliptical galaxies, the gradient of luminosity shows that the gradient in density is much steeper than it is for spirals. However, we have no way of estimating the star density at the very centre of an elliptical, that is, within about 1 parsec of the centre, since at the distance of the Virgo Cluster 1 parsec is approximately 0·01″, which is about two orders of magnitude below the seeing-limited resolving power of the largest land-based telescopes. The luminosity distribution and the velocity dispersion of the stars or gas in large elliptical galaxies such as *NGC* 3379[15] and *NGC* 4278[14] enables us to estimate the average density over a volume contained within a few seconds of the centre. If we take the results for *NGC* 4278 as an example, we find that a mass of $3 \times 10^9\,M_\odot$ is contained within a radius of 85 parsecs, or the average stellar density is 1,200/parsec^3 in this volume. The results obtained by approximating the density curve for an isothermal gas sphere correspond to approximately the same density. However, it is possible that the mean density in the central region is considerably higher than this. Moreover, recent work on *M* 32 suggests that dwarf systems may be considerably denser than large elliptical galaxies. The mean density within the radius containing half of the mass of *M* 32 is 85 times that in *NGC* 3379. If the central density of *NGC* 3379 were about 1,200 stars/parsec^3, similar to that in *NGC* 4278, then if the same model applies to all three galaxies the central density in *M* 32 is at least 100,000 stars/parsec^3.

Let us suppose that the stellar density of the very centre of a galaxy is of the order of $10^7\,M_\odot$/parsec^3. In this case, we might imagine that this density prevails over a radius of 1 parsec from the centre.

Under such conditions, application of the virial theorem shows that the velocity dispersion of the stars is about 400 km./sec. This is comparable with, or less than, the velocity dispersion of the stars in the larger bulk of the nucleus, which lies in the range 400–800 km./sec. Since such a tiny central condensation cannot be observed directly and since its contribution to the integrated light of the nucleus, of radius a few seconds, is negligible, it cannot be said that such a feature could not exist. The formation of a star cluster out of gas ejected from earlier generations of evolving stars, the gas dissipating much of its kinetic energy before stars form, makes such a system possible.

In such a dense region the probability of stellar collisions is quite high. This point has earlier been made by Shklovsky[7]. If such a stellar collision leads to a stellar explosion (and this may well be the case), this will itself provide the trigger instead of a normal supernova explosion which I shall discuss next. However, much depends on how such a dense central condensation forms and whether or not it is a physically separate entity, that is, whether the amplitudes of the orbits of the stars are less than the dimension assumed for it.

It seems fair to conclude that a reasonable extrapolation of the observations suggests that the central densities in elliptical galaxies range upward from 10^3 stars/parsec^3 to values in excess of 10^6 stars/parsec^3. Furthermore, we may speculate that densities in the range 10^6–10^7 stars/parsec^3 may be present in some cases.

It is interesting that Greenstein[19] has suggested that one of the elliptical galaxies in the pair *NGC* 4782, 4783, which is a radio source, is more centrally condensed than is the usual case. Direct plates recently taken by E. M. Burbidge at the prime focus of the 120-in. telescope of the Lick Observatory show that the nucleus of one member of the pair is much more highly condensed than the other.

Supernova Explosions

It is likely that for stars of masses not much greater than the Sun, supernova outbursts will not be of the implosion–explosion type[20], but will stem from the explosion of degenerate material in the way proposed by Hoyle and Fowler[21]. The total energy that is released in such explosions is of the order of 10^{50}–10^{51} ergs.

Energy is ejected from a supernova in γ-radiation, X-radiation, and high- and low-energy charged particles. The amount of degradation of the radiation by the supernova material itself is not known. However, it is reasonable to suppose that a considerable fraction will escape as fairly hard radiation. The comparatively small amount of visible radiation in known supernovæ, as against the total amount of nuclear energy available for release, would also suggest this. When incident on the surface of a nearby star, the major amount of the energy which is carried in γ-radiation will be scattered and absorbed. The total absorption for energies of a few MeV. will be of the order of a few grams. This corresponds to a penetration in a giant, according to the tables of Schwarzschild, of the order of 10^4–10^6 cm. in the convective zone, that is, the top of the convective zone. In a normal solar-type star it represents penetration of only a few metres or perhaps penetration to just below the bottom of the atmosphere. The low-energy charged particles will presumably be stopped from penetrating by the star's magnetic field. The high-energy particles will penetrate considerably farther than the γ-radiation.

If a flux of energy E ergs is emitted in the first supernova explosion then the energy incident on the surface of a star D cm. away will be $E/4\pi D^2$, and the energy absorbed per gram if the radiation passes through x gm. before being absorbed is $E/4\pi D^2 x$. For values of D ranging from 3×10^{17} cm. to 10^{16} cm. and E equal to 10^{50}–10^{51} ergs, with $x \simeq 5$, we find that the temperature of the surface layers of the star will be raised to values from 10^5 to 10^9 degrees.

If the material is heated to values in the upper part of this temperature-range, that is, 10^7–10^9 degrees, nuclear reactions will abruptly commence. The reactions that can occur will depend on the composition of the material present. The most probable reactions will be those involving carbon, nitrogen, oxygen and hydrogen. It appears that what we have here is the generation of a detonation wave which will, in suitable circumstances, be propagated into the interior. Without extensive numerical work it is not possible to determine how the propagation will go. Two effects have to be considered. The first is that the wave must be propagated into regions of higher density, and second, the geometry is such that there will be a focusing effect towards the centre of the star. A sudden generation of energy as the wave passes through the star will lead to explosion. The violence of this will depend on the type of star on which the radiation impinges. The problem is somewhat similar to that encountered by experimenters who are trying to generate thermonuclear reactions in a hot plasma. In their case, some of the limitation on the generation of thermonuclear power is the rate of loss of energy by bremsstrahlung processes and the confining of the plasma. However, in this case the back-scattered radiation may be lost or may simply heat the atmosphere so that it expands, but the forward-scattered radiation will be absorbed further in the star, thus causing pre-heating before the detonation front passes.

If such a chain reaction of stellar explosions occurs, the nucleus of a galaxy will slowly brighten. The rate at which it will brighten is determined by the separation of the stars and the rate at which the light from individual supernovæ fades. Suppose that the radius of the volume over which the event occurs is about 50 parsecs, then the time taken for the whole event to occur will be about 150 years. It will be a little greater than the time for radiation to travel through this volume since a finite time is needed for each star to explode after it is irradiated. The brightening of the galaxy will be very considerable, the maximum being reached just at the point at which the density of stars begins to fall off rapidly. It is this drop in density that will stop the chain reaction. The brightening may amount to something of the order of 10 mag. or greater. However, it is not clear whether such radiation will appear in the normal photographic region of the spectrum, for the following reason.

When such an event occurs, the first supernovæ will brighten the galaxy in a normal fashion, the original radiation being emitted. After a considerable amount of gas has been ejected, however, succeeding supernovæ will irradiate this gas. It will become ionized and will achieve a temperature near 10,000°. It will also have a high random velocity since the gas is ejected from the supernovæ at several thousand kilometres a second. In such a situation all the forbidden lines excited in nova shells will appear. Such a situation is reminiscent of what we see in some of the abnormal radio sources such as Cygnus *A*, *3C*-295, *NGC* 1275, and *NGC* 1068. Very strong emission features such as [O II] λ 3727, [N II] $\lambda\lambda$ 6548, 6583, $H\alpha$, [O III] $\lambda\lambda$ 4959, 5007, [Ne V] λ 3426, etc., will appear. A very large amount of energy will also be emitted in Lyman α. There are, therefore, several stages in the evolution of such a model. In the first stage, the galaxy will apparently brighten in its nuclear region. In the second stage, very strong emission lines excited by electron collisions and high random velocities will appear. In the third stage, the random velocities of the gas will die down and eventually all the gas will disappear since it has velocities considerably in excess of the escape velocity of the galaxy. Throughout all this time the radio emission is increasing in intensity and the high-energy particles are diffusing out through the galaxy. It seems probable that the supernovae give rise to the high-energy particles which move into the regions already containing some magnetic field, where they radiate. Finally, all evidence in the spectra of the explosion will have disappeared and only the radio emission will remain.

It appears that, if the nuclear regions of galaxies explode in the way indicated, they will also be strong sources of cosmic radiation. A very powerful mechanism for acceleration of particles is indicated. Consider, for example, two supernovæ which explode a few weeks or days apart. Many of the particles ejected with the first supernova shell will have a velocity much greater than that of the main body of the material in the shell and will therefore travel ahead of it. However, these particles will very soon collide with the expanding shell from the second supernova and be reflected. A large number of particles can, by this mechanism, be trapped between two supernova shells. At each reflexion they will gain energy in the way first suggested by Fermi. The energy gained through such a mechanism is enormous for particles with the right trajectories.

Conclusions

Although galaxies such as *NGC* 1068 and *NGC* 1275 can be seen to be spirals it is tempting to suppose that the same basic mechanism is operating here as is the case for the radio sources associated with elliptical galaxies. In spiral systems it is possible that there are regions where the star density is very high. Moreover, the more massive supernovæ which may be expected in such galaxies will release more energy and thus make the process more efficient. On the basis of this discussion we may tentatively classify some of the identified radio sources in an evolutionary sequence as follows:

First stage: *NGC* 1068, 1275.
Second stage: Cygnus *A*, *3C*-295.
Third stage: *M* 87, *NGC* 6166, *NGC* 4782–83 and other elliptical galaxies.

Following Bolton[23] we may suppose that *NGC* 5128 is a system in which such an explosion has occurred more than once.

I have described earlier results which suggest that high stellar densities may be present in dwarf galaxies. Let us suppose that a chain-reaction of explosions occurs in a galaxy with the characteristics of *M* 32, but at a distance corresponding to a red-shift of 100,000 km./sec. At this distance the apparent magnitude of *M* 32 would be + 25·5, below the limit of any available optical telescope. Brightening of this galaxy by explosion would bring it well above the limit of observation. However, after the supernova phase has ended the galaxy will fade again but will remain a powerful radio source. Such a phenomenon could account for many of the sources which are apparently not identified with optical galaxies.

If the model outlined here is found to be acceptable, a number of general conclusions can be made.

(1) Strong radio sources will be produced in galaxies where the star density is high. They will not necessarily always occur in the most massive galaxies, but can occur in dwarfs also. The production of a strong source is a chance event since it requires that a star become a supernova while it is in a region of high density.

(2) Elliptical galaxies which are strong sources are comparatively old when this occurs. This is because a star of solar type needs 10–20 × 10^9 years to evolve before it becomes a supernova and triggers a galactic explosion.

(3) Sources which cannot be identified with optical objects may be at great distances. Alternatively they may be centred on dwarf galaxies the nuclei of which have exploded but which now are below the detection limit of the largest optical telescopes.

(4) If strong emission lines are present in identified sources this suggests that the explosion occurred comparatively recently. Since the time-scale for brightening or fading at this period may be only hundreds or thousands of years, a programme of investigation of the optical brightnesses and spectra of such sources should be undertaken, since secular changes may be detectable. Shklovsky[7] has already suggested that such changes be looked for in *M* 87.

There is some tendency for radio sources to be identified with pairs of elliptical galaxies or with galaxies with multiple nuclei. On this picture I must conclude that there is a tendency for the components of such systems to have abnormally high stellar densities.

I am indebted to Dr. E. M. Burbidge for a number of helpful discussions. A more detailed account of these ideas will be published elsewhere.

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OBITUARIES

Dr. J. Unrau

On March 2, 1961, with tragic suddenness, the death of Dr. John Unrau took one of Canada's outstanding geneticists and brought to an end a distinguished career of scientific accomplishment and leadership. Dr. Unrau's death, at the peak of his many challenging scientific activities, is mourned by his colleagues and friends in Canada and throughout the world.

John Unrau was born on January 24, 1916, at Karaguy, Orenburgski Territory, Russia. At the age of ten he moved with his family to Saskatchewan, where he obtained his early education in Mayfair and his high-school courses by correspondence. He entered the University of Saskatchewan and graduated with honours in field husbandry in 1942. In the same year he began graduate studies in genetics and plant breeding and obtained a Master degree in 1945. He then went on to Washington State University, where he undertook studies on wheat genetics under Dr. S. P. Swenson and the late Dr. Luther Smith, and received the Ph.D. degree in 1949.

In 1940, he married Mary Klassen of Mayfair. He is survived by his wife, a daughter, Mary Ann, and three sons, John, Paul and Phillip. John and Paul are students at the University of Alberta.

John Unrau's earliest research was in sunflower breeding at the Forage Crops Laboratory, Saskatoon. This study led to the development of the single-cross hybrid, 'Advance', still widely grown commercially. At Washington State University, he entered into the activities of the Department of Agronomy, and a start was made on his now well-recognized work on monosomic–nullisomic wheat. These studies of F_2 and F_3 crosses between common hexaploid wheats and Sears' chromosome-deficient series, in which he was able to associate specific genes with specific chromosomes, laid the foundation for his latter contributions to cereal genetics.

Dr. Unrau joined the staff of the University of Alberta in 1949 and became head of the Department of Plant Science in 1951. In addition to his intense research activity, he assumed a heavy teaching load and directed the research programmes of nineteen advanced graduates, many of whom have already achieved positions of responsibility and are now making significant contributions to the advancement of plant genetics in Canada. He gave much thought to, and took an active interest in, problems of university teaching and high standards of training. He participated in numerous university committees, and took an active part, as a member of the Prairie Regional Committee of the National Research Council of Canada, in the Associate Committee on Plant Breeding and the Sub-Committees of Common and Durum Wheats, Winter Wheat and Rye, and Cytogenetic Research on Cereals and Related Grasses. At the time of his death he was a member of the Honorary Advisory Council of the National Research Council of Canada and chairman of the Associate Committee on Radiation Biology.

The contributions John Unrau made to genetical research during his short professional career were numerous, leaving a lasting impact on geneticists, plant breeders and cereal cytologists alike. He was a recognized world leader in the exploitation of wheat monosomics and their use in determining gene-effects and linkage-relationships. He demonstrated that substitution lines of whole chromosomes could be used effectively to study the expression and control of quantitative characters. By the development of reciprocal substitution lines he showed that two varieties with contrasting characters could be used in precise genetic studies of character differences. While at the University of Cambridge, during 1957–58, his work led to new evidence on the origin of the *B* genome in wheat. His work on rust-resistant, soft-white, spring wheat culminated in the licensing of the important commercial variety, 'Kenhi', in 1958.

The Genetics Society of Canada owes a very profound debt to John Unrau ; to the present membership he will long be remembered. His initiative, persevering and vigorous leadership were of utmost importance in launching the Society. His wise counsel and active part in soliciting support was decisive in establishing the *Canadian Journal of Genetics and Cytology*. He was a charter member and past director of the Genetics Society of Canada. Successively as vice-president, president and past-president, the Society was indeed privileged to have his outstanding leadership until the time of his death.

John Unrau's research output of some twenty-six articles is an impressive testimony to his rather brief but intensely energetic period of scientific activity. His monument will be his published papers and the men he has trained to add to his personal contributions in genetics. The memory of his life will remain with his colleagues and fellow members of the Genetics Society of Canada as an inspiring example.

T. J. Arnason
L. P. V. Johnson
W. J. White
A. Wilkes

Prof. B. J. Krijgsman

Prof. B. J. Krijgsman, who was head of the Sub-department of General Physiology at the University of Cape Town, died on April 17 at the age of sixty. At the time of his death he was also acting head of the Zoology Department. He had suffered from an incurable complaint of the lungs for some years, but such was his courage and strength of will that he rose above all adversity to remain an active scientist and teacher to within a week of his death. To the very end he maintained a deep humility and sense of responsibility towards his students and colleagues, always managing to find the time and energy to give friendly advice or offer gentle criticism. Though a prolific research worker, Prof. Krijgsman was a man of exceptionally wide interests. He was a great reader, particularly favouring philosophical topics, and was very interested in art. However, his main form of relaxation was listening to music or, when his health allowed, playing the piano, on which he was an accomplished performer. It was indeed as great a privilege to know him socially as it was to work with him in the laboratory.

Prof. Krijgsman was born in Holland in 1901. After studying biology at the University of Utrecht, he became junior lecturer there in 1921, later being appointed lecturer. In 1928, after being awarded the degree of doctor of philosophy with distinction, he emigrated to Java to become head of the Veterinary Research Institute at Buitenzorg. He later combined this post with that of professor of medical zoology at Batavia. In 1935 he returned to Holland to study the physiological effects of insecticides for the Netherlands Council for Scientific and Industrial Research and to lecture at the University of Utrecht. In 1949 he accepted the post of senior lecturer in physiology, in the Zoology Department, University of Cape Town, later being appointed associate professor. At the beginning of 1959 he became the first head of the newly formed Sub-department of General Physiology.

In spite of heavy teaching duties, Prof. Krijgsman published at least one hundred and twelve original papers during his lifetime, wrote four books on zoology and contributed to six others. His wife, Mrs. Nel E. Krijgsman, was a great help to him in this respect, being not only a devoted companion but also undertaking duties as his research assistant. Prof. Krijgsman is, perhaps, best known for his studies on the physiology of the invertebrate heart and on the physiology of parasites, but, in fact, he pioneered research in many fields, and the whole subject of invertebrate physiology would have been the poorer had he turned his talents elsewhere. The world of science has lost an eminent research worker and an original thinker: the University of Cape Town has lost also a valued colleague and friend. A. C. BROWN

Dr. E. de Barry Barnett

EDWARD DE BARRY BARNETT, who died on March 23, will be greatly missed by his many former colleagues and students. He could truly be described as a character. He was held in great affection but was often unpredictable. His instructions to his executors were definitely that he did not wish any obituary notice to be published, but he left a summary of his life among his papers, realizing, no doubt, that it would not be possible to avoid such notice and desiring, as always, to be of help.

He was born on October 20, 1886, and educated at Malvern College and University College, London, graduating with first-class honours in chemistry in 1908. After holding some industrial appointments in the dyestuff and explosives industries, he went back to academic work and was appointed lecturer in organic chemistry at the Sir John Cass Technical Institute in 1919; eventually he became head of the Department and deputy principal, retiring in 1947.

He was the author of a number of well-known books, the latest of which, *Mechanism of Organic Chemical Reactions*, was published in 1956.

His own summary of his life passes very lightly over much that was significant. It does not tell of the extent or the high quality of his published papers, numbering more than a hundred, chiefly in the field of anthracene chemistry. It does not indicate his rather lonely private life as a bachelor, isolated by his deafness in so many ways. It gives no idea of the extent of his help to his students. He had an uncanny knack of selecting as junior laboratory staff those applicants who had the ability to advance. Barnett would teach them himself, at the same time often arranging and paying privately for other tuition to make good defects in their earlier education. Having got them over these hurdles, he would see that they lacked no help in gaining their degrees. There is no way of assessing the generous financial assistance that Barnett gave to so many students in days when grants from public funds were less common than they are now.

He was greatly handicapped by his deafness. His well-known "What?" with his hand cupped to his ear and his "You see what I mean to say", said in the loudest of tones, without any basis for uncertainty and not infrequently to the discomfort of those who overheard his criticism of them, remain as vivid memories. But at all these times, even when he criticized both his students and those in authority. there was a humorous twinkle in his eye and an underlying kindness in all he did.

Sir John Cass College was to him a life-time interest. He worked selflessly for its development throughout the whole of his teaching career. During the War. when much of the building was severely damaged by bombing and fire, he continued his teaching there, chiefly with classes at week-ends and by correspondence. He took more than his full share of fire-watching, spending his nights on the roof to make sure that his deafness did not cause him to miss any incidents that might have escaped his immediate attention had he been in a less-exposed position.

He is remembered by his many friends with a deep sense of loss and also with appreciation of the contribution he made not only to the advancement of chemistry but also to the development of the 'Cass'. His sincere care for the well-being and interests of his many students will not be forgotten. A. M. WARD

Mr. W. E. Lawrence

THE death on April 16 of Mr. W. E. Lawrence came as a great shock to his many friends and admirers who have, from time to time, received his help and been regaled with his varied experiences during his forty-three years of service in the Prosectorium of the Zoological Society of London.

William Edward Lawrence was born May 1, 1900. the son of a London milk-roundsman; his mother was Welsh, but "Billy", as he was affectionately known, was himself—both in physical appearance and temperament—a typical Cockney, with his ready wit, quick repartee, irrepressive sense of humour and rich fund of experiences.

Lawrence received an elementary school education at Princess Road School, Regent's Park (1904–14). Here he proved a good scholar, reaching the highest standard. Under the influence of his teacher, a Mr. Dakin, a Zoo addict, Lawrence's interests were early channelled towards animals and he made many visits with his teacher to the neighbouring Zoological Gardens. Apparently it was through Dakin's influence that "Billy" was recommended to the post in the Society's Gardens when, as was customary at that time, he left school at the age of fourteen.

He entered the 'dead house', as it was then called, as prosector's boy and was placed under the direction of the prosector's assistant, Mr. C. H. Hicks, from whom Billy learned dissection, injection and the preparation of skeletal material, albeit employed mainly on menial tasks until Hicks' promotion to superintendent's assistant in 1925, when Lawrence assumed charge of the technical aspect of the work in the Prosectorium. Apart from a brief interlude of two months in 1916 and two years war service (1917–19), he spent his working life at the Zoo, where he returned in 1920, first for a year as butcher's assistant and thereafter in the Prosectorium, where he remained until retiring on pension on October 1, 1958.

Lawrence's long service was officially recognized by his award in 1953 of the British Empire Medal in the Coronation Honours List, and in 1960 by the conferring of the Zoological Society's Bronze Medal.

One of his most outstanding memories dated from the epidemic in 1926–27 of anthrax in the Zoo's Elephant House. In January 1927, after an autopsy on an elephant, Lawrence developed two small malignant pustules which proved to contain the anthrax bacillus. He was rushed to hospital expecting to be dead in forty-eight hours, but in a few weeks he was back at work, cheerful as ever.

He leaves a widow, son and daughter, to whom the sympathy of his many friends and admirers will be extended in their recent bereavement.

W. C. OSMAN HILL

NEWS and VIEWS

The Queen's Birthday Honours List

THE following names of scientists and others associated with scientific work appear in the Queen's Birthday honours list:

K.C.M.G.: Dr. John C. R. Buchanan, medical adviser to the Secretary of State for the Colonies; Dr. Boris P. Uvarov, lately director, Anti-Locust Research Centre.

Knights: Prof. John F. Baker, professor of mechanical sciences and head of the Department of Engineering, University of Cambridge; Robert R. Blackwood, general manager, Dunlop Rubber Co. (Australia), for services to the University of Melbourne; G. Trenchard Cox, director, Victoria and Albert Museum; Arthur B. Duncan, lately chairman, Nature Conservancy; Prof. Austin Bradford Hill, professor of medical statistics, London School of Hygiene and Tropical Medicine, University of London; Dr. Thomas Malcolm Knox, principal, University of St. Andrews.

C.B.: Dr. R. C. Sutcliffe, chief scientific officer, Meteorological Office, Air Ministry.

C.M.G.: G. C. L. Clark, president of the Australian Institute of Mining and Metallurgy; A. B. Cowen, deputy chairman, Standards Association of Rhodesia and Nyasaland; A. F. MacKenzie, Federal agricultural adviser, West Indies; Dr. J. O. Poynton, director of the Institute of Medical and Veterinary Science, State of South Australia.

C.B.E.: R. E. Beales, deputy director, Central Statistical Office, Cabinet Office; Dr. D. Curran, senior psychiatrist, St. George's Hospital, London; A. K. Briant, director of agriculture, Zanzibar; Dr. W. L. Francis, director (grants and information), Headquarters, Department of Scientific and Industrial Research; Dr. J. W. Gregor, director, Scottish Society for Research in Plant Breeding; Prof. J. E. Harris, professor of zoology, University of Bristol; A. J. Nicholson, formerly chairman, Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Australia; L. F. C. Northcott, principal superintendent, Materials and Explosives Division, Armament Research and Development Establishment, War Office; E. Norton, research director, Yarrow and Co., Ltd., Glasgow; J. D. Parsons, director, Royal Ordnance Factories (Explosives), War Office; Dr. W. H. Penley, deputy chief scientific officer, Royal Radar Establishment, Ministry of Aviation; H. W. Springer, registrar, University College of the West Indies; A. B. Taylor, registrar-general, Scotland; G. A. Whipple, chairman and managing director, Hilger and Watts, Ltd.; R. G. McK. Willan, chief conservator of forests, Nyasaland; Prof. F. C. Williams, professor of electrical engineering, University of Manchester.

I.S.O.: T. W. V. Jones, senior principal scientific officer, Air Ministry (Bracknell, Berks.); A. D. Fraser, director of Wild Life Conservation, Southern Rhodesia; P. Ryan, director of agriculture, State of Victoria; J. H. Griffiths, chief electrical and mechanical engineer, Tanganyika.

Taxonomic Botany at Birmingham:
Prof. J. G. Hawkes

AT a time when it is becoming widely acknowledged that there is a shortage of scientifically trained taxonomic botanists, the election of Dr. J. G. Hawkes to a chair of taxonomic botany in the Department of Botany, University of Birmingham, is a noteworthy event. Dr. Hawkes was educated at Cheltenham Grammar School and Christ's College, Cambridge. After initial research training in cytology, he joined an Imperial Agricultural Bureaux expedition to South America as taxonomic botanist, and was appointed afterwards by the Bureaux to work out the taxonomy and cytogenetics of the potato material collected on the expedition. This was the beginning of the work which has made Dr. Hawkes a world authority on *Solanum*, commanding a unique knowledge of the distribution, variation and ecology of the genus in its principal areas in the New World. In addition to leading further expeditions in Central and South America, the last in 1958, he spent two extensive periods in Colombia at the invitation of the Colombian Government organizing a national potato research programme and training groups of specialists in various aspects of breeding and pathology. Since his appointment as lecturer in flowering-plant taxonomy at the University of Birmingham in 1952, Dr. Hawkes has combined his researches on *Solanum* with a study of the Warwickshire flora. Based on new methods of recording distributional data, this may well become a model of its kind. To his chair of taxonomic botany—the first in Britain to bear the name—Dr. Hawkes thus brings the admirable combination of first-hand acquaintance with tropical and temperate floras and the experience gained from the detailed study of a major genus by modern methods.

A Pioneer of Aerology: Prof. Vilho Väisälä

MODERN weather forecasting techniques are largely based on observations derived from radio-sonde soundings. The name of Väisälä is known to all meteorologists as that of an outstanding pioneer of the radio-sonde. Väisälä's association with aerology goes back to 1916 when he was appointed head of the kite station at Ilmala, Finland. In 1919 he became head of the aerological section of the Finnish Central Meteorological Office. After making a number of improvements to the earlier aerological observing methods he began to experiment with the Väisälä radio-sonde in 1931. This reached a stage of development which could be called entirely satisfactory by 1936. In 1944 Väisälä founded a company for manufacturing radio-sondes on a commercial scale, and at present it exports them to fifteen countries. But apart from his aerological activities Väisälä has pursued an active academic career, having been a teaching assistant and lecturer in meteorology at the Finnish Institute of Physics, and culminating in a personal extraordinary professorship in meteorology at the University of Helsinki, from which he retired in 1958 at the age of sixty-nine. It is announced in the journal of the Geophysical Society of Finland, *Geophysica* (8, No. 1; 1960), that, in order to commemorate the seventieth birthday of this outstanding Finnish scientist, the Society has struck a special medal with the inscription: "The Finnish Pioneer of Aerology".

National Institute of Industrial Psychology : Dr. R. B. Buzzard

Dr. R. B. Buzzard has been appointed research director of the National Institute of Industrial Psychology. The Institute's programme of research is partly financed by grants from the Department of Scientific and Industrial Research. After graduating in physiology and qualifying in medicine, Dr. Buzzard spent ten years as a member of the Medical Research Council's scientific staff at the Experimental Psychology Laboratory in Cambridge and then, in 1953, transferred to the Council's Industrial Psychology Research Unit at University College, London. His work has been concerned with problems of research and application in industry and the armed services. They have included training interviewers, the development of personnel-selection procedures, refresher training of returned prisoners of war, and investigations into industrial injury, sickness and absence and the effects of working environments. He will bring to the Institute wide experience of investigation in fields where the gap between research and practice in industry tends to be greatest. In 1960, he was awarded the Institute's Vernon Prize, which is given in alternate years to the investigator regarded as having carried out the most valuable research in industrial psychology and physiology.

C-E-I-R, Ltd.: Prof. M. G. Kendall

Prof. M. G. Kendall is to join the Board of C-E-I-R (U.K.), Ltd., the computer research services organization. He will be responsible for the Departments of Mathematics, of Statistics and of Operational Research. He will work closely with Dr. Alexander S. Douglas, the director of technical services, whose divisional responsibilities include Departments of Computer Services, of Computer Technology and of Programming. Prof. Kendall is at present professor of statistics in the University of London (London School of Economics) and will vacate his chair on September 30. He received his mathematical training at the University of Cambridge, and entered the Administrative Class of the British Civil Service in 1930 to work in what was then the Ministry of Agriculture and Fisheries, becoming the head of the Economics Intelligence Branch. In 1940 he resigned from the Civil Service to become statistician to the Chamber of Shipping of the United Kingdom. He remained at the Chamber of Shipping until 1949, becoming joint assistant general manager in 1947. Since leaving the Chamber of Shipping in 1949, Dr. Kendall has been professor of statistics in the University of London. Among his activities in the University and at the London School of Economics have been the creation and direction of the Division of Research Techniques. Its work in statistics, econometrics, social surveys, and in operational research is known and followed in every country of the world.

Euratom : U.K. Nuclear Energy Attaché

Mr. G. H. Greenhalgh, of the United Kingdom Atomic Energy Authority, has been appointed nuclear energy attaché to the United Kingdom Delegation to the European Communities, to succeed Mr. D. H. Hill. He will be taking up his appointment in the near future. Mr. Greenhalgh, who is forty and a chemist by profession, was a member of the Extra-Mural Research Division at the Atomic Energy Research Establishment, Harwell, until 1956, when he was appointed scientific attaché to the British Embassy in Stockholm. In his new appointment, Mr. Greenhalgh will act as adviser on nuclear energy matters to the Head of the United Kingdom Delegation to the European Communities and will keep in touch with the various installations on the territory of the Community concerned with the peaceful uses of nuclear energy. The present nuclear energy attaché, Mr. Hill, will be leaving Brussels at the end of May to take up a post with the Authority's Reactor Group.

Co-ordination of Research and Development of Sources of Energy

In a written answer in the House of Commons on May 16, the Minister of Power, Mr. R. Wood, said that his Advisory Council on Research and Development existed to co-ordinate research and development in the whole field of energy. Its terms of reference were: to advise the Minister on research and development in relation to his statutory duty of securing the effective and co-ordinated development of coal, petroleum and other sources of fuel and power in Great Britain, and of promoting economy and efficiency in the supply, distribution, use and consumption of fuel and power; to advise the Minister of new scientific and technical knowledge or application of knowledge which, in the Council's opinion, should be taken into account in the performance of his statutory duties; and to keep the whole field of fuel and power under continuous review with the object of identifying problems needing research and development and advising the Minister of those problems with the view of discussion with the industries concerned.

The members of the Council are: Lord Fleck (*chairman*); Capt. (E.) W. Gregson (*deputy chairman*); Mr. M. A. L. Banks, British Petroleum Co.; Dr. C. M. Cawley, Ministry of Power; Mr. T. B. Clark, Imperial Chemical Industries, Ltd.; Sir Josiah Eccles, Electricity Council; Mr. W. K. Hutchison, Gas Council; Dr. W. A. Macfarlane, National Industrial Fuel Efficiency Service; Sir Harry Melville, Department of Scientific and Industrial Research; Mr. L. Rotherham, Central Electricity Generating

Board; Prof. M. W. Thring, University of Sheffield; Dr. F. A. Vick, Atomic Energy Research Establishment, Harwell; Mr. A. H. A. Wynn, National Coal Board.

Programme of Sounding Rockets

In reply to a question in the House of Commons on May 30, the Parliamentary Secretary for Science, Mr. D. Freeth, said that the experiments in the present sounding rocket programme are devised by United Kingdom scientists and carried out in *Skylark* rockets fired at Woomera under joint Anglo-Australian arrangements. Provision of the rockets, the main element in the cost, is estimated at about £225,000 in the current year. The possibility of enlarging this programme in co-operation with the Commonwealth and Europe is under consideration, and a co-operative sounding rocket programme is one of the first questions which is being explored by the Preparatory Commission set up to plan a possible European organization for space research. The cost could not be assessed pending the outcome of the international discussions now in progress on the co-operative development of a satellite launcher, and on the scale of activities to be carried out by the proposed European Space Research Organization.

Population Statistics in England and Wales

The second part of the *Registrar General's Statistical Review of England and Wales for the Year* 1959 has just been issued (Part 2: Tables, Population. Pp. xi+181. London: H.M.S.O., 1960. 11*s.* 6*d.* net.). There are no major changes in the presentation of the data, or the field covered, which includes population movement, births, marriages, marriage dissolution, fertility and statistics of electoral divisions and local elections. The estimated mid-year population for England and Wales was 45½ millions, of whom 22·8 per cent were children under 15, and 14·7 per cent of pensionable age (men: 65 and over; women: 60 and over). A projection prepared by the Government Actuary for the period up to 1999 predicts a slight decline in the proportion of children, and a rise in the proportion of pensioners to a maximum of 17·9 per cent in 1989. Marriages in 1959 were fractionally higher than in 1958, but the rate per 1,000 unmarried population fell slightly. The number of petitions for dissolution of marriage rose by 88 over the previous year, but at 26,300 was still lower than in any of the years 1945–57. Live births continued rising, and at 748,500 were higher than in any year since 1948. The fertility analysis shows that this is well above the level of replacement. The Registrar General has also issued an illustrated pamphlet *Why a Census* (Pp. 31. London: H.M.S.O., 1961. 1*s.* net) to make the general public familiar with the reasons for the Census taken this year, and to inform them about the questions asked and the techniques used. This is an extremely well-produced guide, which gives an indication of the scope and nature of the census programme and the uses to which the information collected can be put, in simple language.

L'Homme et L'Espace

A new monthly journal, *L'Homme et l'Espace*, made its appearance in April. It is published jointly by Dunod, 92 rue Bonaparte, Paris, VI^e, and Editions Spes, Lausanne (No. 1; Avril 1961. Pp. 56. (Lausanne: Actualité Scientifique et Technique S.A., 4 Place de la Riponne, 1961.) Abonnement annuel (12 numeros): Suisse: Fr. 45. France: N.F. 54. Autres pays: S. fr. 54. Prix du numéro: Suisse: Fr. 5. France: N.F. 6.50. Autres pays: S. fr. 8). Its object is to describe, in not too technical a manner, the present developments in space research and technology, and to discuss their wider implications for man. There is a place for a contemplative journal of this type among the many factual and technical publications on the subject, and *L'Homme et L'Espace* promises to fill that place admirably. The first issue is produced attractively, with a wealth of illustrations, and its contents are informative and varied. In the first section, on sciences, there are articles by P. Auger on European space research, M. Golay on the necessity for space research and results from it, C. de Jager on the Sun and its variability, and J. Eugster on the radiation barriers to space travel. The topics discussed in the second section, "Literature, Law, History", include ancient ideas on the universe, the predictions of Fontenelle, and space travellers in literature. The last section consists of notes on the night sky in April, comments on various space-research events, a table of satellites and a book list. English and German editions of *L'Homme et l'Espace* are planned.

General and Comparative Endocrinology

The first issue of a new international journal, entitled *General and Comparative Endocrinology*, has been published recently (1, No. 1 (April 1961). Pp. 90. (New York and London: Academic Press, Inc., 1961.) Vol. 1 (6 issues), 1961: 18 dollars). The intention of the editors is to publish original work on cellular mechanisms of hormone action, and on functional, developmental and evolutionary aspects of vertebrate and invertebrate endocrine systems. Occasional brief reviews dealing with particular aspects of endocrinology will also be included. The first number contains nine communications which describe work on insects, amphibians, reptiles and mammals. The list of members of the advisory council and board of editors contains the names of scientists of world-wide eminence, and this should help to ensure that a high standard of publication is maintained.

Bibliography of Interlingual Scientific and Technical Dictionaries

The fourth edition of the *Bibliography of Interlingual Scientific and Technical Dictionaries* includes many works published in the U.S.S.R. As in previous editions, it is arranged according to the Universal Decimal Classification and there are author and subject indexes. Nearly 900 new dictionaries have been introduced since the first edition (1951) and attention is directed to 133 new editions of works previously mentioned. It is proposed in future to bring the Bibliography up to date through the publication of supplements. Corrections, and any information or suggestions for improving the Bibliography, are invited by the Department of Natural Sciences, United Nations Educational, Scientific and Cultural Organization. Copies of the Bibliography are obtainable from H.M. Stationery Office, price 17*s.* 6*d.*

The British Museum (Natural History)

The British Museum (Natural History) has just issued a new edition of *A Short Guide to the Exhibition Galleries* (eighth edition. Pp. vi+32. London: British Museum (Natural History), 1961. 1*s.* 6*d.*).

The *Guide* follows the modern format which has now entirely replaced the rather text-book-like editions of earlier days. A useful plan of the ground floor is followed by a short historical sketch of the Museum and a description of the exhibits in the Central Hall including the bays together with an outline of the chief features in the Geological, Zoological and Mineralogical Galleries. As the Botanical Gallery is at present under reconstruction, the only botanical exhibits shown are those in the bays of the North Hall. The *Guide* is well illustrated and includes useful details concerning the library, other publications, the Children's Centre and general information about the Museum.

Science in Antarctica

An interesting assessment of the need for future research programmes in Antarctica has recently been produced by the U.S. Committee on Polar Research of the National Academy of Sciences—National Research Council (*Science in Antarctica*: A Report by the Committee on Polar Research. Part 1: *The Life Sciences in Antarctica.* Pp. xi + 162. Part 2: *The Physical Sciences in Antarctica.* Pp. xi + 131. (Publications Nos. 839 and 878.) (Washington, D.C.: National Academy of Sciences—National Research Council, 1961.) 1.50 dollars each part). The Committee has drawn on leading scientists in the United States to write individual chapters, in each of which the present state of knowledge in a particular discipline is outlined, and possibilities of future developments of research in that field are discussed.

The length of individual chapters, excluding the useful bibliographies, varies from two pages on the "General Ecology and Physiology of Antarctic Fishes" and the "Antarctic Magnetic Programme" to sixteen pages on "Antarctic Glaciology". The report has been published in two parts, and it is of interest to note that Part 1, dealing with "The Life Sciences in Antarctica", covers 162 pages compared with 131 pages on "The Physical Sciences in Antarctica". Although this is due partly to more detailed bibliographies covering the life sciences, it also serves to indicate the increased emphasis which the U.S. Committee on Polar Research would like to place on these disciplines which received little support during the International Geophysical Year. In fact, the introduction points out that the contributions of the United States to Antarctic biology are not great compared with those of other countries, and they hope that the present report will publicize the excellent opportunities in such fields for workers in the United States. The report on physical sciences includes chapters on cartography and geology, two fields of work not included in the programme for the International Geophysical Year. These are included under the section on the "Earth's Crust and Core"; other sections cover the "Heat and Water Budget of Antarctica" and "The Upper Atmosphere". The report has been produced primarily to stimulate interest among scientists within the United States in their Antarctic programme, as well as to emphasize the need for such an effort. It also forms a most useful reference source for scientific administrators connected with Antarctic programmes. The distribution of such reports outlining views of leading scientists on the most promising lines of research during the next five years is in keeping with the best traditions of science and the general atmosphere of international scientific co-operation in Antarctic matters which now prevails.

Nuclear Geochronology

Throughout the world more than a thousand research workers are engaged at present in determining the ages of rocks and minerals by methods based on radioactive decay, and as a consequence of this extensive activity it has of late become increasingly difficult for the average geologist to keep track of the many advances in absolute geochronology. Non-specialists will therefore be indebted to two new works which summarize the latest progress in this field. The New York Academy of Sciences has just published the record of last year's conference on "Geochronology of Rock Systems", edited by Prof. J. L. Kulp of Columbia University, to which more than seventy workers of many nationalities have made contributions (*Annals of the New York Academy of Sciences.* Vol. 91, Article 2. Pp. 159–594. New York; New York Academy of Sciences, 1961). Simultaneously, the Academy of Sciences of the U.S.S.R. has issued a lengthy volume in Russian on *Yadernaya Geokhronologiya* or "Nuclear Geochronology" (630 pp., 1961. Price 3.13 new roubles. Moscow, Academy of Sciences of the U.S.S.R.) which is the work of Dr. I. E. Starik of the Radium Institute in Leningrad. One of the most significant features of these publications is a growing recognition of the extent to which apparent ages are dependent on metamorphic history. A new geochronological time-scale produced by Dr. Starik from Russian evidence is in tolerably good (but far from complete) concurrence with the Holmes–Kulp chronology of the geological systems announced last year, and elaborated lately by Prof. Kulp in a comprehensive review contributed to *Science* (133, pp. 1105–1116, April 16, 1961). Both the American and Russian time-scales agree that the Silurian period, with a duration of only 20 million years, is by far the shortest in Palæozoic time—a conclusion which must surely cause Murchison to turn in his grave.

Conservation of Water

The sixth annual report of the Tweed River Purification Board covers the year ending May 15, 1960, and thus deals with events occurring during the dry summer of 1959 (Pp. 39. Newton St. Boswells: Tweed River Purification Board, 1960). In this connexion the Board is concerned with the increasing demands being made on available water supplies—in particular, the use of spray irrigation by farmers may consume very large quantities, and the Board considers that legislation on this subject should be introduced at an early date. The value of the re-use of water by industry was exemplified at some sand and gravel washing plants in the Board's area—in some cases streams would have been pumped dry and production would have been restricted if the washing water had not been re-used. It is encouraging that the report records that rivers were in a generally cleaner condition, and fish mortality in the main rivers was substantially less than during the dry conditions of 1956–57.

Cambridge Rheology Club

The first discussion meeting of the Cambridge Rheology Club, which was inaugurated on April 24, was held on May 29 at the Technological Research Station, Spillers, Ltd., Cambridge. Dr. J. R. A. Pearson (Department of Chemical Engineering, University of Cambridge), the chairman for the evening, welcomed the two visitors, Dr. M. A.

Payman (Charing Cross Hospital) and Dr. G. W. Scott-Blair (National Institute for Research in Dairying, University of Reading). The discussion on "Some Recent Research Problems encountered in the Study of Blood as a Rheological Fluid" was opened by Drs. D'A. Kok (Department of Medicine) and P. A. G. Munto (Anatomy School). A lively exchange of ideas followed with contributions mainly by the opening speakers and Drs. P. Johnson and A. T. Rowe (Department of Colloid Science), Dr. P. R. Lewis (Anatomy School) and Dr. N. A. de Bruyne (Techne, Ltd.). It was announced that the next meeting would take place at the Technological Research Station, Spillers, Ltd., on September 11, when the Cambridge Instrument Company, Ltd., Grant Instruments (Cambridge), Ltd., and Techne (Cambridge), Ltd., will exhibit and discuss rheological equipment. Further information can be obtained from Mr. H. G. Muller, Technological Research Station, Spillers, Ltd., Station Road, Cambridge.

British Conference on Insecticides and Fungicides

The Association of British Manufacturers of Agricultural Chemicals is organizing a "British Insecticide and Fungicide Conference" at the Grand Hotel, Brighton, during November 6–9. The conference is divided into eleven sessions, which include: (*a*) soil pests and diseases, including "The Eelworm Problem", by Mr. F. Jones, "Insecticide Seed Dressings" by Mr. M. J. Way and "Fungicide Seed Dressings" by Dr. Mary Noble; (*b*) systemic insecticides, including "Systemic Insecticides and Virus Control in Potatoes" by Dr. L. Broadbent and "Control of Sugar Beet Virus Yellows" by Dr. Reitberg; (*c*) forecasting of pest and disease outbreaks (Mr. G. H. Brenchley); (*d*) the control of apple mildew (Dr. R. T. Burchill); (*e*) pests of tree fruits (Dr. G. H. Dicker); (*f*) control of *Botrytis* disease (Mr. J. M. Wey); (*g*) control of fungi and nematodes (Dr. Madel); (*h*) newly developed compounds; (*i*) introduction of insecticides into agricultural practice at home and abroad (Mr. M. Cohen and Mr. G. Ordish). Further information can be obtained from the conference secretary, Mr. W. A. Williams, Association of British Manufacturers of Agricultural Chemicals, Cecil Chambers, 86 Strand, London, W.C.2.

New Universities in Britain

In a written answer given in the House of Commons on May 18, the Chancellor of the Exchequer, Mr. Selwyn Lloyd, said that the University Grants Committee had advised him that, within the scope of the building programme already announced, four new universities, in addition to those which are already being established at Brighton, Norwich and York should be established as the best means of providing places for some of the increased number of students expected. He added that the Committee had advised him that three of the four should be at Canterbury, Colchester and Coventry. The location of the fourth is being considered and the Committee hoped to report on this in the next two or three months. It is the Committee's view that the question of the establishment of any new universities beyond these four should await the report of Lord Robbins's Committee on Higher Education. No new university for Scotland was proposed because it was expected that the expansion of the existing Scottish institutions under the building programme already authorized would provide the additional places needed. Mr. Lloyd said he had accepted this advice, and had authorized the University Grants Committee to enter into discussions accordingly. The detailed proposals would take into account existing provision and plans for other forms of higher education in the areas concerned.

Announcements

Dr. T. D. Day, formerly assistant director of cancer research, Department of Experimental Pathology and Cancer Research, University of Leeds, has been appointed to direct the Tobacco Manufacturers' Standing Committee Research Unit, Harrogate.

The following were elected officers of the Salters' Company for the year 1961–62: *Master*, Sir Alexander Todd; *Upper Warden*, T. G. Hicks; *Second Warden*, Rear-Admiral W. K. Weston.

The 407th meeting of the Biochemical Society will be held in the Department of Physiology and Biochemistry, University of St. Andrews, during June 22–23, starting at 2 p.m., when there will be a colloquium on "The Quantitative Estimation of Amino Acids". Original communications will be presented on the following day.

The National Physical Laboratory, Teddington, is organizing a conference on "The Control of Noise" during June 26–28, in collaboration with the National Engineering Laboratory and the Building Research Station. The programme will include: general problems of the control of noise; hearing and the ear; measuring noise; sources of mechanical noise; propagation of sound in air; effects of noise on performance and productivity, etc. Further information can be obtained from Mr. D. W. Robinson, National Physical Laboratory, Teddington, Middlesex.

The eleventh meeting of Nobel prize winners will be held in Lindau during June 26–30. Further information can be obtained from the Sekretariat der Tagungen für Nobelpreisträger, Lindau (Bodensee), Postfach 11.

Hatfield College of Technology is organizing a summer school on the "Mathematics of Automatic Control" during June 26–30. The object of this school is to enable mathematicians and engineers working on problems of automatic control to study some of the relevant mathematical techniques developed in recent years in Britain, the United States and the U.S.S.R. Further information can be obtained from the Academic Registrar, Hatfield College of Technology, Hatfield, Herts.

The Association for the Study of Animal Behaviour is holding a three-day symposium on "Orientation and Migration" during July 11–13 in the Department of Zoology, University of Cambridge. Further information can be obtained from Dr. J. B. Free, Rothamsted Experimental Station, Harpenden, Herts.

Erratum. In the article entitled "Separation of Two Light Reactions in Noncyclic Photo-phosphorylation of Green Plants", by Dr. M. Losada, Dr. F. R. Whatley and Prof. Daniel I. Arnon, in *Nature* of May 13, p. 606, in Tables 2 and 3, entries 2 and 4, the plus sign should in each case precede the word 'Dye'.

GHANA SCIENCE ASSOCIATION

THE Ghana Science Association held for the first time a conference of all scientists in Ghana at Legon in the University College of Ghana during March 31–April 3. This coincided with the second anniversary of the Association, which was formed from the West African Science Association, after Nigeria had asked to form the Science Association of Nigeria about two years ago.

The conference was opened by the Hon. Kofi Baako, Minister of State for Presidential Matters in Parliament, who is also the Minister responsible to the President for National Research. He deputized for the President, Osagyefo Dr. Kwame Nkrumah. The Minister expressed the Osagyefo's great interest in science and its development in Ghana and appealed to the scientists to channel their efforts through peaceful uses of scientific discoveries. He also promised the help of the Government in the publication of a new scientific journal for the Association. The Minister's speech was followed by the presidential address of the Association, which was given by Mr. R. P. Baffuor, principal of the Kumasi College of Technology and the third and this year's president of the Association.

This conference appeared to have met with approval in Ghana, for although many international scientific conferences have been held in Ghana for some time past much publicity was given to this national conference by both the Press and the radio. The theme of the conference was "Science in the Development of Ghana", and no less than twenty-three papers were read in the three days in various branches of science such as physics, chemistry, botany, zoology, geology and geophysics, engineering, agriculture, medicine and soil science. The actual topics were not necessarily limited to Ghana. Most of the papers were of a general nature, and were presented for the understanding of all scientists irrespective of their speciality, but one afternoon was set aside for specialist papers, when the conference split into biological and physical sciences. Geology and soil science formed the biggest group of papers read.

The most popular paper was on traditional healing methods in Ghana, by Mr. W. S. Mensah-Dapaa, a Ghanaian science graduate who, for about two years, has been living at the shrine of a famous traditional healer at Larteh, a village about thirty-five miles from Accra. In his paper Mr. Mensah-Dapaa described in great detail the ceremony that surrounded the healing at this shrine and gave eye-witness accounts of the spectacular cures that had taken place there. He certainly aroused interest in this field, which has often been relegated to the limbo of non-scientific or quasi-spiritual practices. He appealed to the scientists to shed many of the false ideas about traditional healing, so that the best of this cult, which he claimed to be Africa's version of what prevails in varying forms all over the world, may be successfully studied and integrated into science and medicine. He also called for a department under the proposed medical school for studying the herbs and the methods of traditional healing.

Contributors were selected from all over Ghana, from the University College of Ghana, the College of Technology, research institutions like the West African Cocoa Research Institute, the Government Scientific Civil Service, and private individuals.

One significant and encouraging aspect of the conference was that for the first time an appreciable number of young Ghanaian scientists read more than one-third of the papers.

On the first full-session day, April 1, nine papers were read. The Rev. Dr. J. R. Koster, the first scientist to record sounds from the Russian satellite in 1957, read a paper on "Radio Research in Ghana". He discussed the discovery of some unusual equatorial radio propagation problems in Ghana which affect television and radio broadcasting in Ghana and elsewhere. This was followed by an illuminating talk by Mr. O. W. Snow, adviser on agricultural science to the Government of Ghana, on "Agricultural Research in Relation to Development". With particular reference to the Gezira Cotton Scheme in the Sudan Republic, Mr. Snow showed how the proper utilization of the great potential of the results of agricultural research depended largely on other factors, such as systematic layout, availability of credit and some legalized form of marketing.

After this had been followed by the paper on traditional healing methods referred to above, Mr. A. Foggie, adviser on forestry matters, read his paper on "Conservation of Natural Resources in Ghana". He covered the problems of conservation of water, vegetation, soils and wild-life in Ghana. Another paper of great interest that day was one on "Radio Isotope Unit", by Dr. A. H. Ward, of the Physics Department of the University College, in which were described the various work being carried out in Ghana with radioisotopes and the potentialities of the Radio Isotope Unit installed at the University College. Other papers for the day included the following: "Some Aspects of Genetic Expressions in Agricultural Experimentation in Ghana", by Dr. Agble, deputy chief scientific officer; "Grain Storage Problems in Ghana", by Mr. J. Forsyth, of the Faculty of Agriculture at the University College of Ghana, and finally, "The Role of Geology in the Development of Ghana", by Mr. L. A. K. Quashie, of the Geological Survey Department.

The following day was devoted to the specialist lectures. Under the chairmanship of Prof. W. J. McCallien the Physical Sciences group heard three papers. The first of these, which drew many listeners, was on "Geological and Archæological Evidence for the late Quaternary Climatic Sequence in West Africa", by Dr. O. Davies, a well-known archæologist who continues to make valuable contributions in this field in Ghana. This was followed by a paper by Dr. F. Torto, of the Department of Chemistry, on the structure of gums and mucilages in two Ghana plants, *Fagara xanthoxyloides* and *Afraegle paniculata*. The final paper of the day was on "A Seismic Investigation of the Depth of Bedrock under the Volta River at Tefle", by Mr. F. W. Hales, senior geophysicist of the Geological Survey Department. He showed that the refraction method of analysis corroborated the depth-to-bedrock data obtained by drilling at the site of the proposed bridge across the Volta River at Tefle.

The Biological Sciences group, under the chairmanship of Prof. F. W. Sansome, heard two papers on cocoa—one, "Comparable Growth Rates of Amazon and Amelonado Cocoa and Responses to Fertilizer", and the other, "Problems in the Assessment of Cocoa Varieties". The third paper was entitled "Liver Cirrhosis in Pigs in Ghana", in which the speaker, Mr. D. Zwart, introduced for discussion the problem of liver cirrhosis which attacked the local black pigs, and later also a few of the European ones, when kept under semi-intensive conditions.

The final full session day of the conference, April 3, opened with one of the popular topics, "Tuberculosis in Ghana", by the tuberculosis specialist in Ghana, Dr. A. Koch. Then followed a general paper by Prof. McCallien on "Seas and Oceans", treated with reference to geological problems in recent oceanographic studies.

Quite related to this topic was the next paper on "Outboard-Motor as a Prime Mover in the Development of Fisheries in Ghana", by Mr. E. A. Kwei, of the Government Fisheries Department. Among the other papers for the day were "The Correlation of Field, Laboratory and Analytical Studies of Engineering Structures", by Prof. Royston-Jones, of the Engineering Department, and "Classification of Black and Grey Clays of Ghana using the New U.S. System" presented by three workers led by Mr. H. B. Obeng, of the Scientific Services.

The closing address to round off the conference was given by Mr. H. O. Walker, former chief meteorologist, who made special mention of the many new Ghanaian scientists.

Arrangements are being made to publish the proceedings of the conference in the new scientific journal of the Association. J. Yanney Wilson

SATELLITES AND GEODESY

On March 24, 1961, the Royal Astronomical Society held, at its rooms in Burlington House, a geophysical discussion on "Technique of Optical Observations". The chairman for the meeting was Dr. A. H. Cook (National Physical Laboratory), who opened by remarking that the exploration of the major feature of the Earth's gravitational potential by the conventional methods of geodesy was a topic which had often been discussed at meetings of the Society. This meeting, however, would be concerned rather with the elucidation of small-scale features and the use of observations of Earth satellite positions for this purpose.

The discussion was opened by short papers from four speakers, of whom the first was Brigadier G. Bomford. Geodesists had no difficulty, he said, in deriving the size, latitude and longitude and an appropriate geoid for a piece of land, using a variety of well-known astronomical measurements. The problem was to relate the resulting co-ordinate systems for two or more land masses which were separated by a wide sea. If there were no gaps of more than, say, 500 miles to be spanned, the land masses might be connected in latitude and longitude by radar, but this would not connect the two geoids.

However, the problem might be solved by a sufficient number of simultaneous observations of the position of a satellite from each of the land masses to be connected. It was possible that non-simultaneous non-overlapping observations might be used, but this might also assume a knowledge of the geoid. Brigadier Bomford suggested that such observations would need, in order to be useful, to lead to measurements of position with an accuracy of 30 miles in a horizontal and 10 miles in a vertical direction. Connexions of this kind were required, for example, between Europe and America, across the Mediterranean and between many separated land masses in and around the Pacific.

Dr. R. d'E. Atkinson (Nautical Almanack Office) discussed the astrometric techniques which might be employed, of which there were several. One of the most convenient required the satellite to emit bright, short-duration flashes of light, which would then be photographed against the stars using a camera with an equatorial drive. If the stations were at known longitudes and latitudes, then the time of the flash was in general determined, but if longitude errors in the positions were to be taken out, a timing system with an accuracy of 1 msec. was required.

In this way, accuracies in the star positions of 0·05″ could be obtained, but this was because the long exposure reduced errors due to 'seeing' (that is, irregular variations in tropospheric refraction). At low altitudes this effect might be very important and unfortunately connexions over long distances would usually involve large zenith distances. Thus, at a zenith distance of 70° in a particular case, he had found that 'seeing' increased the angular diameter of the image from 2″ to 4·8″, suggesting that the probable error of a single observation of a satellite might be 2″, equivalent to perhaps ± 60 miles in position. But with multiple observations there would be an improvement.

Alternatively, the conventional method of observing a satellite by reflected sunlight could be used, the observations being made by means of a camera having a shutter which would make timed breaks in the trail. This would be nearly as accurate. The principal problem in this case was to obtain east–west connexions over long distances, as each of the stations could not then be in the dark.

The third paper was read by Dr. A. P. Willmore (University College, London), who pointed out that a principal instrumental difficulty in making optical observations of satellite positions arose from the fact that, viewed from a point on the Earth's surface, a satellite moves with an angular velocity which is neither constant nor accurately predictable. The problem was then to obtain a sufficient exposure to record its position. The sensitivity of a detecting system might in general be limited in any of three ways: by the necessity for recording at least a few photon events in each image element, by statistical fluctuations in the background due to the night sky or by system noise (such as chemical fog, in the case of a photographic emulsion). When these were examined for any but a rather small optical system, it was found that at the high quantum yields characteristic of a photoelectric detector, the limitation always arose from the night sky background, while at the lower quantum yield of the photographic plate, chemical fog determined the sensitivity. It could be said that the practical limits for stationary detectors were at about mag. 9 for a photographic and mag. 11 for a photoelectric detector. It appeared that by tracking the camera at the predicted angular rate of the satellite, a gain of about mag. 5 could be obtained.

In fact, the only precise tracking system of high sensitivity in operation was photographic. This was operated by the Smithsonian Astrophysical Observatory, using the specially designed Baker–Nunn cameras. These have an *f*/1 Super-Schmidt optical system of 20 in. focal length. The cameras are tracked at the predicted angular rate and satellites at a brightness of mag. 13 have been recorded.

However, systems using photoelectric detectors are being studied actively—one, for example, using image intensification by Prof. J. D. Magee at the Imperial College of Science and Technology, London, and another, using photomultipliers, at University College, London.

The advertised speaker for the last paper, Mr. W. H. Guier (Johns Hopkins University), was unfortunately unable to appear, and his place was taken, at rather short notice, by Dr. G. V. Groves (University College, London). This was an account of a radio system which offered some promise as an alternative to optical observations, namely, that employed in the *Transit* series of satellites. This requires on the satellite two or more high-frequency transmitters, with a very-high-frequency stability over the observing period of several minutes. The frequency of the transmission received at the ground is measured as a function of time. Naturally, it is not constant, but diminishes during the satellite passage, on account of the Doppler shift. The principle of the method can be very roughly described by saying that the total change of frequency during the passage is proportional to the velocity of the satellite, while the maximum rate of change depends both on the velocity and the slant range at closest approach, which can then be determined. It is necessary to make a correction for ionospheric effects, which amount to about 0·2 per cent at 100 Mc./s. They are almost inversely proportional to frequency, and can be calculated if two or more transmitters are used.

The method was first applied with encouraging success to the determination of orbital elements of the first satellites by W. H. Guier *et al.* Later, the transit system was tested in a trajectory determination of an *Atlas* rocket with an apogee height of 700 km. where comparison with a trajectory determined by radar showed differences of a few km., to which the errors of the radar would have contributed.

It has now also been used on the *Transit* 1 satellite. Observations were made at six ground stations in the northern hemisphere, and the orbital elements determined, taking tho observations for one day at a time, by reproducing to the best fit the observed frequency-time curves. It was found possible to obtain the coefficient of the third harmonic in the gravitational potential with an accuracy of about 5 per cent, and since this produces oscillations of about 5 km. amplitude, it was concluded that the accuracy of measurement was 0·5 km. It seems possible that an improvement in accuracy of an order of magnitude should be obtainable, resulting in position measurement with an accuracy of about 50 miles.

It is intended to use the *Transit* satellites for navigation, by storing up-to-date orbital elements on a tape recorder in the satellite. These would be read out on command by the ship and combined with Doppler shift observations to obtain the ship's position. During the ensuing discussion, the importance of this for gravity surveys at sea was mentioned by B. C. Browne (Cambridge), as in such observations a principal difficulty is the determination, with adequate precision, of the ship's position.

A. P. WILLMORE

GEOCHEMICAL EFFECTS OF WEATHERING

A JOINT meeting of the Clay Minerals Group of the Mineralogical Society and the British Society of Soil Science, held in the School of Agriculture, University of Cambridge, during April 6–7, was attended by approximately 100 delegates from home and overseas. At the two paper-reading sessions, the chair was occupied on the afternoon of April 6 by Prof. J. White (Sheffield) and on the morning of April 7 by Dr. H. Greene (Rothamsted Experimental Station). The topic, weathering, was discussed from many points of view (for example, mineralogical, geological, pedological and geochemical) and the conference concluded with the alternative of an excursion led by Mr. C. A. H. Hodge (Rothamsted Experimental Station) to the Brecklands area or informal visits to laboratories of the University.

Prof. C. W. Correns (University of Göttingen), in the introductory paper, outlined the experimental work done on the decomposition of mineral powders by aqueous solutions. Plastic was used in the apparatus and factors such as particle size, rate of flow, temperature, and *p*H of solution were shown to be important in determining the nature of experimental reactions with potash-felspar, albite, leucite, muscovite, tremolite, olivine and sideromelane. The results of recent experiments on the decomposition of kaolinite and montmorillonite at *p*H 7 could be of significance in the origin of bauxite. The main findings of the whole study were summarized by Dr. G. Nagelschmidt (Sheffield) to the effect that in open systems and at low temperatures, minerals tend to decompose as ions with formation of amorphous residual crusts, whereas in closed systems and at high temperatures secondary minerals are formed. He also directed attention to the formation in Nature of pseudomorphs of kaolin after felspar, and Prof. Correns commented that in an open system and over a long period this might be possible.

An experimental study of the geochemical alteration of acidic and basic igneous rocks under varying hydrodynamic and physiochemical conditions was described by Dr. G. Pedro (Versailles). Special attention was paid to the secondary formation of iron and aluminium oxides and hydroxides. The value of the ratio of $SiO_2 : (RO + R_2O)$ was found to be greater in the leachates than in the unaltered rocks. Prof. White expressed surprise at this, but Dr. Pedro thought that it might possibly be explained by the high *p*H in the system. Asked whether trace elements had been determined, Dr. Pedro replied that these results would be available very shortly. After several comments on the alteration products, a delegate described an apparatus made of tin which had been found more successful than glass.

In contrast to these experimental studies, two papers were given on natural weathering. In the first, Dr. W. W. Smith (Macaulay Institute) described the weathering of basalts and dolerites from the south-east of Scotland and the mineralogy of the resultant soils. The relative stabilities of the primary minerals in the fine sands were given, and the secondary weathering products in the rocks and soils were discussed. Contamination by glaciation had to be taken into account. With reference to the quantitative analyses of fine sands by X-ray diffraction, Dr. R. M. S. Perrin (University of Cambridge) stressed that, due to limiting factors in the method, the figures should not be taken as exact and care in interpretation is necessary. In the second paper, Mr. D. F. Ball (Bangor) described a deep profile on the Isle of Rhum in which 50 ft. of weathered gabbro is preserved under glacial drift. A thick reddened zone with high content of free iron oxide was likened to the *B* horizon of a podzol and the whole formation was considered to be a relic of interglacial weathering in a tor-like topography. Mr. L. Berry (University of Khartoum) commented that such red zones, because of their frequent discontinuity, can be difficult to interpret, but the tor-like topography is similar to that produced in humid warm climates. Reference was made by Mr. R. H. S. Robertson (Pitlochry) to red Tertiary interbasaltic weathering. Dr. R. Glentworth (Macaulay Institute) contrasted certain deeply weathered (possibly pre-glacial) granite and basic igneous exposures in Aberdeenshire with the buried parts of a granite stone circle which appear to have weathered 3 in. in approximately 3,000 years.

The usefulness of drainage-water analysis in studying the processes of weathering and soil-formation was illustrated by Dr. Perrin. Care in sampling, a knowledge of hydrology, percolation rate, and possibly composition of rain-water are all necessary and applicable in determining absolute loss of material or relative loss of elements. Instances where such analyses could provide useful information include the movement of iron in ground water gleys, problems in salt-marsh reclamation, the rate of weathering of chalk, and the genesis of soils. In addition, Dr. Perrin related the results of chemical analyses to weathering trends in soils formed on Ordovician shales and many of the interesting points which arose in interpreting these results were discussed. Other comments included one by Dr. P. H. T. Beckett (Oxford) on the relative merits of sampling drainage water or water direct from the profile. Awareness of the statistical significance of all results was stressed.

Dr. G. Rosenthal (Würzburg) considered both theoretical and practical aspects of the weathering of porous materials by freezing, and focused attention on the 'freezing-point depression' of water, which is due mainly to high pressure affected by capillary radius, pore surface area and surface tension, etc. The resistance to freezing of sandstones, roofing tiles, and bricks was noted, and it was found experimentally that the freezing temperature of brick was even lower than expected, due possibly to the inclusion of meta-kaolin with free valence-bonds which could hinder re-orientation of water into ice. Further work on surface effects was urged before tackling rocks. Asked about the effect of ion concentration, Dr. Rosenthal stated that in brick this lowered the freezing point only very slightly, but in soils and clays it would be more important. Calculations for soils would also be complicated by the wide range in pore size, and when dealing with rocks, climatic effects would have to be taken into account.

The origin of English fuller's earths was proposed by Mr. Robertson to be marine rather than volcanic and to be due to a transgression of the sea during the Upper Aptian. The heavy- mineral suite above and below the clay in the Sandgate beds was shown to be derived from the old land surface of the London Platform. Uniformity in composition of various widespread montmorillonite deposits was considered as evidence of similar conditions of formation. In reply to Dr. Nagelschmidt, Mr. Robertson suggested that the seas would have been mainly calcium ion-saturated and that any sodium ions could have been absorbed by the adjacent more porous formations. A possible tie-up in conditions with those in the Piedmont areas of the south-east of the United States was proposed by Dr. A. Muir (Rothamsted Experimental Station) and Dr. Perrin directed attention to the general absence of mica in the fuller's earth deposits.

Finally, two papers were given by Mr. J. E. G. W. Greenwood (Directorate of Overseas Geological Surveys, Tolworth) and by Mr. Berry and Mr. B. P. Ruxton (University of Khartoum) on the weathering of rocks in hot climates. The first showed how the effects of chemical weathering and thermal disintegration on rocks in arid areas can be of significance in the interpretation of aerial photographs. For a variety of igneous and metamorphic rocks, theoretical orders of response to both processes were detailed but disagreed with the observed order since, for example, thermal disintegration is apparently more effective on granite while chemical weathering is more effective on gabbro and diorite. Rock properties such as grain size, jointing, porosity, and texture were shown to have an important influence on weathering processes. In discussion, Mr. Berry advocated experimental work on the effects of thermal disintegration. In the second paper, examples of tropical weathering of granite in humid, monsoonal, semi-arid, and arid climates were chosen to illustrate different weathering features. Generally, factors such as topography, rainfall, and ground water movement were shown to influence profile development. Illustrations included photographs of 'abandoned' hills and foothill zones of laterization and intense weathering.

During the excursion to Breckland, a series of three soils developed from Gipping Till was demonstrated. The first, a very shallow rendzina, was seen at Lakenheath Warren. The other two, situated within Forestry Commission woodland, were a brown calcareous soil and a brown earth with textural *B* horizon at 95 cm. Although time was too short for a visit to a fourth profile, it was obvious that the afternoon trip had provided adequate food for thought.

The symposium, as a whole, was commendable for the wide scope given to the discussion of weathering. The importance of laboratory experiments as an aid to elucidating problems in the field was emphasized, and among recommendations during discussion were included care in choice of apparatus. methods of sampling, and assessment of significance of results. Undoubtedly, the possibilities of future research in the field of weathering are enormous.

WILMA W. SMITH

THE ARTHUR D. LITTLE RESEARCH INSTITUTE

THE annual report for 1960 of the Arthur D. Little Research Institute* records an average expansion since 1957 of about 20 per cent per annum. Sixty-five per cent of its income comes from industry and 35 per cent from Government and related agencies, about half its sponsors being British and European and the rest American. Practically all the Institute's work is classified as oriented fundamental research, and has so far been predominantly concerned with the chemistry of plant materials. The Biochemistry Section was established in 1960 to undertake the study of biochemical problems where newer techniques, such as starch-gel and high-voltage electrophoresis and paper and column chromatography with automatic densitometry, are already having their impact. Work has been initiated on the separation of the wheat gluten gel complex into its components.

The Organic Chemistry Section continued work on the polymerization of glucose and its derivatives, and already polyamides with viscosities comparable with those of nylon have been obtained using the new technique of interfacial polycondensation. In the work on the chemical modification of wood, a low-temperature catalytic acetylation process gave wood of permanent shrinkage and only slightly improved dimensional stability. Promising results have been obtained by impregnating wood with monomers and polymerizing *in situ*, and attempts have been made to reduce flammability of wood by introducing non-leachable compounds containing phosphorus. Work was initiated on the release of corrosive volatile vapours from wood at high humidity and high ambient temperatures, that is, greater than 40° C., and also to explore the properties of ethylene sulphide.

In physical chemistry, the Polymer Section continued to study the bulk properties of polyolefins, particularly the kinetics of crystallization of polyethylene, with the view of obtaining a better understanding of the effect of crystal structure on the physical properties, and has shown that the initial crystallization behaviour depends markedly on molecular weight. Work was also commenced on the kinetics of the reaction between molten aluminium and water.

The Metallurgy Section completed a three-year study of corrosion involving the screening of 470 organic compounds, to ascertain any correlation between chemical structure and inhibitory power, and although no clear-cut correlation emerged, some compounds were found to favour the inhibition of corrosion of mild steel and cast iron in acid solution while others opposed inhibition. A study of the hydrogen embrittlement of certain steels is planned. A list of publications of the Institute is appended with abstracts of those published in 1960.

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* Arthur D. Little Research Institute. Annual Report for 1960. Pp. 44. (Inveresk, Midlothian: Arthur D. Little Research Institute, 1961.)

THE WOOL INDUSTRIES RESEARCH ASSOCIATION

IN addressing the forty-second annual general meeting of the Wool Industries Research Association on April 19, the chairman of the Association, Mr. B. A. Smith, after referring to the opening of the new Worsted Processing Block at Torridon on March 22 (see *Nature*, 190, 313; 1961) by the Right Hon. R. G. Menzies, stressed the need for more research and technical education in the industry. The Association was just ending a quinquennial period in which the grant from the Department of Scientific and Industrial Research had been based on £170,000 of industrial income; under the new £70,000 arrangements this grant would be £59,000 on £170,000 of industrial income, but with a further £75 for every £100 supplied by industry up to a total of £25,000, and Mr. Smith asked for support for any proposal in the near future for an increase in levy.

The report of the director of research, Dr. A. B. D. Cassie, for 1960* refers to progress in constructing an apparatus for the automatic quantitative analysis of mixtures of amino-acids by separating them on a cationic exchange–resin column, converting to copper complexes, and determining the copper content polarographically. Excellent results have been obtained in the automatic assay of basic amino-acids, but citrate buffers cannot be used for chromatography because of the formation of copper-citrate complexes, and new buffer systems are being developed for amino-acid separation. Several protein components of oxidized wool have been isolated by percolating columns of the material with 5 *M* urea solution, gradually changing in *p*H from 3·0 to 5·8, and determinations by Sanger's method of the N-terminal groups of the components isolated from wool following treatment with acid gave average chain-weights of 96,500 and 20,500 for component I and component III, respectively.

Search for a method of determining the moisture content of raw wool continued, and a general survey being made of the diffusion constants of a wide range of organic molecules, from toluene and benzyl alcohol to a non-ionic detergent of mol. wt. 558, indicates that the mobility of a molecule in keratin depends more on its hydrophobic–hydrophilic balance than its size. Work completed on the breaking-time and dynamic elastic modulus of nylon confirms that changes in moisture content affect both the elastic properties and the strength of the structural elements as well as the internal viscosity.

Recent work has shown that at least one of the causes of yellowing of woollen yarn on storage is the presence in the olein of small amounts of polyunsaturated acids: linolenic acid causes permanent yellowing, but the effect of linoleic acid is less important, while pure oleic acid has little deleterious action. Laboratory and works trials have shown that the moth-proofing agent 'Dielmoth' can be applied to worsted yarn during normal bleaching processes, but the treated yarn had only poor washing fastness. Fabric construction has been shown to play an important part in the production of fabrics with durable pleats or creases.

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* Wool Industries Research Association. Report of the Director of Research for 1960. (Publication No. 215.) Pp. 39. (Torridon, Headingley, Leeds: Wool Industries Research Association, 1961.)

THE BENGUELA CURRENT

IN 1953 two interesting articles appeared in *Nature*. The first was by Currie[1] and described the process of upwelling in the Benguela current. He showed that in addition to the upwelling inshore, a convection cell develops at the edge of the continental shelf which separates the cold upwelled waters from the warm oceanic waters further out to sea. This line of demarcation is used in a recent report* to define clearly what is meant by the Benguela current: "We have restricted the use of the name Benguela current to the coastal circulation in which upwelling movements occur and have referred to the oceanic circulation as the 'south-east trade wind drift'."

The second article was by Hart[2] and dealt with the preliminary results of the phytoplankton surveys. He stressed the distinction between oceanic species of the south-east trade-wind drift and the coastal forms in the rich upwelled waters. In the latter he introduced the new conception of ageing of upwelled water, by which was meant the succession of species as the characteristics of the water mass change.

In the present report the two authors have enlarged on these preliminary findings. New data on winds, surface drift, bottom topography, water temperatures, salinities, dissolved oxygen, phosphate and the species composition, density and distribution of the microplankton have been analysed and co-ordinated to form an impressive picture of the oceanography of the seas off south-west Africa.

After summarizing the results of earlier investigations, the two cruises of R.R.S. *William Scoresby* off south-west Africa are described. The first was in the southern autumn (March 3–14, 1950) and the second was in spring (September 21–October 12). They go on to show that the winds are the driving force behind the currents. Off the shelf, the south-east trades cause a drift which Defant[3] has shown to be north-north-west, parallel to the coast. Nearer the coast and on the shelf itself, there are coastal winds which are mainly south-south-west and these induce a current away from the shore and upwelling results. Many charts and sections show that this upwelled water differs from the oceanic water in temperature, salinity, dissolved oxygen and phosphate concentration. In spite of complexities due to swirls and interlocking tongues of water, the dividing line lies approximately over the edge of the continental shelf. Moreover, the vertical sections show clearly that on this line the isotherms and isohalines above the 300-m. level have an upward kink and that water from the 200–300-m. level reaches the surface inshore. The kink in the isotherms is the result of the convection cell which separates the two water masses and is well illustrated in a section which shows the distribution of the anomaly of specific volume off the mouth of the Orange River during the spring survey when active upwelling was in progress. It is suggested that conditions in the Benguela current are similar to those in upwelled waters off California where Sverdrup[4] has described a similar convection cell.

The evidence that the upwelled water comes from the 200–300-m. level denies once and for all the vague idea that the Antarctic intermediate current is concerned, for the latter lies at the 600–800-m. level. The water at the 200–300-m. level is part of the South Atlantic central water and its origin is controversial. Possibly it is the result of sinking at the sub-tropical convergence. Off south-west Africa there is a general movement to north-north-west at this level, but there are also indications, based on admittedly slender evidence, of a southerly movement along the edge of the shelf. "It is postulated that this is a compensatory movement replacing the water which has been drawn up to the surface inshore and we have therefore called it the compensation current." Vertical sections of salinity, temperature and phosphate show no discrete body of water at this level, nor is the existence of this southerly current essential to the main argument, and I doubt its existence. Indeed, the distribution of the oxygen minimum layer in the South Atlantic shown in Fig. 47 is more readily understandable as a north-westerly drift of the products of decay from the highly productive areas off south-west Africa.

The second part of the report deals with the microplankton as sampled by *N.50.V.* nets. The bulk of the catch was diatoms and peridinians. After dealing with the vexed question of terminology in which the term 'panthallassic' is revived to cover species which at different times are both neritic and oceanic, the authors give a full list of the species identified and select from this the dominants for further discussion. Their distribution is charted and their density discussed in relation to distance from the shore. In broad outline they found a dense growth of chætocerids and some other neritic species in the area of upwelling, a scant growth of *Planktoniella sol* and other oceanic forms off the shelf and a poor flora of panthallassic species in between. It is stressed that the many differences between the spring and autumn surveys may not be purely seasonal effects but the result of ageing of upwelled waters: "The more extensive coastal production in autumn (survey 1) with numerous resting spores of the dominant chætocerids is thought to represent a late stage in the succession of waters earlier enriched by upwelling . . . the succession may be repeated several times annually." This section ends with valuable notes on the ecology and distribution of the more important species and most ecologists would agree with the final warning that it is not the presence or absence of a particular species which defines a particular water mass but the differing proportions of the species within them. Statistical methods for the discrimination of floristic groups are now being developed.

Finally, the authors come to the controversial question of the mass mortality of fish in the Walvis Bay area and the cause of the anaerobic deposits which extend along the shelf for some 400 miles.

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* Discovery Reports, Vol. XXXI. *The Benguela Current*. By T. John Hart and Ronald I. Currie. Pp. 123–298. (Cambridge: At the University Press, 1960.) 75*s.* net.

Brongersma-Sanders[5] has suggested that the mass mortality is due to poisonous blooms of dinoflagellates such as *Gymnodinium*, but this is discounted. It is argued that the decay of dense plankton on the shelf leads to a depletion of oxygen at the lower levels. Under normal meteorological conditions the currents bring in new supplies of oxygenated water, but during periods of calm, stagnation occurs and anaerobic conditions develop at the bottom which favour the growth of sulphate-reducing bacteria and the evolution of poisonous sulphuretted hydrogen. Oxygen depletion actually extends to the surface inshore and there are press reports from Swakopmund of the sea bubbling with hydrogen sulphide and mud islands rising to the surface. This, the authors infer, is the cause of the mass mortalities of fish, and certainly their meteorological evidence and vertical sections of dissolved oxygen tie in neatly with the theory.

The authors have shown that the Benguela is one of the richest currents in the oceans of the world. This impressive report provides a firm basis for its further study.

J. H. DAY

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[1] Currie, R. I., *Nature*, 171, 497 (1953).
[2] Hart, T. J., *Nature*, 171, 631 (1953).
[3] Defant, A., *Landerkdl. Forsch. Festschr. N. Krebs.*, 52 (1936).
[4] Sverdrup, H. U., *J. Mar. Res.*, 1, 155 (1938).
[5] Brongersma-Sanders, M., *Arch. Needrl. Zool.*, 7, 291 (1945).

NATIONAL FORESTS OF AGRICULTURAL CROPS FOR THE WET HUMID TROPICS

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THE natural vegetation of the wet humid tropics consists of extensive forests of large and valuable timber trees. These large trees are of paramount importance in protecting the soil of the hillsides from erosion and its consequences, and they make the best use of the leached and acidic soils commonly found in the wet humid tropics. This forest of large trees has been, and is being, selectively destroyed to make way for 'planned forests' of agricultural crops such as rubber and oil palm, which are also able to make good use of such soils. There has been, however, a certain amount of misuse of such land in certain areas where food crops, mostly annuals, such as vegetables and short-lived perennials, such as papayas, have been cultivated. Such crops do not provide adequate cover to the soil, which is exposed to the elements and gives rise to serious soil erosion and unproductivity of the land. Such land has often been abandoned to fallow, and a system of shifting cultivation has been practised.

An ecological and economic study of the wet humid forests of certain agriculturally important large tree crops will show that there is a definite place for developing national forests of certain agricultural crops, which can grow under semi-wild conditions and which need occasional attention for satisfactory growth and crop-production. In Malaya, such agricultural crops are *Durio*, *Nephelium*, *Bertholletia*, *Garcinia*, *Lansium*, *Baccaurea* and *Metroxylon* species.

Most of the tree crops mentioned have the following characteristics :

(*a*) A number of them to this day is grown successfully under semi-wild conditions, and it is doubtful whether it is more economical to grow them under refined cultural techniques.

(*b*) The trees grow to a large size and would function effectively as binders of the soil and efficient utilizers of leached and acidic soils, as the natural forest-trees do.

(*c*) It is questionable whether such crops should be grown by smallholders who have only small pieces of land.

(*d*) The trees take 7–15 yr. to mature and are therefore not ideal for smallholders who lack capital for growing perennial crops. On the other hand, perennial crops are ideal from the point of view of the little work required to maintain crop production.

(*e*) The trees are seasonal in bearing, although there is one season of heavy bearing and another negligibly light one annually.

(*f*) There is negligible damage done to the trees in the process of harvesting the produce and is unlike rubber-tapping, which is done daily, and where inexperienced labourers can damage the trees permanently.

Therefore seasonality of fruiting, semi-wild conditions of growth to large trees and negligible damage done to such trees during the harvest provide conditions which not only find employment for labour that is occasionally unemployed at certain times of the year in the country, and prevent erosion of soil which is a national asset, but also permit the public to harvest the produce on annual lease, which can be called by tender.

Some of these trees have good durable timber, and should the trees go out of production (and this will only occur after several decades) the timber produced would give some remuneration.

At this point a question may be raised about finding planting material and the need for ensuring that planted trees bear high quality and quantity of produce. This is possible by adopting the following theoretically sound but yet unproved technique :

Seeds from trees selected for quality and quantity of produce will, in most cases, be satisfactory as planting material since this technique is practised to this very day in most of these tree crops. However, the individuals will vary in quality and quantity of the produce. In order to minimize the variability it is suggested that in planting the seedlings, twice or thrice the usual number of plants, at closer spacing, can be planted within a unit area, so that it would be possible to destroy progressively half the number of the poorer trees within a unit area, from the time the trees start bearing, to permit good growth of the remaining better trees.

By these methods, therefore, it is theoretically possible to create national forests of agricultural crops in the wet humid tropics where large acreages are still under forests. However, capital will be the problem and this may be found should the national policy-makers be convinced that the idea of national forests of agricultural crops in the wet humid tropics is sound not only theoretically but also practically.

GAS-LIQUID CHROMATOGRAPHY OF STEROL METHYL ETHERS

By R. B. CLAYTON

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SEVERAL reports have appeared recently of the separation of sterols and steroids by gas-liquid chromatography on a variety of liquid phases[1-4]. Up to the present time, however, no correlation has been reported between the structures of steroids and their behaviour on the gas-liquid chromatogram which might allow an accurate prediction of retention times on the basis of molecular structure[5]. Such a correlation is being attempted with the methyl ethers of neutral sterols chromatographed on polyethylene glycol succinate as the liquid phase and some preliminary findings are discussed here.

Preparation of sterol methyl ethers on a 'micro' scale (5–500γ) was carried out in 78–90 per cent yield by reaction of the sterols or their esters with methyl iodide in ether solution in the presence of potassium *t*-butoxide.

Gas-liquid chromatography was carried out using 6 ft. × 4 mm. metal columns packed with 5 per cent polyethylene glycol succinate on 'Chromosorb *W*'. The column was conditioned at 202° for 48 hr. with a slow argon flow. Operating conditions were: column 195°, vaporizer 350°, ionization detector 212°. Gas flow: 220 c.c. per min. (argon). Retention times, tabulated below, are relative to that of cholestane (retention time, 8·5 min.).

The values recorded in Table 1 show that good separations can be achieved with a number of sterols of biochemical interest and with retention times usually between 30 and 60 min. The technique has been applied to the analysis of sterols and their esters isolated from tissues by established methods.

Table 1. RELATIVE RETENTION TIMES OF STEROLS AS METHYL ETHER

	Cholestane	1·00	XVIII	$\Delta^{7,22}$-Ergostadienol	5·55
I	Cholestanol	3·60	XIX	$\Delta^{5,7}$-Ergostadienol	7·30
II	epi-Cholestanol	2·61	XX	$\Delta^{5,7,22}$-Ergostatrienol	6·52
III	Coprostanol	2·36	XXI	$\Delta^{5,24(28)}$-Ergostadienol	6·08
IV	epi-Coprostanol	3·08	XXII	Stigmastanol	6·03
V	Cholesterol	3·90	XXIII	Δ^5-Stigmastenol	6·60
VI	Δ^6-Cholestenol	4·15	XXIV	$\Delta^{5,22}$-Stigmastadienol	5·80
VII	Δ^7-Cholestenol	4·70	XXV	$\Delta^{5,24(28)}$-Stigmastadienol	7·86
VIII	Δ^8-Cholestenol	3·88	XXVI	Δ^{22}-Stigmastenol	5·40
IX	$\Delta^{8(14)}$-Cholestenol	3·73	XXVII	Lanosterol	5·32
X	$\Delta^{8,14}$-Cholestadienol	4·56	XXVIII	24,25-Dihydrolanosterol	4·00
XI	$\Delta^{5,22}$-Cholestadienol	3·83	XXIX	Agnosterol	5·60
XII	$\Delta^{5,7}$-Cholestadienol	5·50			
XIII	$\Delta^{5,24}$-Cholestadienol	5·40			
XIV	Ergostanol	4·81			
XV	Δ^5-Ergostenol	5·12			
XVI	$\Delta^{5,22}$-Ergostadienol	4·43			
XVII	Δ^7-Ergostenol	6·05			

The values shown in Table 1 are most readily interpreted in terms of the operation of three distinct factors: (1) the molecular weight; (2) the polarity; and (3) a 'conformational restriction' factor. These are considered in turn.

(1) *Molecular weight.* The stanols I, XIV. XXII form a series in which the increase in relative retention time is proportionate to the increase in molecular weight. They deviate from the log relationship of true homologous series[6].

(2) *Polarity.* The simplest polarity effects (analogous to those found in adsorption chromatography) are seen in comparing the stanols I and III with their epimers II and IV respectively. Clearly, the more hindered, axial orientation of the three methoxyl group renders the compound more mobile than its epimer in which this group is equatorial. In certain circumstances the weight and polarity factors can largely offset each other. The 4 : 4-dimethyl grouping of XXVII, XXVIII and XXIX (C_{30}) is well known to hinder the 3β oxygen function. Hence these compounds have greater mobilities than XXII (C_{29}).

Compounds having olefinic bonds in the nucleus show greater retention times than their parent stanols. The Δ^7-bond exerts the greatest effect (cf. I and VII, XIV and XVII) of any isolated double bond so far studied. The homoannular, 5,7-diene increases the retention time still further (cf. I and XII, XIV and XIX).

Table 2. RETENTION RATIOS FOR UNSATURATED STRUCTURES OF DIFFERENT MOLECULAR WEIGHT

Retention ratio*	Cholestanol	Ergostanol	Stigmastanol	Lanostanol
Δ^7/stanol	1·3	1·26	+	+
$\Delta^{5,7}$/stanol	1·52	1·52	+	+
$\Delta^{5,7}/\Delta^7$	1·17	1·2	+	+
Δ^5/stanol	1·08	1·07	1·09	+
$\Delta^{5,22}/\Delta^5$	0·98	0·87	0·88	+
$\Delta^{5,7,22}/\Delta^{5,7}$	+	0·89	+	+
Δ^{22}/stanol	+	+	0·90	+
$\Delta^{8,24}/\Delta^8$	+	+	+	1·33
$\Delta^{5,24}/\Delta^5$	1·38	+	+	+
$\Delta^{5,24(28)}/\Delta^5$	+	1·18	1·19	+

$$* \text{ Retention ratio} = \frac{\text{relative retention time of compound with } \Delta^x}{\text{relative retention time of analogue lacking } \Delta^x}$$

+ Compounds necessary for comparison not available.

Table 2 lists the ratios ('retention ratios') of the relative retention times of various compounds having certain features of unsaturation to those of their analogues which lack these features. These results show that the introduction of an isolated double bond, Δ^x, into a stanol, s, will change its relative retention time, r_s, to a new value $r_{(s+x)}$, according to the relationship:

$$r_{(s+x)} = r_s \times k_x$$

where k_x is a constant ('retention constant') for the double bond in position x, which is virtually independent of molecular weight. Further, for two non-interacting double bonds at positions x and y (for example, Δ^7 and Δ^{22} in the ergostane series) the relative retention time of the new $\Delta^{x,y}$-dienol is given by:

$$r_{(s+x+y)} = r_s \times k_x \times k_y$$

It is apparent that the 5,7-diene must be treated as a unit system in these calculations and the contribution of the Δ^{22}-bond shows an anomaly which is discussed below.

(3) '*Conformational restriction*'. Double bonds originating at C_{24} in the side-chain raise the retention time considerably, but the Δ^{22}-bond fails to increase the retention time. A scarcely measurable negative effect is seen in the cholestane series (V and XI), but in the ergostane and sitostane series the presence of a double bond at C_{22} markedly reduces the retention time. This apparent anomaly becomes intelligible from an examination of molecular models. In compounds without substitutents at C_{24}, the Δ^{22}-bond only slightly restricts the movement of the side-chain, whereas in C_{24}-substituted compounds this restriction is far more pronounced. Such a restriction could lead to some curtailment of solute-solvent interaction and prevent the dissipation of thermal energy in certain modes of intramolecular vibrations which

are available when the side-chain is saturated and hence less restricted. These effects would tend to increase the mobility of these compounds and apparently are great enough to overcome the opposing polarity effect of the Δ^{22}-bond. In Δ^{22}-cholesterol, it must be assumed that the restriction imposed on the side-chain approximately cancels out the increase in polarity which the additional double bond might otherwise confer on cholesterol.

Probably a similar conformational restriction is responsible for the difference between the retention times of cholestanol (I) and epi-coprostanol (IV).

It appears that some advance is being made in this work towards a rationalization of the behaviour of polycyclic compounds on the gas-liquid chromatogram. The concept of 'retention constants' for variously situated functional groups, such as those derived for the double bonds in this work, is an attractive one and may be of considerable practical and theoretical value. However, such constants obviously can only be used with scrupulous attention to the details of the intramolecular environment of the functional groups in question. Moreover, the influence, if any, of the nature of the liquid phase on the values of these constants remains to be determined.

I wish to thank Prof. Konrad Bloch for his interest in this work, which was supported by a grant from the American Heart Association and was conducted during tenure of an established investigatorship of the American Heart Association.

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THE 'LAG-PHASE' IN SALT UPTAKE OF STORAGE TISSUE

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WHEN various dormant storage tissues are washed in aerated water, a characteristic delay in the generation of an absorption capacity occurs for which a wide variety of explanations has been offered. Rees and Skelding[1] suggested that a specific water-soluble inhibitor was present in the tissue which would prevent salt absorption until it had been leached from the tissue. In a later publication, Skelding[2] further supported this view and suggested that the inhibitory substance was formed by dark fixation of accumulated respiratory carbon dioxide. An alternative view, that salt uptake was stimulated as a result of continuous removal of electrolytes from the washed tissue, has been quite persistent[3–5], but was recently disproved by MacDonald *et al.*[6].

Unfortunately, the matter at issue is confounded by the consistent use of tap water, which may vary widely in its mineral contents, and in which the calcium ions present have the property of reducing leakage[7,8]. Dale and Sutcliffe[9], finding that certain organic acids, and also beetroot extracts containing the same organic acids, would stimulate potassium uptake of disks of beetroot tissue washed for a relatively short period (48 hr.), claimed that the lag-phase in potassium uptake was an artefact, and merely a reflexion of a time lag in the development of a chloride accumulatory system. Their results, however, showed that disks which had been washed for 2 hr. could not be stimulated. Probably the most predominant contention at present is that the lag-phase is a period during which a gradual revival of metabolic activity provides the energy required for the formation or activation of carrier molecules participating in the accumulation process[10,11], or for the protein synthesis which is thought by Steward *et al.*[12,13] to be the first requirement for salt uptake.

During the course of testing various compounds for their effect on the lag-phase of beetroot tissue, it was found that 2-amino-2(hydroxymethyl)-propane-1 : 3-diol (*tris*-(hydroxymethyl)aminomethane, or *tris*) had the remarkable property of inducing a rapid rate of uptake of potassium ions immediately after cutting the disks of tissue.

Disks of beetroot (var. Crimson Globe) prepared in the usual manner (15 mm. diameter, 1 mm. thick, average weight approximately 180 mgm.), were rapidly washed in three rinses of distilled water to remove materials released from the cut cells before commencing the experimental treatment. Batches of 25 disks (4·5 gm.) were added to each of the 150 ml. of aerated solutions to be tested, kept in 200-ml. boiling tubes and placed in a thermostat. Each treatment was done at least in triplicate. *Tris* solutions were adjusted to the required *p*H with hydrochloric acid. At required intervals, 5-ml. samples of the solutions were drawn off, and replaced by an equal volume of the original solutions, which caused a negligible change in salt concentration. Potassium and sodium contents were determined with an *EEL* flame photometer. The technique is based on the principle that changes in the absorptive capacity of the tissue are detected much more easily at very low than at high salt concentrations.

Fig. 1,*A* and *B* include typical curves for the potassium ion content of the external solution when disks of beetroot were incubated in distilled water. An initial rapid rise of potassium ions in the solution must be expected to originate from the apparent free space and Donnan free space of the tissue. However. an equilibrium should have been reached within the first hour[14], and the continued leaching of potassium ions up to 16 hr. must come from other sources, most likely the cytoplasm and/or the vacuole. The subsequent slow loss of potassium ions from the external solution occurred in the majority of cases; however, with quite a few roots, especially the older ones, leakage surpassed absorption at this stage, resulting in a net loss of potassium ions from the tissue. A marked change always set in at approximately 30–40 hr. from the start of the experiment, resulting in a period of rapid absorption, quite often causing the concentration of potassium ions of the external

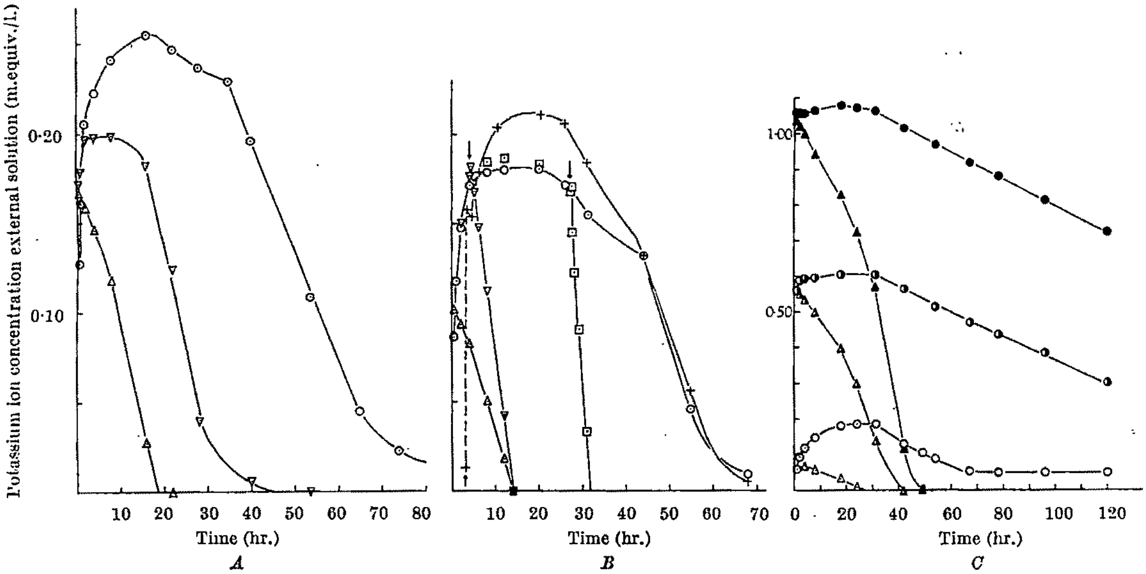

Fig. 1 *A*, *B* and *C*. Potassium ion content of the external solution (m.equiv./l.) due to leaching and subsequent re-absorption of calcium ions by red beet slices. *A*: ⊙, distilled water *p*H 6·3–6·4; △, 10^{-2} *M tris* *p*H 7·9–8·0; ▽, 10^{-2} *M tris* *p*H 6·0–6·3. Temp. 20° C. *B*: ⊙, distilled water *p*H 6·2–6·5; △, 10^{-2} *M tris* *p*H 7·9–8·0; ▽, 10^{-2} *M tris*, 4 hr. from start of experiment, added as 7·5 ml. 2×10^{-1} *M* solution, *p*H 7·85–8·0; ⊡, 10^{-2} *M tris*, 27 hr. from start of experiment, *p*H 8·0; +, 10^{-2} *M tris* for 2 hr., *p*H 8·0, followed by two rinses of ½ hr. duration each (*p*H 6·7 and 6·1, respectively), 1 ml. 0·019 *M* potassium chloride added to make up for lost potassium ions during rinsing of tissue. Temp. 21–22° C. *C*: ⊙, distilled water, *p*H 5·55–6·15; △, 10^{-2} *M tris*, *p*H 7·80–7·95; ◑, $0·5 \times 10^{-3}$ *M* potassium chloride solution, *p*H 5·50–5·95; ◭, $0·5 \times 10^{-3}$ *M* potassium chloride + 10^{-2} *M tris*, *p*H 7·80–7·90; ●, 10^{-3} *M* potassium chloride, *p*H 5·50–6·00; ▲, 10^{-3} *M* potassium chloride + 10^{-2} *M tris*, *p*H 7·50–7·80. Temp. 23° C.

solution to drop to less than 10^{-6} *M*. With an average potassium ion concentration in the tissue of close to 10^{-1} *M*/kgm., this meant that under these experimental conditions accumulation ratios of 10^5 and more were observed for potassium ions, which is much higher than any of the values recently quoted and observed by MacDonald *et al.*[6].

When 10^{-2} *M tris* (*p*H 8·0, adjusted with hydrochloric acid) was applied, more potassium ions appeared in the external solution during the first half-hour; presumably *tris* ions replaced a proportion of the potassium ions in the Donnan free space. However, this was immediately followed by a rapid uptake at a linear rate.

When the *p*H of the *tris* solution was adjusted to 6·0–6·3, leakage continued over a longer period (up to 2 hr.), and *tris* was less effective in reducing the lag-phase. A very similar curve to that at *p*H 6·0–6·3 was obtained when *tris* was applied at 10^{-3} *M* and *p*H 8·0. *Tris* has a pK_a value of 8·14 (ref. 15), and is 98·6 per cent ionic at *p*H 6·3; assuming that the base of *tris* is more effective than its ionic form, the reduced effect of *tris* at *p*H 6·0–6·3 could be ascribed to the lowering of its effective concentration.

Tris added at 4 and 27 hr. respectively from the start of the experiment had an immediate and greatly accelerating effect on uptake apart from freeing some potassium ions of the Donnan free space during the first 10 min. (Fig. 1,*B*). In another treatment, disks were immersed for 2 hr. in *tris* and were then washed with two quick rinses in water. On replacement in water containing potassium ions, equivalent to the amount lost during the rinses, it can be seen (Fig. 1,*B*, curve — + —) that the effect of *tris* on the net influx was not maintained after the *tris* had been washed away.

It yet remained possible that part of the difference between *tris* and control treatments could be due to the presence or absence of suitable anions in the respective solutions. Hence, the results of an experiment in which either $0·5 \times 10^{-3}$ *M* or 10^{-3} *M* potassium chloride was added to the external solutions are presented in Fig. 1,*C*. It can be seen that the presence of an initial amount of potassium chloride had no effect on the length of the lag-phase, or on the rate of uptake in control solutions, while it substantially increased the rate of uptake in the presence of *tris*.

Sharp contrasts were provided by the effect of *tris* on the lag-phase of other tissues (Fig. 2). Although extremely effective on carrot and parsnip tissue, it had no such effect on artichoke, swede, potato and turnip

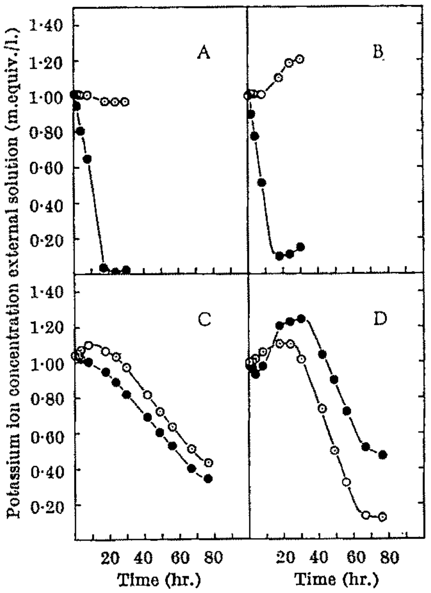

Fig. 2. Change in potassium ion content of the external solution, due to uptake or release of potassium ions at 23° C. by *A*, carrot phlœm tissue; *B*, parsnip phlœm tissue; *C*, artichoke tissue (*Helianthus*); and *D*, swede tissue. ⊙, disks in 10^{-3} *M* potassium chloride solution, *p*H 6·05–6·85, 6·00–6·70, 6·10–7·20 and 6·00–7·10 for *A*, *B* *C* and *D*, respectively; ●, disks in 10^{-3} *M* potassium chloride + 10^{-2} *M tris* solution, *p*H 7·65–7·95, 7·50–7·95, 7·85–8·00 for *A*, *B*, *C* and *D*, respectively

tissue. Hence, the effects of *tris* are quite specific, and in the case of beetroot, parsnip and carrot the characteristics of the molecule seem to fit certain requirements to make a rapid and immediate uptake of potassium ions possible.

Experiments with some readily available analogues of *tris* showed that the presence of OH-groups were essential for activity, *tris*, 2-amino-2-methyl-1 : 3-propanediol, 2-amino-2-methyl-1-propanol and 2-amino-2-methyl-propane (tertiary butylamine) form a series of declining activity (Fig. 3,*A*). In fact, tertiary butylamine had no effect on the lag-phase, and the slightly better intake of potassium ions over the distilled water treatment could have been partly due to the difference in *p*H of the solutions.

Triethanolamine and *tris* were equally effective, indicating that the nature of substituents rather than degree of substitution at the N-level was of prime importance, namely, triethanolamine was much more effective than ethanolamine while N,N-dimethyl-aminoethanol had no effect on the lag-phase (Fig. 3,*B*).

It was observed on an earlier occasion that the addition of 10^{-2} *M* calcium ions shortened the lag-phase to approximately 12 hr.[16]. This effect, rather remarkable in itself, could most probably be related to the so-called 'Viets' effect[17], that is, the stimulation of potassium ion absorption by the presence of moderately high concentrations of calcium ions, which has received considerable attention, because of its rather paradoxical nature[18,19]. Because some relationship might exist between the shortening of the lag-phase induced by the presence of calcium ions and that induced by *tris*, experiments were carried out to study the interaction of these effects. Results presented in Fig. 3,*C* show unequivocally that the effect of calcium ions prevailed when disks were immersed in a mixture of 10^{-2} *M* calcium chloride and 10^{-2} *M tris*, while, on the other hand, additions of 10^{-5}–5×10^{-5} *M* ethylenediaminetetraacetic acid (EDTA) to the medium have consistently produced small but significant enhancements of the *tris* effect (Fig. 3,*C*). Hence, it is feasible that *tris* and calcium ions compete for a common site, the bivalent calcium ions being much more efficient in this respect than the univalent *tris* ions.

The mechanism of the *tris* and calcium effects cannot be defined at present at all closely. It has been shown that calcium ions are essential for membrane formation or maintenance[20], and there is little doubt that the calcium ion status of the membrane plays an important part in the characterization of its physical and chemical properties[21,22]. It would be premature to make a pronouncement whether *tris* operates as a carrier molecule, or indirectly exerts its influence through its adsorption to the membrane structure: however, the absence of a sustained effect when *tris* is removed externally, the fact that calcium ions also can have a shortening effect on the lag-phase, and that *tris* and calcium may compete for a common site in that respect, make the second alternative more likely at present. In the presence of *tris*, accumulation ratios for potassium ions were of the order of 5×10^5, the highest ever observed. Hence, it appears that *tris* caused such change as to facilitate the approach and incorporation of potassium ions from the external solution into the membrane. For example, if a carrier operated across the plasmalemma, it could only be effective as such when the active part of the molecule is exposed at the external side of the membrane. The presence of *tris* may condition the membranes in such a way, possibly through the unfolding or swelling of proteinaceous material[23,24], as to facilitate this exposure. According to Klotz and Ayers[23] suggestions, the latter effect would increase with an increase in *p*H.

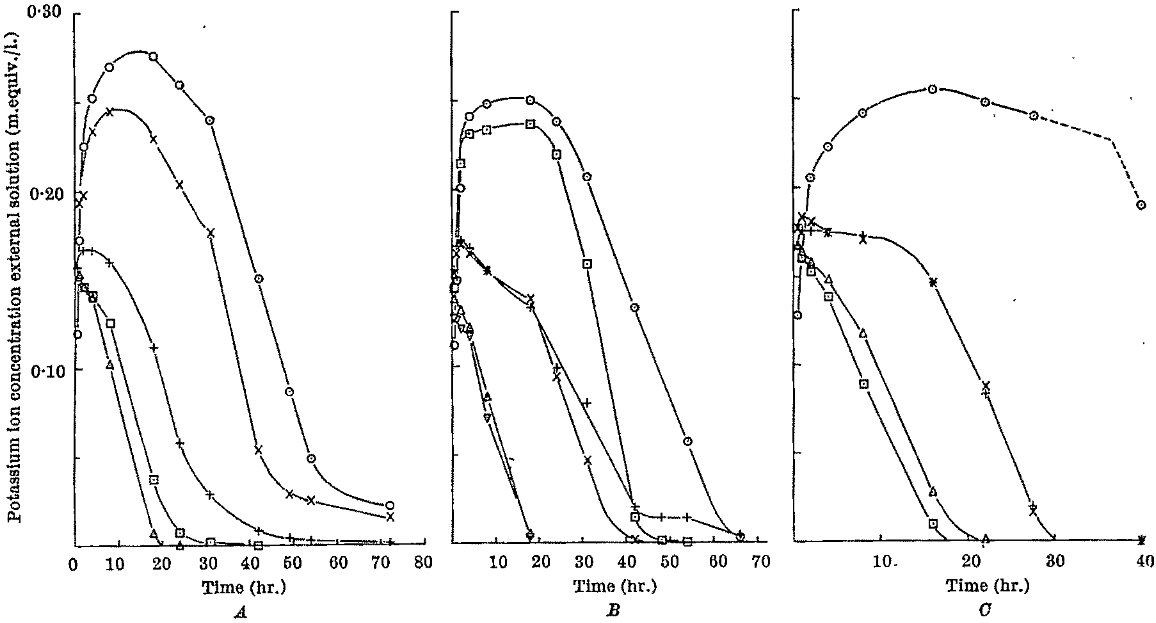

Fig. 3 *A*, *B* and *C*. Potassium ion content of the external solution (m.equiv./l.) due to leaching and subsequent re-absorption of potassium ions by red beet slices at 24° C. *A*: ⊙, distilled water, *p*H 6·35–6·70; △, 10^{-2} *M tris*, *p*H 8·20–8·35; ⊡, 10^{-2} *M* 2-amino-2-methyl 1 : 3 propanediol, *p*H 8·05–8·30; +, 10^{-2} *M* 2-amino-2-methyl 1-propanol, *p*H 8·00–8·10, *p*H adjusted with base during the course of the experiment; ×, 10^{-2} *M* 2-amino-2-methyl propane (tertiary butylamine), *p*H 7·80–7·95, *p*H adjusted with base. *B*: ⊙, distilled water, *p*H 6·25–6·55; △, 10^{-2} *M tris*, *p*H 8·1–8·2; ▽, tri-ethanolamine 10^{-2} *M*, *p*H 7·8–8·2, *p*H adjusted with base during the experiment; ⊡, N,N-di-methyl amino-ethanol 10^{-2} *M*, *p*H 7·8–8·0 (adjusted); ×, methyl amino-ethanol 10^{-2} *M*, *p*H 7·7–8·2 (adjusted); +, ethanolamine 10^{-2} *M*, *p*H 7·4–8·1 (adjusted). *C*: ⊙, distilled water, *p*H 6·3–6·4; △, 10^{-2} *M tris*, *p*H 7·9; +, 10^{-2} *M* calcium chloride, *p*H 8·0 → 7·0 → 7·5 (adjusted with saturated calcium hydroxide); ×, 10^{-2} *M* calcium chloride + 10^{-2} *M tris*, *p*H 7·8; ⊡, 10^{-2} *M tris* + 10^{-5} *M* ethylenediaminetetraacetate, *p*H 7·9

The existing data[25–28] show little effect of *p*H on uptake of potassium ions between 6 and 8 except for Hurd's[29] results, which, however, apply to uptake in aged disks and which show stimulation of uptake at *p*H 8·5. Hurd's results did show the importance of HCO_3^- with aged disks, and he regarded *tris* as an indifferent buffer, which is certainly not true for uptake during the lag-phase.

The present results do not support the views that leaching of inhibitors or of cations are pre-requisites for ion-uptake. Nor do they suggest that accumulation of carrier molecules in metabolism is essential.

I am much indebted to Prof. T. A. Bennet-Clark for his help and encouragement during the course of the investigations. This work was carried out during the tenure of New Zealand National Research Fellowship.

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PHAGOCYTIC ACTIVITY IN SQUAMOUS EPITHELIA AND ITS ROLE IN CELLULAR SUSCEPTIBILITY TO FOOT-AND-MOUTH DISEASE

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EXPERIMENTAL foot-and-mouth disease in the guinea pig is characterized by epithelial necrosis, and sometimes vesiculation, of squamous epithelium in certain rather sharply localized regions of the body, of which the buccal mucosa, the prepuce and the hairless parts of the feet are the most consistently involved[1]. Skin-grafting experiments have indicated that the mild environmental trauma to which these sites are constantly exposed plays an important part in rendering them susceptible to the virus, and that it probably does so principally through some direct effect on the epithelial cells[2]. Since this might be effected by an influence on the cell membranes, a study has been made in guinea pigs of the response of squamous epithelial cells in the skin and tongue to inert particles. The purpose of the present article is to report the marked phagocytic activity of injured epithelium, and its relevance to the distribution of lesions in foot-and-mouth disease.

The animals used were albino guinea pigs, weighing 500–600 gm., housed and maintained as previously described[2]. The metatarsal pads, palmar pads of the fore-feet, outer surface of the ear, skin of the flank and margin of the prepuce were inoculated intradermally with 0·02–0·05-ml. volumes of particulate suspensions, or tattooed with a sewing needle through a drop of the material on the epithelial surface. In the metatarsal pads, and in grafts of pad on the chest, injections were made via longitudinal intra-epithelial tracks. Injection of the tongue was done under nembutal anæsthesia, and inocula (0·02–0·05 ml.) were introduced into the corium underlying the dorsal epithelium via the lower surface of the tongue. The materials injected were: (1) indian ink (G. T. Gurr, Ltd.); carbon particle size < 0·5–2·0μ; this was heated for 5–10 min. at 100° before use; (2) a 5 per cent suspension of lamp-black (prepared by combustion of benzene) in reconstituted guinea pig serum; particle size < 0·5–6·0μ; (3) suspensions of ground carmine in reconstituted guinea pig serum; particle size 0·5–3·0μ; (4) saccharated iron oxide ('Colliron'; Evans Medical Supplies, Ltd.). This was injected as a clear solution. In each case, an equal volume of hyaluronidase was usually added (final concentration 75–125 turbidity-reducing units/ml.) but it was not essential. The animals were killed 6–18 hr. later, and their tissues were excised, fixed in 10 per cent neutral formalin or in carnoy, embedded in paraffin wax and sectioned at a thickness of 6μ. Sections were stained with hæmatoxylin and eosin for demonstration of carbon, with hæmatoxylin alone for carmine, or with Perl's method for saccharated iron oxide.

In all sites, a proportion of epithelial cells around the needle tracks had usually phagocytized the inert particles. The reaction was most marked in the apparently undamaged cells surrounding the zone of traumatized and necrotic tissue produced by the needle and sometimes cells up to ½ mm. from the edge of this zone contained particles. Cells from the basal layer to the upper part of the stratum spinosum showed an apparently equal capacity for phagocytosis. The amount of material engulfed varied considerably, from occasional cytoplasmic particles, often lying near the nucleus, to dense aggregates forming nuclear caps (Figs. 1 and 2). All the materials were taken up to some extent but the cells appeared to phagocytize the indian ink and saccharated iron oxide rather more readily than the other suspensions. Intra-epithelial injections in the metatarsal pads were frequently accompanied by some hæmorrhage from the dermal capillaries and, in several instances, free erythrocytes had entered the intercellular spaces of the viable epithelium around the track and been phagocytized by cells of the stratum spinosum. Some cells contained both inert particles and erythrocytes.

At the periphery of some of the inoculation sites (hairy skin, prepuce, fore-feet), where the inoculum was in contact with the basal layer remote from the needle track, cells containing particles were very much rarer. They were mostly basal cells with occasional prickle cells. In the tongue dorsum, there was marked phagocytosis by local basal and spinous cells in areas of the epithelium inadvertently damaged during inoculation, but when the suspensions were injected without epithelial damage, no phagocytosis was found.

These results demonstrate that squamous epithelial cells under the influence of local trauma are capable of considerable cell surface activity. This probably arises through activation of their amœboid mechanisms under the influence of reparative stimuli and local loss of contact inhibition[3,4]. The phagocytic activity of the epithelial cells is thus a reflexion of their cell-membrane instability.

These findings are relevant to the pathogenesis of foot-and-mouth disease, and also possibly other virus diseases in which mild traumatization plays a part in the initiation of infection[5]. In the epithelium of the mouth, feet and other sites, where continual low-grade trauma is experienced, cell-membrane instability is likely to be present in some degree. In addition, the dislocation of intercellular contacts resulting from the mechanical stresses to which these epithelia are exposed may also be expected to increase cell-membrane activity, since cells not making good all-round contact display more active pinocytosis[6]. In regions where these conditions exist therefore, the squamous epithelial cells are more likely to take up small particles such as viruses non-specifically, and the degree of stimulation needed for this may be much less than is required for the uptake of relatively gross particles such as carbon, for which an actual wound appears to be necessary. It is likely, therefore, that the distribution of lesions in foot-and-mouth disease is partly attributable to enhanced cell-membrane activity at the predilection sites, thus facilitating the entry of the virus into the local cells.

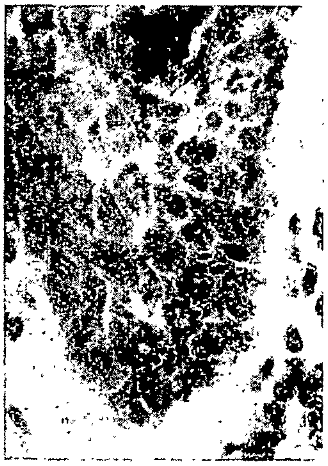

Fig. 1. Metatarsal pad; showing a rete peg with a needle track containing indian ink (above), and phagocytosis of carbon particles by local epidermal cells (hæmatoxylin and eosin, × 500)

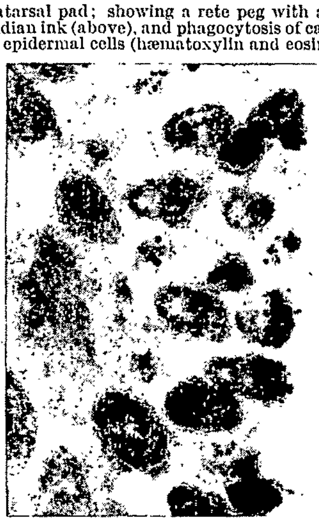

Fig. 2. As Fig. 1; showing dense aggregates of carbon in epidermal cells of a rete peg (× 1,220)

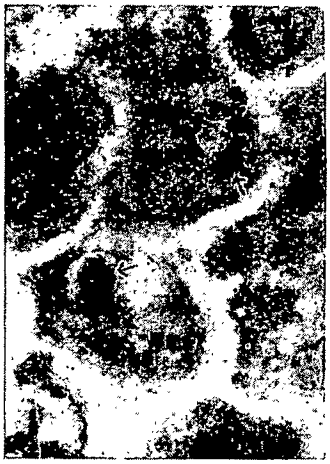

Fig. 3. Metatarsal pad; phagocytized erythrocytes (arrowed) and carbon granules in epidermal cells (hæmatoxylin and eosin, × 900)

The ability of the virus to multiply after cellular penetration presumably depends on highly specific cytochemical conditions within the host cell. In this respect, the cells at the predilection sites appear to provide a more favourable environment for multiplication of the virus than elsewhere in the skin. Thus, injection of foot-and-mouth disease virus directly into the hairy skin of the flank, or into pad grafts on the chest, produces only minimal lesions[1,2], yet the present experiments indicate that virus thus injected would be taken up by local epidermal cells around the puncture. The failure to develop typical lesions suggests that these cells are metabolically insusceptible. The cytochemical conditions necessary for multiplication of foot-and-mouth disease virus are unknown, but it is relevant that cells exhibiting active pinocytosis will take up more nutrients and fluid than their less-active neighbours[6]. Reparative influences may also play some part in determining metabolic patterns favourable to multiplication of the virus in areas of skin and mucous membrane exposed to persistent traumatization.

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THE NUCLEOLUS

Formation of the Nucleolus in Animal Cells

In most animal cells the nucleoli disappear as visible entities during the early stages of mitosis and reappear in the daughter cells when mitosis is completed. The question arises whether the nucleolar material, which is for the most part protein, is synthesized *de novo* after mitosis, or whether it is dispersed at the beginning of mitosis without being degraded and reassembled when mitosis is at an end. In the present article an experiment is described which partially resolves this question for one cell type: the connective tissue cell of the rat.

Dispersed cultures of connective tissue cells were prepared from rat hearts and cultivated in small coverslip chambers[1]. If such cells are incubated for 30–60 min. in medium containing tritium-labelled valine and autoradiographs of the fixed preparations are made, the nucleoli in almost all the cells can be seen to be heavily labelled. (Tritium-labelled valine was prepared by an exchange reaction between tritium gas and the vapour of an ethyl ester of valine on a platinized platinum electrode at 50° C.[2]. The purified compound had a specific activity of 1·5 c./m.-mole.) It has been shown that in these cells exogenous valine is not converted to any significant extent to other compounds but is incorporated almost entirely into the valine residues of protein[3]. The autoradiographs therefore reflect the distribution of radioactive valine which has been incorporated into the proteins of the cell. Amino-acids incorporated in this way are released only very slowly over several days and thus form a relatively stable label for protein. When cells which had been incubated in the presence of radioactive valine are transferred to non-radioactive medium containing unlabelled valine, the specific activity of the valine in the free amino-acid pool of the cells falls within half an hour to negligible levels. Therefore, if cells which have been exposed to tritium-labelled valine are transferred to non-radioactive medium containing unlabelled valine and incubated in this medium until they have all undergone mitosis, autoradiographs of the daughter cells should show unlabelled nucleoli if the nucleolar protein is synthesized *de novo* from free amino-acids immediately after mitosis, and labelled nucleoli if the reappearance of these structures after mitosis involves the aggregation of material which was synthesized earlier.

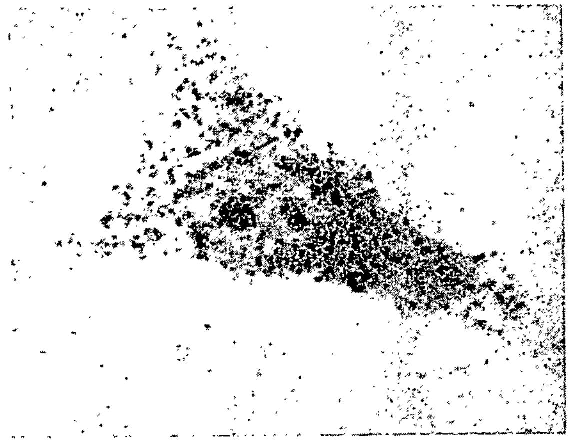

Fig. 1. Autoradiograph of a connective tissue cell incubated for 1 hr. in medium containing tritium-labelled valine. Note the heavy labelling over the nucleoli

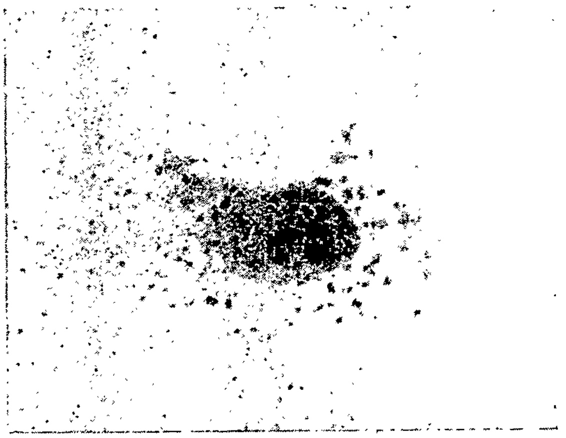

Fig. 2. Autoradiograph of a connective tissue cell from a culture incubated for 1 hr. in medium containing tritium-labelled valine and then for 40 hr. in non-radioactive medium. The nucleolus is still heavily labelled relative to the rest of the cell

The following experiment was undertaken. Replicate cultures of connective tissue cells were incubated for 1 hr. in medium containing tritium-labelled valine at a concentration of 5 μc./ml. At the end of this period some of the preparations were fixed in methanol and then extracted with 0·3 N trichloroacetic acid at 90° C. for 30 min. Autoradiographs of the fixed extracted cells were made by the usual stripping-film technique and developed after 3 days. Fig. 1 shows an autoradiograph of a cell from one of these preparations; it will be noted that the nucleoli are heavily labelled.

Other preparations were washed repeatedly with physiological saline after the period of incubation in radioactive medium and then incubated for 40 hr. in non-radioactive medium containing L-valine at a concentration of 5 mM. The mean generation time of the cells under these conditions was about 24 hr., so that by the end of 40 hr. almost all the cells would have passed through at least one mitosis. The preparations were then fixed and extracted with trichloroacetic acid. Autoradiographs were made and were again developed after 3 days. Examination of these autoradiographs showed that in the great majority of the cells the nucleoli were still heavily labelled relative to the rest of the cell, and unlabelled nucleoli were very rare. Fig. 2 shows an autoradiograph of a cell from a culture incubated for 1 hr. in radioactive medium and then for 40 hr. in non-radioactive medium.

This finding eliminates the possibility that all the nucleolar protein is formed *de novo* from free amino-acids immediately after mitosis. The ratio of the mean number of grains over the nucleoli to the mean number over the rest of the nucleus shows no great change during the 40-hr. cultivation in non-radioactive medium, thus indicating that the nucleolar proteins are not broken down and resynthesized at a rate very different from the rest of the proteins in the nucleus. The reappearance of nucleoli at the end of mitosis must therefore involve the aggregation into a visible structure of proteins which were synthesized before mitosis. The present experiment does not, of course, demonstrate that these proteins are necessarily derived from the pre-mitotic nucleoli, but this does seem to be a likely possibility.

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Evidence for the Continuity of Nucleolar Material in Mitosis

IN a previous article[1] evidence is presented that in pollen grains of maize and agave, basic protein of the pre-mitotic nucleolus was divided without loss, but unequally, between the nucleoli of the resulting vegetative and generative cells. These experiments were repeated in another species and extended to include measurements on the ribonucleic acid (RNA) and the dry mass of the nucleolus. The species studied was *Strelitzia reginae* Banks, which has large pollen grains easily divested of their walls. Each nucleus has only one nucleolus and this is large (about 7μ in diameter at prophase) and not obscured by the relatively small amount of chromatin.

Measurement of Dry Mass. Anthers in which there were both pre- and post-mitotic pollen grains were fixed in acetic-alcohol (1 : 3) for 6 hr. and stored in 70 per cent alcohol at 4° until required. 2-mm. segments of anther were treated by the method of Warmke[2] and squash preparations of the pollen grains were made in 45 per cent acetic acid. With this treatment the walls of the pollen grains were separated from the contents, which could then be flattened. Photometric measurement of azure *B*-binding[3] in sectioned material indicated that the treatment in Warmke's solution did not remove a significant amount of RNA from the nucleoli. The coverslips with the squash preparations were then floated off in distilled water, rinsed twice, mounted in distilled water on other slides and ringed with rubber solution. Each preparation was examined and the positions of all cells suitable for measurement—those which were intact, flat, not folded and not obscured by debris—were noted. In all such cells the path differences of the nucleolus or nucleoli were measured at 5460 Å. with the Baker interference microscope.

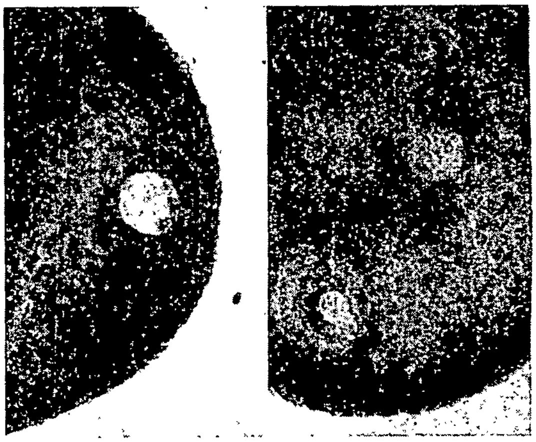

Fig. 3. Pollen grains of *Strelitzia reginae* divested of their walls, flattened and photographed through the Baker interference microscope to show nucleoli. Left, pre-mitotic; right, post-mitotic with vegetative nucleolus top, generative nucleolus bottom (× 550)

The blank reading was made on an area immediately adjacent to the nucleolus. Typical cells are shown in Fig. 3. Cells on which measurements were made were photographed, and the areas of the nucleoli were computed with a planimeter on a drawing of the projected image of the negative.

The coverslips were then floated off the slides and, because cells adhered to the slides and the coverslips, both were treated with ribonuclease at 35° for 3 hr. (0·2 per cent (w/v) in distilled water at *p*H 6). The ribonuclease (L. Light and Co.) was free from protease activity as judged by its failure to release free amino-acids from serum albumen. The slides and coverslips were washed and mounted separately in distilled water. In order to make it easier to find the cells again, the coverslips were placed on fresh slides in the same relative positions as they had occupied on the slides from which they had been removed. Path differences and areas were again measured. The preparations were then stained with azure *B*. Complete absence of stain in the nucleoli confirmed the effectiveness of the ribonuclease.

Results were obtained from two experiments and, because they did not differ significantly, they have been combined in Table 1. There is good agreement between the measurements on the two post-mitotic nucleoli for the total dry mass and the reduction in dry mass resulting from treatment with ribonuclease. It appears that in all three types of nucleoli, RNA

Table 1. MEANS AND STANDARD DEVIATIONS OF MASSES (GM. × 10^{-12}) OF NUCLEOLI IN 45 PRE-MITOTIC AND 23 POST-MITOTIC POLLEN GRAINS

Nucleolus	No.	Total dry mass	Removed by RNA-ase	Remaining after RNA-ase	Mean percentage removed by RNA-ase
Vegetative	23	115 ± 33	26 ± 13	89 ± 31	22·9
Generative		29 ± 10	6 ± 4	23 ± 9	22·3
Sum of veg. + gen.		144 ± 39	32 ± 15	112 ± 35	
Pre-mitotic	45	145 ± 29	33 ± 19	112 ± 25	22·4

Table 2. MEANS AND STANDARD DEVIATIONS OF AMOUNTS OF STAIN IN ARBITRARY UNITS (WHICH ARE DIFFERENT FOR EACH STAIN) IN NUCLEOLI BEFORE AND AFTER POLLEN GRAIN MITOSIS

Nucleoli	Azure *B*		Fast green	
	No. of cells	Amount	No. of cells	Amount
Vegetative	30	84·4 ± 11·8	16	35·3 ± 6·0
Generative		20·0 ± 4·9		9·1 ± 2·5
Veg. ± gen.		104·4 ± 13·0 } $0·6 > P > 0·5$		44·4 ± 6·0 } $0·2 > P > 0·1$
Pre-mitotic	30	102·0 ± 15·9	13	41·2 ± 6·5

accounts for about 22 per cent of the dry mass after fixation (95 per cent fiducial limits of the mean are 20·2 and 24·8 per cent).

Measurement of Basic Protein and Ribonucleic Acid. An anther from the same bud as that used in the previous experiments was fixed in 10 per cent neutral formalin, washed overnight, embedded and sectioned at a thickness of 15μ. The sections were heated in water at 90° for 10 min.[4] and then incubated for 2 hr. at 35° in 0·1 per cent (w/v) deoxyribonuclease. They were then stained in azure *B* bromide by the method of Flax and Himes[3], which is thought to be specific for RNA. The sections were differentiated until nucleoli were the only densely stained cell constituent and then mounted in thick cedarwood oil. Positions of all cells suitable for measurement—those in which the nucleoli were not cut or obscured—were noted. Measurements were made on the cells seriatim across the slide until 30 pre-mitotic and 30 post-mitotic nucleoli had been measured. The amount of stain in each nucleolus was measured at 5050 Å. with an integrating microdensitometer[5]. The blank reading was made on an area immediately adjacent to the nucleolus. The coverslip was then removed, and the slides were hydrated (and destained in the process). They were then stained with fast green *FCF* by the method of Alfert and Geschwind[6], which is thought to be specific for basic protein. During this procedure about half the cells were lost. In those remaining, the amount of stain in the nucleoli was measured at 5800 Å. (The photomultiplier was not sensitive at 6280 Å., the absorption maximum.) The results are shown in Table 2.

Again, the sum of the values for the two post-mitotic nucleoli does not differ significantly from the value in the pre-mitotic nucleolus.

Measurements were made on the amounts of azure *B* and fast green *FCF* in thirteen pre-mitotic nucleoli, and a correlation was found between the amounts of the two dyes bound ($r = 0{\cdot}663$, $0{\cdot}01 < P < 0{\cdot}02$). A similar correlation was found in measurements on sixteen vegetative nucleoli ($r = 0{\cdot}694$, $0{\cdot}01 > P > 0{\cdot}001$), but in sixteen generative nucleoli, which were considerably smaller and more difficult to measure accurately, a significant correlation was not detected because of the magnitude of the errors of measurement. A possible interpretation of this correlation is that the two stains reflect different components of what, in the intact cells, is a single structural entity, for example, ribonucleoprotein.

The demonstration by interferometry that the sum of the dry masses, after fixation, of the post-mitotic nucleoli was equal to the dry mass of the pre-mitotic nucleolus makes it possible to test the validity of an independent method of estimating a nucleolar component. For example, the fact that the amount of azure *B* bound by the two post-mitotic nucleoli together was statistically equal to the amount bound by the pre-mitotic nucleolus tends to confirm the specificity of the staining procedure and also the validity of the method of estimation. Further evidence that the different methods yield similar results is obtained from a comparison of the ratio of the mean values for the vegetative nucleoli to the mean values for the generative nucleoli. In the interferometry experiments the ratio was 3·98 for total dry mass, 4·02 for the mass removed by ribonuclease and 3·97 for the remainder; in the photometry experiments it was 4·22 for azure *B* and 3·88 for fast green *FCF*.

The results presented here give support to the view that the nucleolar material is not degraded during mitosis, but simply dispersed and re-assembled. If the nucleolar material were degraded and then re-synthesized, rather complex regulatory mechanisms would have to be postulated to explain the fact that the total amount of material in the two post-mitotic nucleoli, although these differ in size, is equal to the amount in the pre-mitotic nucleolus. In the light of the autoradiographic results reported in the preceding article, the simplest and most plausible explanation is that a substantial part of the nucleolar material maintains a structural continuity throughout mitosis. It is, of course, possible that only one component of the nucleolus, for example, protein, remains intact and that the amounts of other components, for example, ribonucleic acid, are regulated by the amount of protein present.

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HUMAN HÆMOGLOBIN A_2: CHEMISTRY, GENETICS AND EVOLUTION

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THE normal individual has at least three types of hæmoglobin. In fœtal life and in early infancy he has a specific fœtal hæmoglobin (*F*). Within a few months of birth this hæmoglobin has been replaced by adult hæmoglobin (*A*), together with a small proportion of another hæmoglobin (A_2)[1]. This hæmoglobin A_2 normally amounts to about 2·5 per cent of all hæmoglobins; however, in many instances of thalassæmia minor the proportion is doubled. We wish to report briefly the results to date of chemical studies of hæmoglobin A_2 which shed light on the possible role in thalassæmia of this form of hæmoglobin and on its evolution.

Hæmoglobin A_2 is readily distinguished from the major adult component (A) by its characteristic electrophoretic mobility ; it moves with the abnormal hæmoglobin E at pH 8·6 and pH 6·5 (ref. 1). Like hæmoglobin E, hæmoglobin A_2 shows the same unusual shift in relative electrophoretic mobility at the two pH values. At pH 8·6, hæmoglobin A_2 is close to hæmoglobin C, while at pH 6·5 it is relatively slower and moves close to hæmoglobin S.

Kunkel *et al.*[2] showed that A_2 is truly a separate hæmoglobin and not a chemical derivative of hæmoglobin A. In the ultracentrifuge, hæmoglobins A and A_2 could not be distinguished[2], which implied that their molecular weights are similar ; their spectra in the visible and ultra-violet were also indistinguishable[2]. As a result, the only difference which could be found between the hæmoglobins A and A_2 was their electrophoretic mobility, in contrast to hæmoglobin F, which differs from hæmoglobin A in its ultra-violet spectrum[3] as well as in electrophoresis[4].

Characteristically, hæmoglobin F differs also from A in having four *iso*leucine residues per molecule[5], an amino-acid notably absent from A. Our quantitative amino-acid analysis on the Spinco model Moore and Stein automatic analyser of carefully purified hæmoglobin A_2 showed the absence of *iso*leucine ($< 0·2$ residues per molecule). This finding is in contrast to the report by Rossi-Fannelli *et al.*[6], who recently published analyses of hæmoglobin A_2 containing > 6 residues of *iso*leucine per molecule. However, their preparation of hæmoglobin A_2 involved fewer purification steps than ours and might be contaminated with the non-hæm-containing protein known to have an electrophoretic mobility similar to hæmoglobin A_2.

Fingerprinting of Hæmoglobin A_2

Hæmoglobin A_2 was prepared from normal blood and from the blood of people with thalassæmia minor. The hæmoglobin solution from washed cells was carefully freed from stromal material by centrifugation at 30,000g at 2° C. Hæmoglobin A_2 was obtained by starch-block electrophoresis in 0·06 M veronal buffer pH 8·6 followed by adsorption on a short column of carboxymethyl–cellulose in 0·01 M phosphate buffer at pH 7·0. The non-hæm protein is not adsorbed under these conditions, and could therefore be separated from the hæmoglobin. The hæmoglobin was eluted with 0·2 M phosphate buffer at pH 7·0 or with 0·06 M veronal buffer at pH 8·6. After dialysis, the hæmoglobin A_2 was submitted for a second time to starch-block electrophoresis, eluted, and reconcentrated on carboxymethyl–cellulose. The protein was dialysed against 0·001 M phosphate buffer, pH 7·0, before use.

Comparison of hæmoglobin A_2 with A was made in the first instance by fingerprinting[7,8] tryptic digests of the two proteins (Fig. 1). In what follows a peptide will be named as follows: tryptic peptide number 26 (ref. 7) from hæmoglobin A is 'peptide AT-26'; similarly, peptide A_2T-26 is peptide number 26 from tryptic digests of hæmoglobin A_2, and so forth.

Several differences have been found in the fingerprints: the same peptide AT-26 in which hæmoglobin E differs from A (ref. 9) has an amino-acid substitution in hæmoglobin A_2 also ; a glutamic acid residue has been replaced by alanine in peptide A_2T-26 (Fig. 2). However, determination of the amino-acid sequence of peptide A_2T-26 showed that

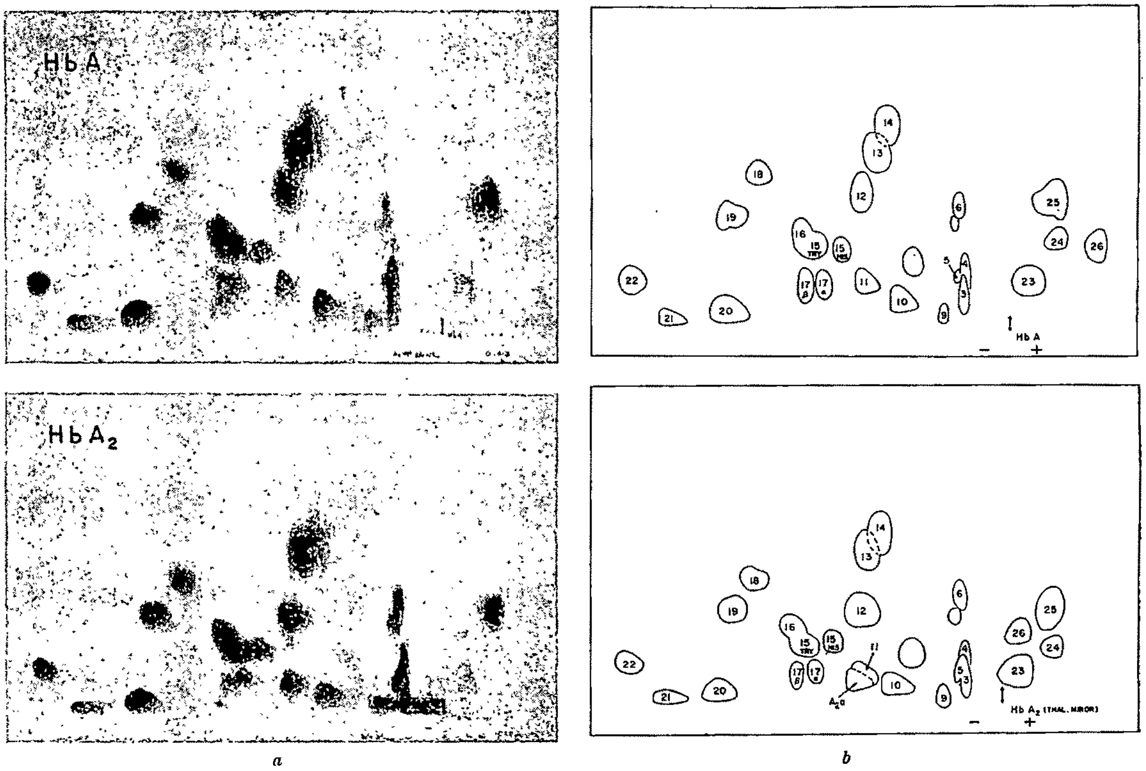

Fig. 1. Fingerprints (refs. 7, 8) of tryptic digests of hæmoglobins A and A_2; a, photograph; b, tracing

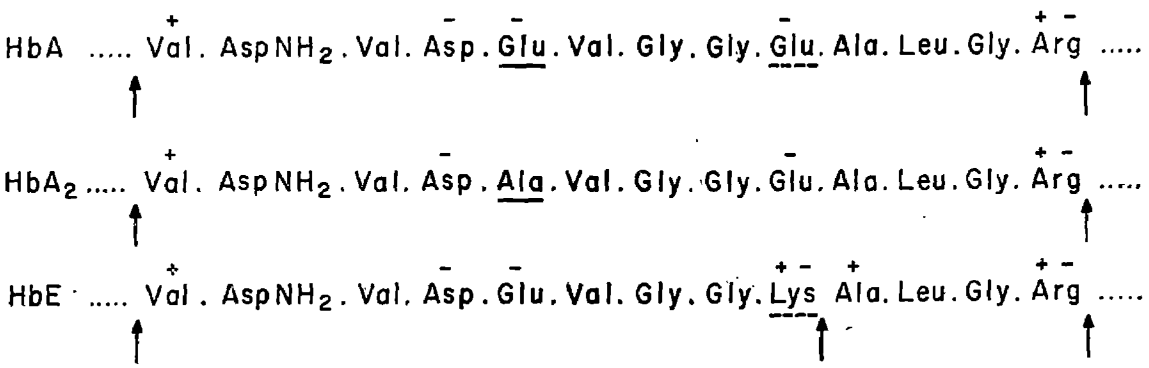
HbA ..... Val. AspNH2. Val. Asp. Glu. Val. Gly. Gly. Glu. Ala. Leu. Gly. Arg .....
HbA2 ..... Val. AspNH2. Val. Asp. Ala. Val. Gly. Gly. Glu. Ala. Leu. Gly. Arg .....
HbE ..... Val. AspNH2. Val. Asp. Glu. Val. Gly. Gly. Lys Ala. Leu. Gly. Arg .....
+
-
+ -

Fig. 2. Amino-acid sequences of tryptic peptide 26 from hæmoglobins *A*, A_2 and *E*. The arrows indicate points of cleavage by trypsin

it was the first of the two glutamic acid residues of peptide *AT*-26 which had changed to alanine. In hæmoglobin *E* it is the second glutamic acid which is replaced by lysine[9].

Another difference between the fingerprints of hæmoglobins *A* and A_2 is found in the peptides *AT*-12 and A_2T-12, which have the same electrophoretic mobility but differ in behaviour during chromatography. Peptide A_2T-12 is definitely slower. This difference was confirmed by the technique of dissected fingerprints[10]. The quantitative amino-acid composition of the two peptides, determined as above, was as follows: *AT*-12: $\mathrm{Ser_1,Thr_1,Gly_1,Ala_2,Val_1,Leu_1,Lys_1}$; A_2T-12: $\mathrm{Asp_1,Thr_1,Gly_1,Ala_2,Val_1,Leu_1,Lys_1}$. The basic nature of peptide A_2T-12 at *p*H 6·4 suggests that the aspartic acid residue is in the amide form. Both peptide *AT*-12 and peptide A_2T-12 also contain tryptophan, but this is destroyed on acid hydrolysis. As expected, the difference did not involve a charged amino-acid, but seemed to indicate a replacement of serine by asparagine. However, end-group determination by the dinitrophenol-procedure[11] showed that the situation was more complex than had been realized. Peptide *AT*-12 gave serine and peptide A_2T-12 gave threonine as N-terminal amino-acids. Clearly, we are dealing here with two substitutions, serine → threonine and threonine → asparagine. Further experiments on the sequence of the two peptides are in progress.

Examination of peptide *AT*-25 and A_2T-25 showed a further amino-acid difference. These peptides are indistinguishable in paper electrophoresis; a slight chromatographic difference was observed, when the isolated peptides were compared by paper chromatography in butanol/acetic acid/water (4 : 1 : 5). Amino-acid analysis of the peptides *AT*-25 and A_2T-12, carried out as above, showed a threonine (*AT*-25) to serine (A_2T-25) change in this nonadecapeptide. It is remarkable that such a small difference, involving a single methyl group in a peptide of molecular weight of more than 2,000, should show at all in chromatography. No further studies have yet been made on peptides *AT*-25 and A_2T-25 to determine whether this substitution is the only one in the peptide.

Finally, there is an additional peptide, A_2a. To date, the peptide of hæmoglobin *A*, which corresponds to the tetrapeptide A_2a, has not been found in spite of a careful search. Many other tryptic peptides of A_2 have been analysed quantitatively and found to be similar to their hæmoglobin *A* analogues (Table 1). The peptides examined account for at least 90 amino-acid residues, or about two-thirds of the δ-chain. Of these, only 4 or 5 amino-acids appear to be altered, as discussed above, assuming that for the rest similar amino-acid composition implies similar sequence.

Examination of the trypsin resistant 'core' of hæmoglobin A_2 by chymotryptic digestion and fingerprinting[13] suggested that the core might be different in several of its chymotryptic peptides. However, not all these differences were reproducible; although most of the 'core' fingerprint is similar for hæmoglobins *A* and A_2, the degree of similarity is by no means certain.

An important conclusion to be drawn from the present chemical studies is that the hæmoglobin A_2 from individuals with thalassæmia minor is indistinguishable from that of normal people, within the limits of the methods employed. Most of the analyses and sequences reported in this article were in fact made with thalassæmic hæmoglobin A_2, since this was less laborious to prepare. However, careful comparison of fingerprints and 'dissected fingerprints'[10] of hæmoglobin A_2 from normal and thalassæmic individuals gave us confidence that the two proteins are identical. Furthermore, if the thalassæmia hypothesis[14], discussed here, is correct, then the donor of the thalassæmic blood should be suffering from a β-chain thalassæmia, and the genes controlling his hæmoglobin A_2 would not be affected by this mutation.

We can summarize the chemical findings by saying that the tryptic fingerprints of hæmoglobins *A* and A_2 show four definite amino-acid substitutions and, in addition, the peptide A_2a.

The peptide chains of hæmoglobin A_2 were next separated to ascertain how these five differences are

Table 1. COMPARISON OF TRYPTIC PEPTIDES FROM THE δ CHAIN OF HÆMOGLOBIN A_2 WITH THOSE FROM THE β CHAIN OF HÆMOGLOBIN *A*

Peptide No.*	No. of amino-acids present	No. of amino-acids found to be different in hæmoglobin *A* and A_2
		By amino-acid analysis
22	1	—
20	4	—
19	2	—
12	9	2
4	8	—
5	12	1 (?)
6	16	—
25	19	1
26	13	1
	By specific staining only†	
14	3	—
15	2	—
17	2	—
	90	4–5

* See ref. 7 for the numbering of peptides.

† Detecting only arginine, tyrosine, histidine, tryptophan and sulphur-containing amino-acids (ref. 12).

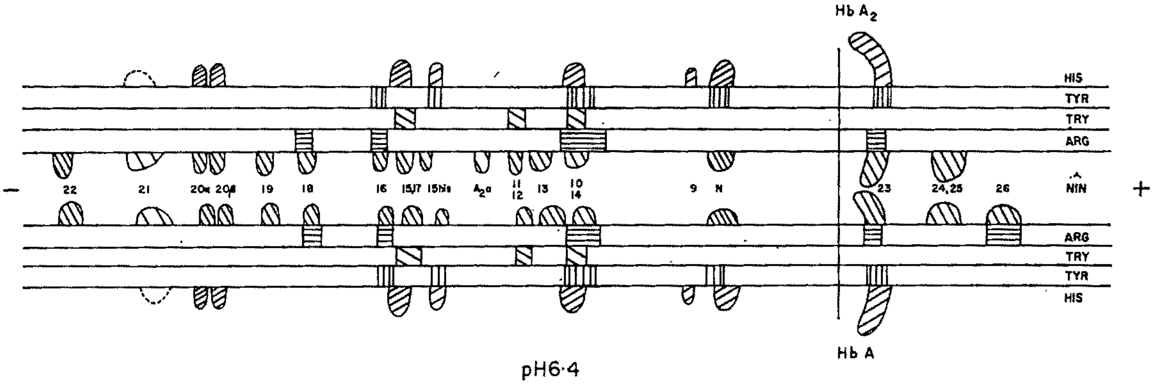

Fig. 3. One-dimensional electrophoresis at pH 6·4 and specific stains of tryptic digests of hæmoglobins A and A_2

distributed between them. For this purpose, we used a small-scale modification of Hill and Craig's countercurrent distribution technique[15], employed by them to separate the two types of peptide chain (α and β) of hæmoglobin A. Hunt[16] had shown that in fœtal hæmoglobin, the molecule of which is written $\alpha_2^A\gamma_2^F$, the two α chains are similar, if not identical to the α chains of hæmoglobin A—$\alpha_2^A\beta_2^A$. We find that none of the differences between hæmoglobins A_2 and A is in the α chain, but they are confined entirely to the other type of chain of A_2. This chain should be called the δ^{A_2} chain and the whole molecule, HbA_2 = $\alpha_2^A\delta_2^{A_2}$.

Genetic Control of Hæmoglobin A_2

The reasons for introducing the concept of separate δ chains in hæmoglobin A_2 are several. The observed amino-acid substitutions are at least four in number and probably more numerous. This fact distinguishes these chains from the β chains of the series of abnormal human hæmoglobins, such as the β^S chain of Hb-S the β^C of Hb-C, the β^E chain of Hb-E, etc., all of which differ from the normal β^A chain of Hb-A by a single amino-acid substitution[17]. On the other hand, the chemical similarity between β and δ chains is considerable as evidenced by the possession of the same N-terminal sequence Val.His.Leu . . .[18], by the same C-terminal sequence . . . Tyr.His[8], by the absence of *iso*leucine and by the apparent similarity in many tryptic peptides.

The necessity for their designation as 'δ chains' is confirmed by the genetic evidence that these chains are under genetic control which is separate from that of the β chain. For example, a mutation of the β gene, as in Hb-S or Hb-E, does not result in an altered δ chain ; the hæmoglobin A_2 component remains normal[2].

In addition, it appears that there is an independent series of alleles of hæmoglobin A_2. Ceppellini[19] has discovered an abnormal minor component, hæmoglobin B_2, which has a slower electrophoretic mobility than hæmoglobin A_2. In individuals carrying the B_2 component, the proportion of A_2 is decreased from the normal 2·5 to 1·2 per cent, and B_2 is present in the same amount, that is, the sum of hæmoglobins A_2 and B_2 is the usual 2·5 per cent. Furthermore, in individuals who also have thalassæmia, the level of A_2 and B_2 together was increased to 5 per cent, each being present at a level of 2·5 per cent.

Another abnormally slow A_2 component, described by Huisman and Meyering[20] as A_2', has an electrophoretic mobility which is similar to that of the hæmoglobin B_2 mentioned above. In the family in which it was discovered, it was found always together with thalassæmia, and the sum of A_2 and A_2' was also elevated above the normal level of A_2.

Shooter *et al.*[21] have evidence that the α chains of hæmoglobins A and A_2 are under the control of the same α gene. In a person homozygous for the abnormal hæmoglobin G_{Ibadan}, which differs from hæmoglobin A in the α chain[22], no normal hæmoglobin A_2 was found. Instead there was a new minor component, G_2, which differed in electrophoretic mobility from hæmoglobin A_2 in the same way that hæmoglobin G differs from hæmoglobin A.

We are, therefore, left to conclude that the genetic control of the production of hæmoglobin A_2 is exercised by α and δ genes, whereas hæmoglobin A is controlled by α and β genes. It appears[19], in addition, that the β and δ genes are linked. The hæmoglobins of a normal individual are therefore under the control of four genes: α, β, γ and δ. The α gene is concerned in the manufacture of all three types of hæmoglobin : $F = \alpha_2^A\gamma_2^F$, $A = \alpha_2^A\beta_2^A$, $A_2 = \alpha_2^A\delta_2^{A_2}$. These various hæmoglobins are produced depending on whether or not the β, γ or δ genes are 'active', which in turn seems to depend on the stage of development of the individual.

Hæmoglobin A_2 in Thalassæmia

The work of Kunkel *et al.*[2] and of Gerald and Diamond[23] has shown that hæmoglobin A_2 is involved in some of the thalassæmia diseases, since in many instances the proportion of A_2 rises to about 5 per cent.

We have recently proposed a hypothesis[14] (and an alternative) which suggests that the thalassæmia mutations are similar to those which lead to the single amino-acid substitutions in the recognized abnormal adult hæmoglobins (for example, Hb-S, C, E, etc.). However, thalassæmia is thought to differ from the abnormal hæmoglobin diseases in that the proposed thalassæmia mutations lead to amino-acid substitutions which do not affect the electrophoretic mobility of the hæmoglobin produced, but merely its rate of synthesis. An immediate consequence of this idea is the distinction of different thalassæmias as affecting either the α or the β chain of Hb-A. It has been suggested[24] that the occurrence of high proportions of fœtal hæmoglobin is compensatory in cases which we now describe as homozygous for a β chain thalassæmia. This could not happen for an α chain thalassæmia, since fœtal hæmoglobin uses α chains. Similarly, the observed rise of hæmoglobin A_2 in some thalas-

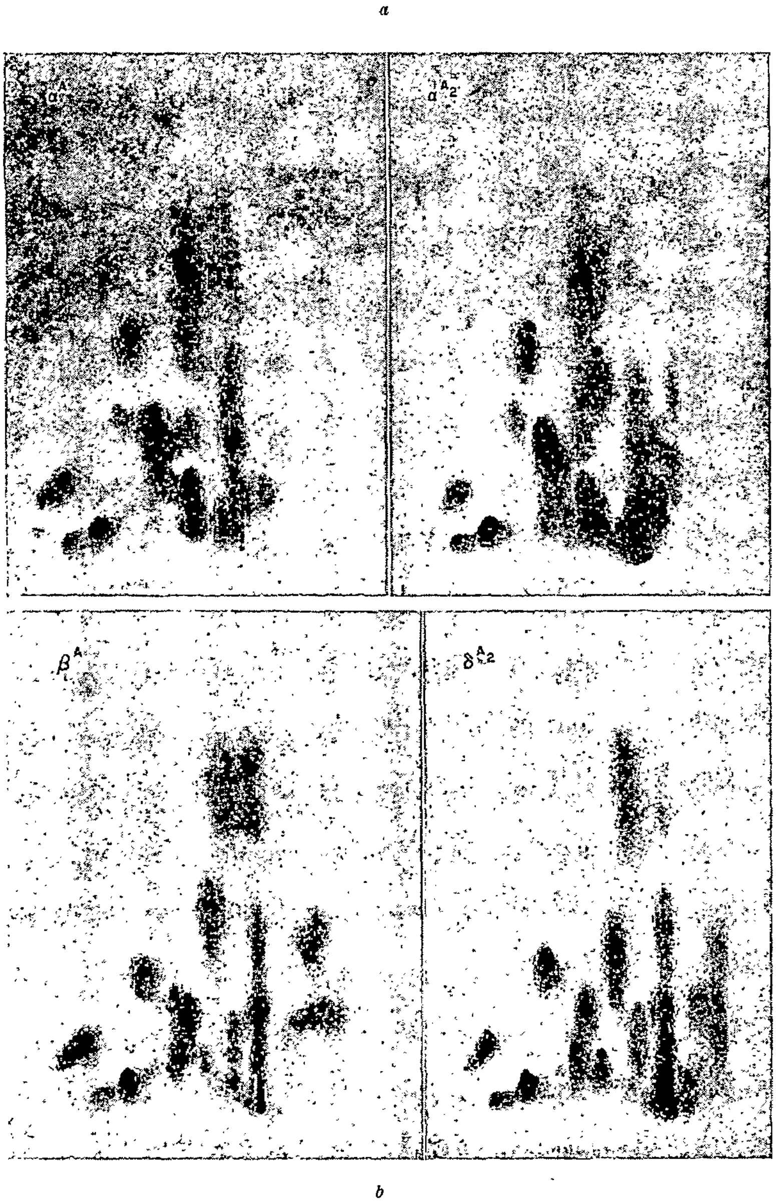

Fig. 4. Fingerprints of tryptic digests of: *a*, α chains of hæmoglobins *A* and A_2; the extra spots in the fingerprint of the α^{A_2} chain are known to be due to contamination with the δ^{A_2} chain; *b*, β chain of hæmoglobin *A* and δ chain of hæmoglobin A_2

sæmia minors can be ascribed to the presence of a β chain defect. In certain cases, this classification of the thalassæmia is borne out of a study of the pedigree[14]. Again, the cases of thalassæmia which do not have increased levels of A_2 (refs. 2, 25, 26) would be classified as α chain thalassæmias.

In the case of an α chain thalassæmia, a rise in hæmoglobin A_2 would not be expected since the relative rates of synthesis of β^{A} and δ^{A_2} chains should be normal. On the other hand, a β chain thalassæmia would decrease the rates of synthesis of the β^{A} chain relative to that of the δ^{A_2} chain, and therefore lead to an increase in the proportion of hæmoglobin A_2.

Evolution of Hæmoglobin A_2

Kunkel *et al.*[3] showed that blood specimens from cattle, horses, pigs, dogs and rabbits failed to show a minor component similar to A_2 hæmoglobin.

However, when they examined specimens from primates, in several cases a hæmoglobin A_2-like component was found. Ten chimpanzees showed such a component, comprising 2·4 per cent of the total hæmoglobin; the mobilities of the major and the minor components were indistinguishable from those of the human counterparts.

The New World monkey, *Cebus capucinus*, also showed the presence of A_2, but at levels of 4–5·5 per cent. No hæmoglobin A_2 was found in several Old World monkeys: *Macacus rhesus*, *M. cynomolgus* and *M. cercopithecus*.

The absence of A_2 in the Old World monkeys is at first sight surprising, since they are often held by zoologists to be more advanced than the New World monkeys. However, it might be that the particular examples studied were exceptions, which had lost the A_2 component during evolution. If all Old World monkeys lack A_2, perhaps this can be taken as a taxonomic criterion, along with the anatomical criteria at present used.

It would be of interest to extend this survey to other primates, to see whether the presence or absence of A_2 has a phylogenetic significance, as is suggested by these preliminary studies. Among the apes, the gibbon, orang-utan and gorilla have yet to be examined. An examination of primitive races of humans, such as aborigines, would also be of interest in this respect.

Although, on the basis of the limited survey that has been made, the A_2 component appears to be confined to the primates, other animal species are known to possess more than one hæmoglobin component. For example, it is possible that the two horse hæmoglobins[27] are related in a similar way as are the human hæmoglobins *A* and A_2.

A satisfactory explanation of the system of genetic control of hæmoglobin A_2 is that comparatively recently in evolution the β gene underwent duplication and that the two genes evolved independently from the original type by mutation to give the presently existing β and δ genes[28]. Gene duplication has been demonstrated in *Drosophila* both by genetic analysis and by cytological investigation of salivary chromosomes (for example, the 'bar' duplication)[29].

A process of duplication followed by divergence has long been considered by geneticists as a possible mode of origin of new genes[30]. While cases of duplication are known, no instance of subsequent independent evolution has been reported. It is believed that hæmoglobin A_2 provides an illustration of this process and that it furnishes evidence as to the way in which genes evolve.

We wish to thank Dr. Park Gerald for supplies of thalassæmic blood and for stimulating discussions.

This work was supported by a grant from the National Science Foundation. One of us (A. O. W. S.) thanks the National Institutes of Health for a fellowship under a training grant.

Addendum. Muller and Jonxis[31] have recently discussed their fingerprints of hæmoglobins A and A_2. Although there is a large measure of agreement between their results and ours, our results differ in that we find no arginine in the neutral tyrosine-containing peptide 5 (which they denote as peptide 2). Also, we find the additional peptide A_2a, which they do not report.

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ESTIMATION OF PERCENTAGE NATURAL CROSS-POLLINATION : EXPERIMENTS ON RICE

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IN work on the amount of cross-pollination which occurs under natural conditions in crop plants, the term 'percentage cross-pollination' has not always been clearly defined; consequently it is not always easy to assess the meaning of some of the published values. In many cases this is because the nature of the experimental design used has made it impossible to give a simple definition. Percentage cross-pollination is defined here as the number of seeds produced by all the plants in a plot—excluding those plants at the edge—which are the result of a cross-pollination, expressed as a percentage of the total number of seeds produced by those plants. Our definition of cross-pollination is the fertilization of one plant by another. (Fertilization of one flower by another on the same plant can be considered as self-pollination from the genetical and practical point of view.)

The basis of our design is as follows. Two pure-line varieties are used which have the same duration from sowing to flowering and are similar in most other respects (such as height, tiller number, etc.) except for one character which is genetically dominant in one variety and recessive in the other. We used the rice variety *BG*79, which has a red-pigmented leaf-sheath in the seedling stage (dominant), and Lead 35, which has a green leaf-sheath (recessive). The two varieties are planted as shown in Fig. 1. Assuming that the chance of cross-pollination is the same between plants at the same distance, an examination of Fig. 1 (see also Table 1) shows that any plant has an equal chance of being cross-pollinated by either variety when the eight nearest plants to it are considered. When plants farther away than the nearest eight are considered, it may be assumed that the chance of cross-pollination is less, but the chance of either variety effecting this is still approximately the same. (No information is available concerning the relationship between distance and the chance of cross-pollination; but if one makes the assumption that pollen dispersal is comparable to certain forms of spore dispersal[1], then the chance of cross-pollination would be inversely proportional to the square of the distance; if this is so, then the chance of cross-pollination in any plant near the centre being caused by any plant of the same variety as against any plant of the other variety is approximately 0·485 : 0·515.)

When the plot is ripe, a number of plants of the variety which has the recessive character is gathered from the centre of the plot (in Sierra Leone we chose the nine plants included in the central square shown in Fig. 1). The seed (F_1) from these plants is grown and the number of plants showing the dominant and recessive character noted. Those showing the dominant character provide an estimate of half the number of cross-pollinations which took place.

The main disadvantage inherent in this design is due to the fact that plants with the same character are aligned in rows. In Fig. 1, if the top of the

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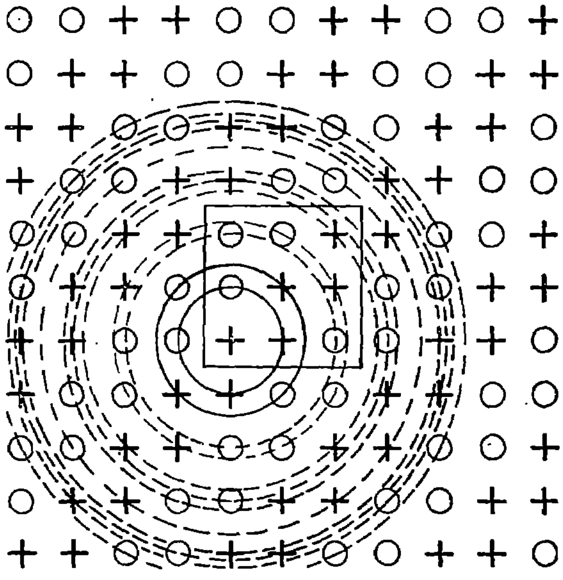

Fig. 1. Planting diagram of cross-pollination experiment. Each small circle represents a plant with the dominant character and each cross represents a plant with the recessive character. Concentric circles have been described round one of the 'recessive' plants to demonstrate those plants which are equidistant from it for the twelve innermost circles of plants. In the immediate neighbourhood of the central plant (solid circles), there is an equal number of each variety equidistant from it

page is considered as north, these lines run from south-west to north-east. Because of this alignment, directional factors are likely to distort the result. (This criticism also applies to a number of popular designs such as those based on a chess-board pattern.) For example, if wind is an important agent for carrying pollen then a prevailing south-westerly or north-easterly wind at the time of pollination would result in an underestimate of the amount of cross-pollination taking place, whereas a wind at right-angles to this would result in an overestimate. However, this apparent disadvantage can be made use of providing replicate trials are laid down with the diagonal rows of plants orientated at right-angles to one another. Such replication should compensate for any directional influences; furthermore, comparison of the differently orientated trials should give an indication as to whether directional influences are important.

Table 1. DISTRIBUTION OF PLANTS OF EACH VARIETY AROUND ANY GIVEN PLANT NEAR THE CENTRE OF THE DESIGN, SHOWING THE NUMBER OF PLANTS OF EACH VARIETY AND THEIR DISTANCES FROM THE GIVEN PLANT

The circles 1–12 referred to in the first column are shown in Fig. 1

Position of circle of plants numbering from the innermost outwards	Radius of circle (that is, distance of plants from the central plant) in feet	No. of plants on circumference of circle	
		Same variety as central plant	Different variety from central plant
1	1·00	2	2
2	1·41	2	2
3	2·00	0	4
4	2·24	4	4
5	2·83	4	0
6	3·00	2	2
7	3·16	4	4
8	3·61	4	4
9	4·00	4	0
10	4·12	4	4
11	4·24	2	2
12	4·47	0	8
13	5·00	6	6
14	5·10	4	4
15	5·38	4	4
16	5·66	4	0
17	5·83	4	4
18	6·00	0	4
19	6·08	4	4
20	6·32	8	0
21	6·40	4	4

We have carried out experiments on rice at two sites near the coast of West Africa—at Rokupr (9° 01′ N., 12° 57′ W.) in Sierra Leone and at Koba (9° 55′ N., 14° 04′ W.) in Guinea. One experiment was carried out at Koba and two at Rokupr within the normal season but planted on different dates. At Koba 75 out of the 14,021 seedlings in the F_1 were found to have a red leaf-sheath; this gives a value of 1·07 per cent cross-pollination. At Rokupr, 1 red leaf-sheath out of a total of 5,210 seedlings was obtained in the first experiment and 1 out of 5,004 in the second. As these results at Rokupr are very similar, they have been grouped together to give a value of 0·04 per cent cross-pollination.

These experiments indicate that the percentage natural cross-pollination in rice in West Africa is low but may vary considerably under different environmental conditions.

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PRIMARY METABOLIC DEFECT SUPERVENING ON VITAMIN B_{12} DEFICIENCY IN THE SHEEP

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Résumé of Experimental Findings which led to the Present Investigations

WHEN the concentration of cobalt within the paunch contents of a sheep falls below 40 mμgm. cobalt/gm. (20 μμgm./ml. of the bacteria-free supernatant) a manifest change occurs in the nature of the delicately poised associative growth of micro-organisms which constitutes the rumen flora. Although the rate and course of fermentation of carbohydrates within the rumen remain unaltered by this change, production of vitamin B_{12} and its analogues by the newly established microbial population decreases very materially. In such circumstances, bacterial synthesis of vitamin B_{12} itself falls precipitately from the level of 600–1,000 μgm./day, which normally prevails in the presence of adequate cobalt, to less than 50 μgm./day.

As the efficiency of absorption of the vitamin B_{12} produced by the rumen micro-flora is not more than

3 per cent, the amount of the vitamin that becomes available to the animal's tissues at this lower level of production is insufficient to make good the normal metabolic wastage of the functional vitamin B_{12}-containing complexes. Depletion then ensues.

The sheep suffers no obvious impairment of its physiological function until its complement of the vitamin as indicated by the concentration of vitamin B_{12} in its liver is reduced below 0·15 μgm. vitamin B_{12}/gm.

This is a critical level; depletion beyond it results in a marked loss of metabolic efficiency. The animal, then, can deal no longer with the fermentation products arising from a full feed; its appetite fails progressively as depletion proceeds, food consumption reflecting with remarkable precision the extent of the diminishing vitamin B_{12}-status, until death from inanition supervenes.

At any stage of the syndrome, provision of adequate vitamin B_{12} arrests further decline and dramatically restores normal health.

In the search for this defect of intermediary metabolism, the fact that a shortage of vitamin B_{12} imposes a much more profound metabolic stress on ruminants than on other animals, prompted the experiments reported here.

Unlike other animals, the ruminant derives the major part of its energy requirements from acetic and propionic acids produced by fermentation of the carbohydrates of its fodder. The course of this fermentation is remarkably consistent. The relative concentrations of the volatile fatty acids present in the rumen contents of our experimental sheep vary somewhat, but not greatly, throughout the 24-hr. cycle of rumination. The molecular ratios of the three main fatty acids present in the rumen contents 4 hr. subsequent to feeding are typical. At this stage, acetic acid comprises about 57 per cent, propionic acid 27 per cent and butyric acid 13 per cent of the total volatile acids; the remaining 3 per cent is made up of traces of formic, *iso*butyric and higher acids arising from secondary fermentations of other substrates.

The earliest of our investigations of the metabolic efficiency with which a sheep deals with individual fatty acids injected into its bloodstream demonstrated, unequivocally, that a state of vitamin B_{12}-deficiency seriously impairs the animal's capacity to utilize propionic acid, whereas its capacity to utilize formic and acetic acids remains unchanged.

Normal capacity to catabolize acetic acid becomes reduced occasionally in sheep that have been confined for two years or more to cobalt-deficient rations supplemented by parenteral injection of vitamin B_{12}, and then induced to become vitamin B_{12}-deficient by removal of the supplement. In these very rare cases, a massive parenteral dose of folic acid immediately reinstates normal metabolic function in this respect, leaving unaltered the seriously impaired capacity to metabolize propionic acid as the primary consequence of uncomplicated vitamin B_{12}-deficiency.

The site of the metabolic disability imposed in animal tissues by vitamin B_{12}-deficiency was located, unequivocally, by our work on the relative capacities of homogenates from the livers of normal and of vitamin B_{12}-deficient sheep to effect the catabolism of propionate. The metabolic defect is in the pathway of transformations, described by Flavin and Ochoa[1], through which, in animal tissues, propionate is launched into the Krebs cycle. Precisely, the breakdown arises from failure of the transfer reactions responsible for the intermediate isomeric conversion of methyl malonyl co-enzyme-A to succinate.

Tagging of the functional vitamin B_{12}-containing component by injection of cobalt-58 vitamin B_{12} into deficient sheep facilitated its isolation from their livers. This complex functions as a co-enzyme essential for the conversion of methyl malonate to succinate.

Finally, we have shown this co-enzyme to be identical with the 5 : 6-dimethyl-benziminazole cobamide co-enzyme separated from the cells of micro-organisms. We are very grateful to Dr. H. A. Barker, Department of Biochemistry, University of California, and to Dr. D. Perlman, Squibb Institute for Medical Research, New Brunswick, for gifts of crystalline 5 : 6-dimethyl-benziminazole cobamide co-enzyme prepared respectively from the cells of *Clostridium tetanomorphum* cultured in media containing free 5 : 6-dimethyl-benziminazole, and from *Propioni-bacterium shermanii*.

Our latter experiments were influenced and facilitated by the work of Barker *et al.*[2], on the isomeric conversion of L-glutamate to α-L-β-methyl-aspartate by *Clostridium tetanomorphum*, a transformation shown by them to be dependent normally on a co-enzyme which contains the analogue, Pfiffner's ψ vitamin B_{12} (adenyl cobamide).

Whatever its action *in vitro*, the adenyl cobamide co-enzyme is unlikely to function in animal tissues either in the isomeric conversion of methyl malonate to succinate, or in the metabolism of glutamic acid, which latter takes a course entirely different from that in *Clostridium tetanomorphum*. The animal organism either rejects vitamin B_{12}-analogues or destroys them after absorption. The total vitamin B_{12}-activity in the tissues of a sheep, for example, is due to vitamin B_{12} itself, although very considerable quantities of its analogues are produced along with it in the rumen contents.

Whether the 5 : 6-dimethyl-benziminazole cobamide co-isomerase present in animal tissues is specific only for the transfer reactions involved in the conversion of methyl malonate to succinate is a moot point, however. Whereas the main symptoms of the vitamin B_{12}-deficiency syndrome in the sheep may be attributed unquestionably to the impairment of propionate metabolism, there are subsidiary symptoms that occasionally become evident, especially in long-standing cases of partial deficiency. Outstanding among these are a blood dyscrasia due to a reduced capacity to mature the red cells, and, on occasions as a terminal symptom, a fatty infiltration of the liver, attributable to an impairment of methionine metabolism. Both could arise from partial failures to translocate single carbon atoms, processes dependent either on the vitamin B_{12}-containing methyl malonate co-isomerase or upon similar vitamin B_{12}-containing complexes essential in other transfer reactions. In some of these, folic acid is involved.

The megaloblastic anæmia, which in sheep is a frequent, though not invariable, symptom of vitamin B_{12}-deficiency, responds spectacularly to treatment with either vitamin B_{12} or folic acid, as it does in the human malady, Addisonian pernicious anæmia. Vitamin B_{12} completely reinstates normal metabolic function; in the sheep, apart from reinstating normal erythropoiesis, folic acid has no effect on the course of the deficiency syndrome which progresses to its fatal termination irrespective of the improved oxygen-carrying capacity of the blood.

Details of the experimental work upon which the above outline is based will be submitted for publication elsewhere. Those conducted, *in vivo*, which demonstrated the primary disturbance in the vitamin B_{12}-deficient sheep to be an impaired capacity to metabolize propionic acid, were referred to in the eleventh Annie B. Cunning Lecture delivered to the Royal Australasian College of Physicians in July 1958 (ref. 3). The studies, *in vitro*, which revealed the origin of this disability to be in the intermediary conversion of methyl malonate to succinate, and proved the dependence of this isomeric change on a vitamin B_{12}-containing co-isomerase, were reported in a section of the Tercentenary Exhibition of the Royal Society in July 1960. The following résumés are of some of our findings which concern directly the function of vitamin B_{12} in animal tissues.

H. R. MARSTON

Demonstration, *in vivo*, of the Site of the Metabolic Defect

As the volatile fatty acids are well tolerated, even when injected in considerable quantities into the blood stream; as the sheep's kidney threshold for them is high; and as equilibrium is quickly established between their concentrations in the blood and in the extra-cellular fluids, the criterion adopted to assess the effect of vitamin B_{12}-deficiency on the animal's metabolic efficiency in respect to them was the rate of their disappearance from the circulation after increasing materially their concentration by infusing their sodium salts into the jugular vein.

The best recommendation for this simple approach is the nature of the information it provided. The findings led directly to recognition of the metabolic defect that supervenes on vitamin B_{12}-deficiency.

Merino sheep were employed as experimental animals. They were confined in metabolism cages and fed on cobalt-deficient fodder which provided adequate nutrition in all respects other than the supply of vitamin B_{12}, which was considerably short of normal requirements. In the course of other experiments, we have determined that about 1·5 μgm. vitamin B_{12}/day is absorbed by sheep consuming the full ration (*cf.* legend to Fig. 6 for its composition). A sheep confined to this fodder loses close to 7·5 μgm. vitamin B_{12}/day in the normal course of its metabolism. Thus the overall deficit in these circumstances is about 6 μgm./day.

In some of the determinations, sheep of virtually the same age, size and previous nutritional history were selected and pair-fed. One of each pair was treated, parenterally, with abundant vitamin B_{12}, and its food intake restricted each day to that of the other animal, which was allowed to become depleted over the course of 35 or more weeks until it was in an advanced stage of the deficiency syndrome (Fig. 1). In other determinations, the effects were assessed by measurements made before and after the administration of massive amounts of vitamin B_{12} to deficient animals, food intake being regulated at a constant level during the short (4–7 days) interval between the tests. As vitamin B_{12} status was the only variable, both series of observations provided critical comparisons.

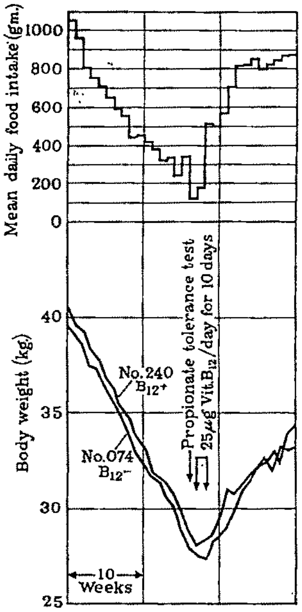

Fig. 1. Performance of pair-fed sheep
The performance of ewes No. 240 B_{12}^+ and No. 074 B_{12}^- used for the experiment illustrated in Fig. 4 is typical of that of the pairs referred to in Figs. 2 and 3. Food consumption of No. 240 was restricted throughout to that of No. 074

For each test, 3·5 m.mol. of the particular fatty acid per kgm. body-weight of the animal fasted for 24 hr., neutralized with sodium hydroxide and diluted to 1 molar concentration, was warmed to 38° C. and transfused steadily over the course of 5 min. into the blood stream via a fine plastic tube implanted temporarily into one of the external jugulars. A series of blood samples was collected at intervals through this tube, and the volume of the blood withdrawn replaced with physiological saline. Coagulation was prevented by washing the tube thoroughly with a solution of 1 : 10^5 heparin in physiological saline between each operation. Volatile fatty acid was determined in aliquots of the tungstic acid filtrates by constant-volume distillation and micro-titration in a stream of nitrogen. Gas–liquid partition chromatography was employed to determine the nature of the volatile acids in the blood.

The results from repeated overall determinations were within a very small experimental error, and left little doubt of the consistency of the underlying phenomena which they reflected. Those reported here are typical.

When raised artificially to abnormally high levels in the blood stream, simple fatty acids rapidly establish equilibrium with the *milieu interieur*, and afterwards their concentrations decrease exponentially to the low levels which normally prevail in the circulation of the fasting sheep.

The rate of this fall in the concentrations of formic acid (Fig. 2) and of acetic acid (Fig. 3), measured in this way, is unaffected by vitamin B_{12}-deficiency, even when the uncomplicated deficiency syndrome has progressed to its terminal stages. The metabolic capacity of the sheep to deal with propionic acid, however, is manifestly impaired by a state of vitamin B_{12} deficiency (Fig. 4). A significant decrease of this function is detectable even in the early stages of the deficiency syndrome, and thereafter it becomes progressively worse as depletion proceeds. Throughout the course of the supervening syndrome the extent of this defect correlates closely with the diminishing vitamin B_{12} status of the animal's tissues.

We conclude, therefore, that vitamin B_{12} is an essential constituent of the enzyme system which constitutes the intermediary channel through which propionic acid passes in normal metabolism.

In ruminants, which, of necessity, deal with relatively large quantities of propionic acid, this metabolic consequence of vitamin B_{12}-deficiency is particularly serious. In the mixed-feeders it is also important, for these animals have to deal with considerable quantities of propionic acid derived from cæcal fermentation of refractory carbohydrates that escape hydrolysis and absorption in the upper levels of the alimentary canal. Moreover, catabolic utilization of protein adds to this. The metabolic degradation of the amino-acids from 100 gm. of wheat gluten, for example, yields 4·1 gm. of propionic acid, which arises as an intermediary product in the degradation of the glucogenic amino-acids, L-valine and L-*iso*leucine.

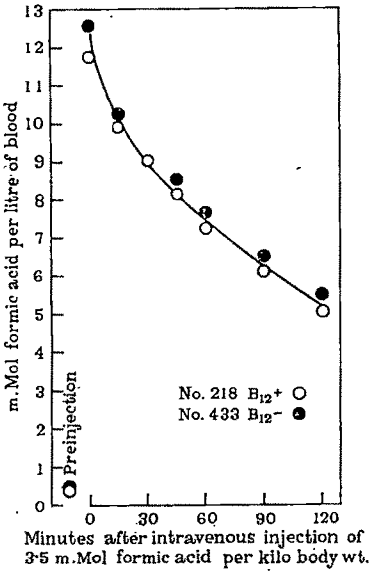

Fig. 2. Lack of effect of vitamin B_{12}-deficiency on the metabolism of formic acid

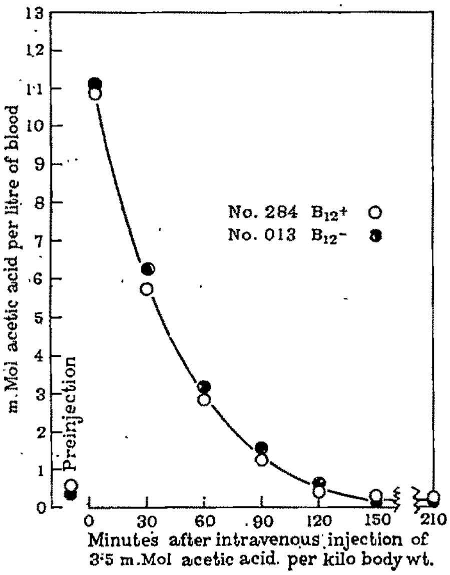

Fig. 3. Lack of effect of vitamin B_{12}-deficiency on the metabolism of acetic acid

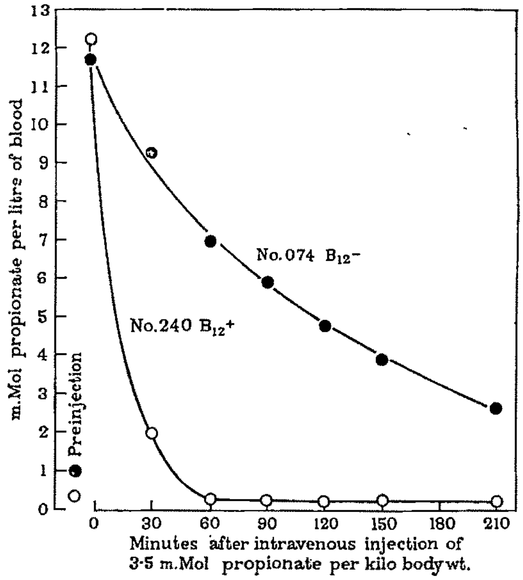

Fig. 4. Effect of vitamin B_{12}-deficiency on the metabolism of propionic acid

The metabolic defect briefly discussed here has been reported[4] as an integral part of a comprehensive investigation of the effects of cobalt deficiency on Merino sheep.

S. H. Allen
H. R. Marston
R. M. Smith

Localization, *in vitro*, of the Metabolic Defect, and Separation of the Vitamin B_{12}-containing Methyl Malonyl Co-isomerase

If provided with carbon dioxide, adenosine diphosphate, and extra cytochrome *c*, homogenates of the livers of normal sheep, prepared to ensure that the mitochondria remain intact, metabolize propionate at approximately the same rate as malate and succinate. Under identical conditions (cf. legend to Table 1) homogenates, prepared in this way from the livers of vitamin B_{12}-deficient sheep, retain their ability to deal with malate and succinate but lose their ability to deal with propionate. Within the small experimental error of the observations, propionate added as a substrate remained unaltered, and there was no increase in oxygen consumption (Table 1).

The capacity of homogenates from deficient livers to metabolize propionate, *in vitro*, however, is strikingly different when the mitochondria are fragmented. Such homogenates, prepared to ensure liberation of the mitochondrial enzymes into the reaction mixture, readily convert propionyl coenzyme *A* to methyl malonyl coenzyme *A* if provided with carbon dioxide and adenosine triphosphate. In these circumstances, methyl malonyl coenzyme *A* accumulates (Fig. 5 (*a*)). Addition of the vitamin B_{12}-containing coenzyme, isolated (*vide infra*) from the livers of normal sheep, reinstates the capacity to effect the isomeric conversion of this intermediate, and as the efficiency of the Krebs cycle is reduced by dispersal of its enzymes in the reaction mixture, succinate accumulates (Fig. 5 (*b*)).

Table 1. METABOLISM OF PROPIONATE, MALATE AND SUCCINATE BY HOMOGENATES PREPARED FROM LIVERS OF NORMAL AND VITAMIN B_{12}-DEFICIENT SHEEP UNDER CONDITIONS IN WHICH THE MITOCHONDRIA REMAIN INTACT

Rate of propionate consumption, μequiv./mgm. N/hr.

Normal livers		Vitamin B_{12}-deficient livers	
Exp. No.		Exp. No.	
17	2·1	18	0·3
23	2·0	19	0·6
25	2·9	20	0·0
27	2·7	28	0·0
29	1·6	30	0·0
31	3·0	33	0·0
35	2·7	36	0·5
Mean	2·4	Mean	0·2

Rate of oxygen consumption, μl. O_2/mgm. N/hr.

Exp. No.	No Substrate	Propionate	L-Malate	Succinate
Normal livers				
25	48	100	103	168
26	27	60	83	125
27	28	76	85	125
35	50	110	120	173
Mean	36	87	98	148
Vitamin B_{12}-deficient livers				
28	60	44	125	223
30	54	63	130	—
33	54	45	—	218
36	—	40	98	164
Mean	56	48	118	202

The liver homogenates used in the above experiments were prepared in 0·25 molar sucrose immediately after slaughter of the sheep. Nuclei and larger particles of cell debris were removed at 600 *g* for 10 min. The reaction mixtures comprised: potassium phosphate buffer *p*H 7·4, 2×10^{-2} *M*; sodium bicarbonate, $2·5 \times 10^{-2}$ *M*; potassium chloride, $9·0 \times 10^{-3}$ *M*; adenosine triphosphate, $1·3 \times 10^{-3}$ *M*; cytochrome *c*, $1·1 \times 10^{-5}$ *M*; added substrate, 5×10^{-3} *M*; sucrose added in homogenate about 4×10^{-2} *M*. The mixture, 5 ml., plus 0·5 ml. homogenate (2–3 mgm. nitrogen) was equilibrated in Warburg vessels with 95 per cent oxygen and 5 per cent carbon dioxide, which remained as the gaseous phase, then 0·5 ml. of substrate was added from the side arm. The reaction, at 37° C. for 36 min. with rapid shaking, was terminated by addition of tungstic acid. Volatile acid was estimated in the protein-free filtrate by micro-titration of the distillate in a rapid stream of nitrogen. Oxygen consumption was measured by Warburg's indirect method

The site of the impairment of propionate metabolism is indicated by the failure of the liver homogenate from the vitamin B_{12}-deficient sheep to effect efficiently the conversion of propionate to succinate. This metabolic defect, which, as illustrated in Fig. 5 (*a*), leads to an accumulation of the intermediate, methyl malonyl coenzyme *A*, is made good (Fig. 5 (*b*)) by the addition of a vitamin B_{12}-containing methyl malonyl co-isomerase, isolated from the livers of sheep (*vide infra*). The experimental conditions were essentially as follows.

The homogenate, from the liver of the cobalt-deficient sheep, *A*7–507 (Fig. 6) was prepared in *tris*-hydroxymethylamino-methane buffer under conditions which fragment the mitochondria and disperse their constituents into solution.

Reaction mixture (*a*) comprised propionyl coenzyme *A*, 10^{-3} *M*; *A*7–507 liver homogenate (containing 1·53 mgm. protein); magnesium ions, adenosine triphosphate and glutathione, the whole buffered at *p*H 7·4, *tris*-buffer 0·05 *M*. Two μc. of $^{14}CO_2$ in 10 μmol. carbon dioxide were distributed between the solution and the gas phase above it. Total volume of reaction mixture, 1 ml. Incubated at 30° C. for 20 min.

Reaction mixture (*b*) was identical with (*a*), but contained in addition 5×10^{-7} *M* co-isomerase isolated from the livers of normal sheep. Volume and treatment as above.

At the end of the incubation period, the coenzyme *A* thioesters were hydrolysed with 0·2 ml. 2 *N* potassium hydroxide. The reaction mixture was then adjusted to *p*H 2·0 with sulphuric acid, carrier acids were added, and the whole exhausted with ether. The ether extract was taken to dryness, the residue dissolved in a drop of water and chromatographed on Whatman No. 1 paper with *iso*amyl alcohol saturated with 4 *N* formic acid.

The tracings of radioactivity by a scanning device (Fig. 5) are roughly comparable in arbitrary units.

The critical quantitative determinations of radioactivity due to $^{14}CO_2$ in each spot, reported there as c.p.m. observed, were effected by cutting round the spots developed by brom-phenol blue, and igniting the disks in a combustion furnace from which the whole of the effluent gases was transferred to a high-vacuum manifold essentially of the type described by Glascock[12]. Therein the total carbon dioxide was isolated and, after diluting with the requisite volume of inactive carbon dioxide and the addition of carbon disulphide, transferred quantitatively into a Geiger tube and total radioactivity estimated by modern scaling instruments. Determinations effected by this sensitive procedure are accurate and strictly comparable.

Under the same conditions, homogenates, prepared by this method from the livers of normal sheep, metabolize propionyl coenzyme *A* more readily to succinate without additional co-isomerase, the rate of clearance of the intermediate, methyl malonyl coenzyme *A*, depending on the concentration of co-

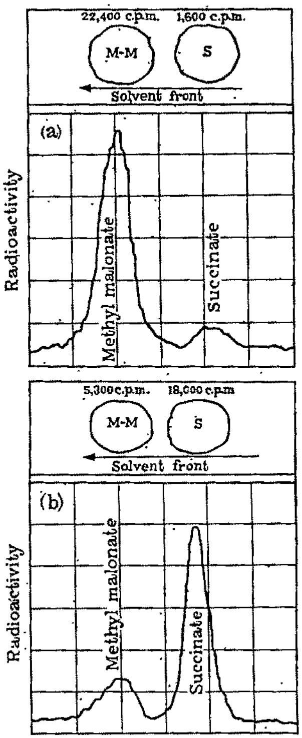

Fig. 5. Influence of added methyl malonyl co-isomerase on the metabolism of propionate by a homogenate from the liver of sheep *A*7–507 (Fig. 6) prepared to ensure fragmentation of the mitochondria. Reaction mixtures (*a*) and (*b*) were identical, other than the addition of 5×10^{-7} *M* co-isomerase to (*b*). The accumulation of methyl malonate in (*a*) indicates the metabolic lesion. For details see text

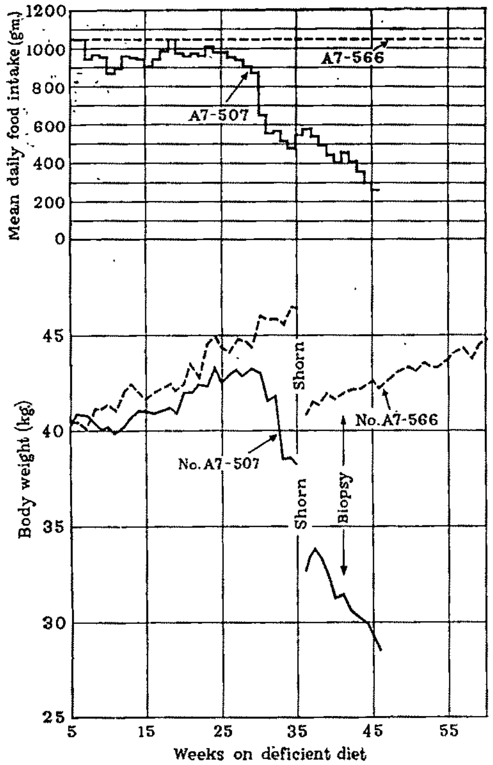

Fig. 6. Course of the vitamin B_{12}-deficiency syndrome in sheep *A*7-507

Criteria employed to assess the state of deficiency of sheep *A*7-507 were, failure of appetite, and the concentration of vitamin B_{12} in the liver and blood.

This animal and its control, *A*7-566, which was treated with adequate cobalt *per os*, were confined in pens and offered each day a cobalt-deficient ration composed of 1 kgm. of chaffed wheaten hay plus 50 gm. of gluten; the mixed fodder contained 0·023 μgm. cobalt/gm. The control animal avidly consumed the whole of the ration throughout. The appetite of the unsupplemented animal, unsteady after 8 weeks, failed progressively after 25 weeks, and thereafter reflected the degree of depletion below the minimum level commensurate with normal function. At 41 weeks, aspiration biopsy samples of the livers of the deficient and control animals contained respectively 0·08 and 1·12 μgm. vitamin B_{12}/gm. wet weight. The rate of depletion indicated by the vitamin B_{12} concentration in the blood was as follows:

Sheep No.	m.μgm. vitamin B_{12}/ml. blood at week number 26	32	41	56
*A*7-507 (deficient)	0·17	0·16	0·032	—
*A*7-566 (cobalt supplemented)	1·9	2·2	2·4	3·4

Sheep No. *A*7-507 was thus in an advanced stage of vitamin B_{12}-deficiency when it was slaughtered at 46 weeks to obtain its liver for the *in vitro* experiments reported in Fig. 5.

isomerase in the liver, as indicated by microbiological assays of the vitamin B_{12} content.

Some measure of further change occurs in both instances. When a $^{14}CO_2$-enriched atmosphere is employed, traces of fumarate and malate, both labelled with carbon-14, may be detected in the reaction mixtures.

As the total carbon dioxide bound in the combined methyl malonate and succinate fractions was observed in all instances to be closely similar, irrespective of additional co-isomerase—in the experiment reported in Fig. 5 they were, perchance, identical—it is clear that the potential efficiency of the propionyl carboxylating enzymes, described by Flavin and Ochoa[1], is not impaired in vitamin B_{12} deficiency.

The virtual stasis in the capacity of the intact mitochondria from the livers of vitamin B_{12}-deficient sheep to catabolize propionate invites comment. In the absence of a necessary quota of methyl malonyl co-isomerase, it is conceivable that conversion of propionate to succinate by an orientated array of enzymes within the mitochondria is stemmed back by local high concentrations of methyl malonyl coenzyme *A* at the reaction site. Such local accumulations of this intermediate as could greatly impede clearance through the propionate → methyl malonate → succinate channel would be relatively minute in terms of concentration within the reaction mixture as a whole. Certainly, sensitive chromatography failed to demonstrate the presence of either methyl malonate or its coenzyme *A* thioester in these reaction mixtures.

The methyl malonyl co-isomerase, employed in the first experiments (Fig. 5), was isolated from sheep's liver by a procedure similar to that employed by Barker and his colleagues[2] for the isolation of the adenyl cobamide coenzyme from *Clostridium tetanomorphum*.

As it was feasible for us to separate only a small quantity of the active vitamin B_{12} component from the liver, the precursor vitamin B_{12} was tagged with cobalt-58 to facilitate its identification during fractionation. On each of four consecutive days, 25 μgm. of vitamin B_{12}, containing 2·92 μc. cobalt-58 vitamin B_{12} was injected subcutaneously into a vitamin B_{12}-deficient sheep. The animal was slaughtered 24 hr. after the last injection. Its liver, which contained 14·3 per cent of the total radioactivity administered as cobalt-58 vitamin B_{12}, was finely ground, together with sufficient normal sheep's liver to yield 2 kgm. of minced tissue. After extraction with 80 per cent ethanol and preliminary purification, the major radioactive peak of the *p*H 6·4 eluate of the material adsorbed on a 'Dowex-50' *p*H 3·0 column was collected, extracted with phenol and re-extracted finally into 18·6 ml. of water. This solution contained 30 per cent of the cobalt-58 present in the original liver-mash. Addition of potassium cyanide to a sample of it changed the absorption spectrum to a curve, with maxima at 350, 367 and 581 mμ, that is characteristic of the cobamides. Microbiological assay (*Lactobacillus leichmannii*) of this sample indicated a concentration of $7 \cdot 9 \times 10^{-6}$ molar co-isomerase in the final solution.

The relationship between the concentration of the coenzyme and the rate of conversion of propionate to succinate by homogenates of the livers of vitamin B_{12}-deficient sheep under the conditions outlined in the legend to Fig. 5 agreed tolerably well with the requirements, *Km.*, of the Michaelis–Menten equation[5]. This criterion was employed for the comparison of the sample separated from liver with the two samples of 5:6-dimethylbenziminazole cobamide coenzyme kindly presented by Dr. Barker and Dr. Perlman. In the experiments employed for this purpose the concentration of the former was assessed from its vitamin B_{12} content: the concentrations of the crystalline samples were determined spectrophotometrically[6]. The apparent *Km.* values of all three ranged closely about 9×10^{-9} *M*. Thus there is little doubt that the coenzyme reported in Fig. 5 was in fact identical with the crystalline preparations.

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Lack of Influence of Vitamin B_{12}-Deficiency on the Sheep's Capacity to metabolize Glutamate

Barker *et al.*[7] fixed attention on the essential function assumed by derivatives of vitamin B_{12} and its analogues in transfer reactions concerned with isomeric change by demonstrating that *Clostridium tetanomorphum* metabolizes L-glutamate via α-L-β-methylaspartate to mesaconate, and proving that the isomeric conversion of L-glutamate to α-L-β-methylaspartate is normally dependent on a coenzyme form of pseudo-vitamin B_{12} (adenyl cobamide).

In animal tissues L-glutamate is usually considered to be catabolized by simple oxidative deaminization to α-ketoglutarate[8], with which it forms an equilibrium, thence via the Krebs cycle. An alternative pathway in which aspartate and oxaloacetate are involved has recently been suggested[9].

The sheep's capacity to deal with glutamate is uninfluenced by vitamin B_{12} deficiency.

The procedure adopted in the experiment reported here is typical of the series in which comparative determinations were made on the same animal before and after correction of the imposed deficiency by injection of vitamin B_{12} (Fig. 7).

Glutamate in the blood plasma was estimated, after precipitation of the proteins[10] with picric acid and removal of the excess picric acid from the filtrate on a column of the chloride form of 'Dowex 2-X8', by separation[11] from other amino-acids on a column of the acetate form of 'De-Acidite *FF*' and estimation with ninhydrin.

As the peak of the concentration of glutamate in the blood stream immediately subsequent to the infusion of 3·5 m.mol. of glutamate/kgm. body-weight was somewhat above renal threshold, about 20 per cent of the glutamate injected was passed in the urine during the course of 5 hr. The findings, however, leave little doubt that, in sheep, vitamin B_{12} does not enter into any of the metabolic transactions involved in the catabolism of glutamic acid (Fig. 8). Under the conditions of the experiment the defect in the metabolism of propionate is clearly revealed (Fig. 9).

S. H. Allen
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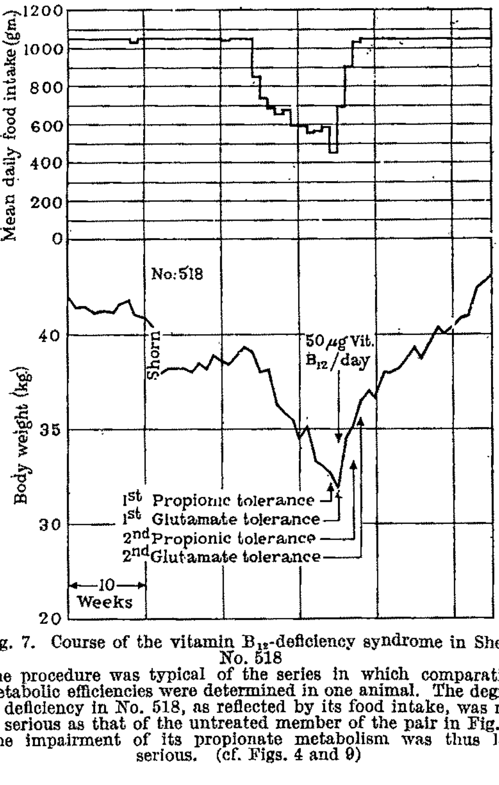

Fig. 7. Course of the vitamin B_{12}-deficiency syndrome in Sheep No. 518

The procedure was typical of the series in which comparative metabolic efficiencies were determined in one animal. The degree of deficiency in No. 518, as reflected by its food intake, was not so serious as that of the untreated member of the pair in Fig. 1. The impairment of its propionate metabolism was thus less serious. (cf. Figs. 4 and 9)

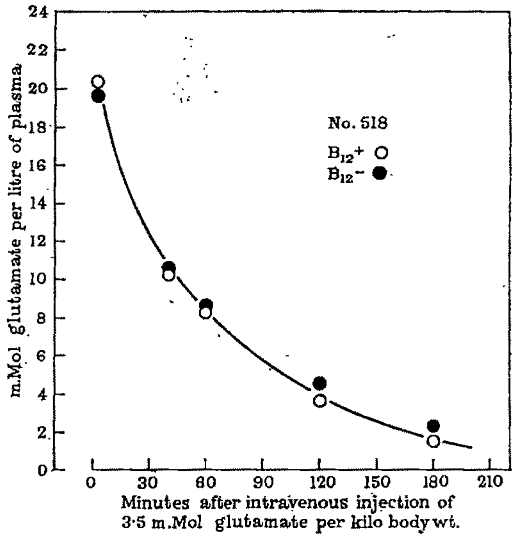

Fig. 8. Lack of effect of vitamin B_{12}-deficiency on the metabolism of glutamate

The concentrations of glutamic acid prior to injection were about 0·07 m.mol. per litre of plasma

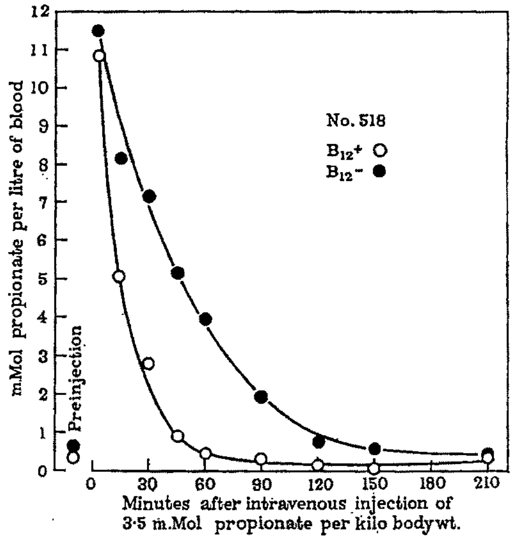

Fig. 9. Effect of vitamin B_{12}-deficiency on the metabolism of propionate

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LETTERS TO THE EDITORS

HISTORY OF SCIENCE

Observation in the Year 1648 of Diffraction through a Lattice

In the first half of the seventeenth century there worked in Bohemia the naturalist, physician and philosopher, Ioannes Marcus Marci (1595–1667), professor of the Faculty of Medicine in the University of Prague. It is known that Marci published in 1639 his book "De proportione motus", wherein he defined the laws governing impact of spherical bodies.

Besides other works in the field of physics, mathematics, medicine and philosophy he also wrote a book *Thaumantias. Liber de arcu coelesti deque colorum apparentium natura, ortu et causis* in the year 1648, in which he dealt with the origin and qualities of rainbow and "fictitious" (spectral) colours.

The book is remarkable in that Marci described in it certain observations of phenomena in the field of physical optics, about which published reports appeared only much later. Marci described, but without correct knowledge of the causes of these phenomena, the colour of thin plates (p. 241) and diffraction (pp. 103, 111, 119, 138, 178). In these chapters Marci did not speak only of diffraction on a wire, edge and aperture, but spoke directly (p. 119) about the system of apertures, which he called the lattice ("reticulum"):

"If you put the lattice before a light source, you will see as many rainbows, as there are apertures", as translated from the original, namely, "... si reticulum opponas luminoso, nam totidem irides, quot foramina, videbis".

Marci spoke of the lattice also on p. 138. "Itaque cancelli reticulatim intersecti ad singula foramina suas irides habent proprias."

Furthermore, Marci also mentioned the observation of spectral colours on the apertures formed by meshes of wires or threads (p. 103):

"Or plate with the apertures in the form of grating or twisted wires (threads) develops as many rainbows as one has apertures or slits through which light penetrates. Individual glasses in window frames behave in a similar way. But if we put a net in front of one of them, again there appears as many rainbows as there are apertures", from the original, namely: "Quin lamella in modum reticuli pertusa, aut filamenta convoluta totidem irides procreant, quot foramina seu rimae luci perviae. Ita quoque orbiculi vitrei fenestrarum singuli suas irides habent. Atque si illorum uni reticulum obtendas, rursum totidem irides quot foramina procreabis".

These quotations from Marci's book "Thaumantias" allow us to date the first known report about diffraction through lattices to the year 1648, that is, to the year of issue of his book.

I thank Dr. Ladislav Křivský, scientific worker of the Czechoslovak Academy of Sciences, for his support.

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PHYSICS

Concentration of Atmospheric Radon and Wind Direction

It is usually assumed that atmospheric radon comes from the Earth's crust by a slow diffusion process[1,2]. We may expect that radon concentration will change with wind direction at the time of sampling, according to the geographical and geological configuration near the sampling station.

The situation of our laboratory, in Lisbon, near the sea, is therefore excellent to test any probable relationship between radon concentration and wind directions since only half the possible wind directions are continental ones, therefore corresponding probably to higher radon concentrations. In order to study any correlation, the concentration of radon in air has been measured, by a method described in ref. 3. The wind directions have been recorded as is usually done in meteorology (sixteen directions).

Table 1 shows the number of observations for each direction and radon concentration data grouped in ranges of 5 × 10^{-14} c./l. In Fig. 1 the mean value of radon concentration corresponding to each wind direction is plotted in polar co-ordinates centred in Lisbon, over a chart of Portugal. From this we can conclude:

(1) Continental winds are generally related to higher concentration-levels of atmospheric radon.

(2) Sharp maxima are observed in those directions passing over the regions where uranium ores are present in great quantities.

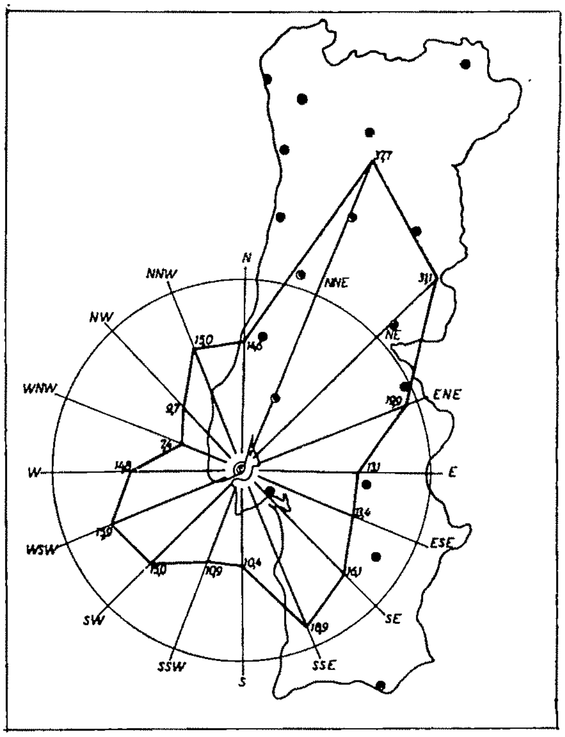

Fig. 1

Table 1

	0–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Total	cm.
N	57	54	31	19	10	12	6	6	2	3	0	—	—	1	1	2	1	205	14·6
N.N.E.	3	3	9	5	3	3	2	3	7	2	4	3	1	—	2	—	4	54	37·7
N.E.	4	8	10	[illegible]	15	11	6	8	9	5	3	3	1	1	4	1	1	100	31·1
E.N.E.	1	5	6	5	5	1	—	—	1	1	—	2	—	—	—	—	—	27	19·9
E.	—	3	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	5	13·1
E.S.E.	—	1	1	2	1	—	—	—	—	—	—	—	—	—	—	—	—	5	13·4
S.E.	—	4	1	1	—	1	—	—	1	—	—	—	—	—	—	—	—	9	16·1
S.S.E.	—	—	—	3	2	—	—	—	—	—	—	—	—	—	—	—	—	5	18·9
S.	9	5	3	1	3	1	—	—	1	—	—	—	—	—	—	—	—	23	10·4
S.S.W.	15	16	10	3	2	1	2	—	—	—	—	2	—	—	1	—	—	53	10·9
S.W.	17	18	15	4	5	1	1	1	1	—	1	—	—	—	1	—	2	57	15·0
W.S.W.	27	24	12	9	3	4	2	1	3	1	1	1	1	1	—	1	1	94	15·9
W.	3	4	—	3	1	—	—	—	—	—	—	—	—	—	—	1	—	11	14·8
W.N.W.	9	9	—	1	1	—	1	—	—	—	—	—	—	—	—	—	—	21	7·4
N.W.	6	8	4	4	1	1	—	—	—	—	—	—	—	—	—	—	—	24	9·7
N.N.W.	33	30	28	12	9	9	3	1	2	3	1	2	1	1	—	—	—	135	15·0

Variations of radon concentration with wind direction have already been observed by Tanaevsky and Vassy[4], and Stierstadt[5]. In the present work we can, furthermore, correlate higher concentration-levels with those directions corrsponding to well-established situations of uranium ores.

The above conclusions should be kept in mind when we intend to use the variation of radon concentration in the atmosphere as a sign of temperature inversion, particularly in those places where the geographical situation may explain radon variations by any other reason, as for example in Lisbon, as we found.

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Electrical Conductivity and Thermoelectric Power of Calcium Oxide

During recent years a number of experimental investigations of the physics of the oxide-coated cathode have taken the form of determinations of the thermionic and electrical properties of the individual alkaline earth oxides. The work reported in this communication represents part of a programme of research on calcium oxide, a component of some commercial mixed oxide coatings which has received but little attention.

The electrical conductivity of samples of calcium oxide has been determined in an experimental arrangement in which the oxide, in a layer about 100μ thick, is contained between two nickel cathode buttons. Each button is provided with an independent heater and the temperature of the nickel base may be determined by the use of a tungsten–nickel thermocouple attached to it. The assembly is attached to a glass pinch and is contained within a glass envelope, attached to a vacuum system. The oxide sample is prepared by the pyrolysis of a carbonate suspension, and after the usual techniques of breakdown, outgassing and activation (by applying 4 V. d.c. between the nickel buttons at 1,100° K., with frequent reversals of polarity, for a period of up to 12 hr.) the tube is sealed off from the system and an active barium getter is fired.

Measurements of the electrical conductivity of the calcium oxide sample as a function of temperature are made by setting both nickel buttons to the desired temperature T and determining the conductivity current when potentials of up to 500 mV. are applied between the buttons. For small applied voltages, the current-voltage characteristics are linear, the gradient providing a measure of the conductivity σ of the sample. Graphs of log σ versus $1/T$ show the characteristic form associated with conduction in the porous sample: a typical curve is shown in Fig. 1. Following the interpretation of Loosjes and Vink[1] the linear portion at lower temperatures (up to about 800° K. in this case) is associated with semiconduction through or over the surface of the crystallites making up the sample; the gradient of the low-temperature portion corresponds to an activation energy of 0·72 eV. for this process, for the particular state of sample activation under investigation. A second linear portion occurring between about 800° K. and 1,000° K. is associated with the thermionic emission of electrons within the pores of the sample. The activation energy of this high-temperature process, corrected for the parallel low-temperature mechanism, is 1·27 eV. Deviation from linearity at higher temperatures may be due to the current density in the pores becoming large enough for space-charge effects to be important. The most active samples gave an average low-temperature activation energy of about 0·7 eV., and an average corrected high-temperature activation energy of about 1·2 eV. Measurements in the low-temperature region were often hampered by the effects of thermal

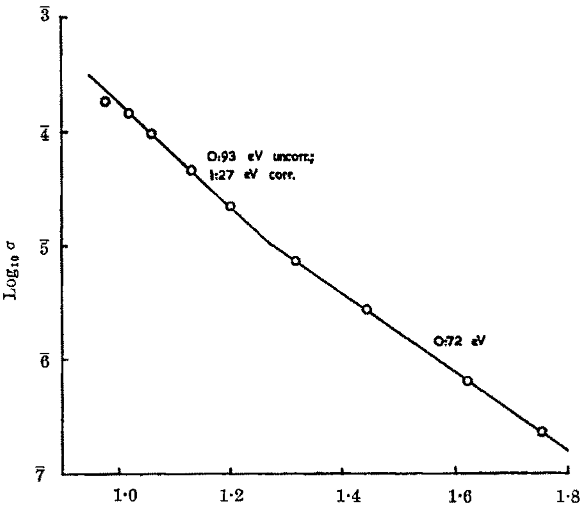

Fig. 1. Electrical conductivity of activated calcium oxide as a function of temperature. The conductivity σ is expressed in ohm^{-1} cm.$^{-1}$

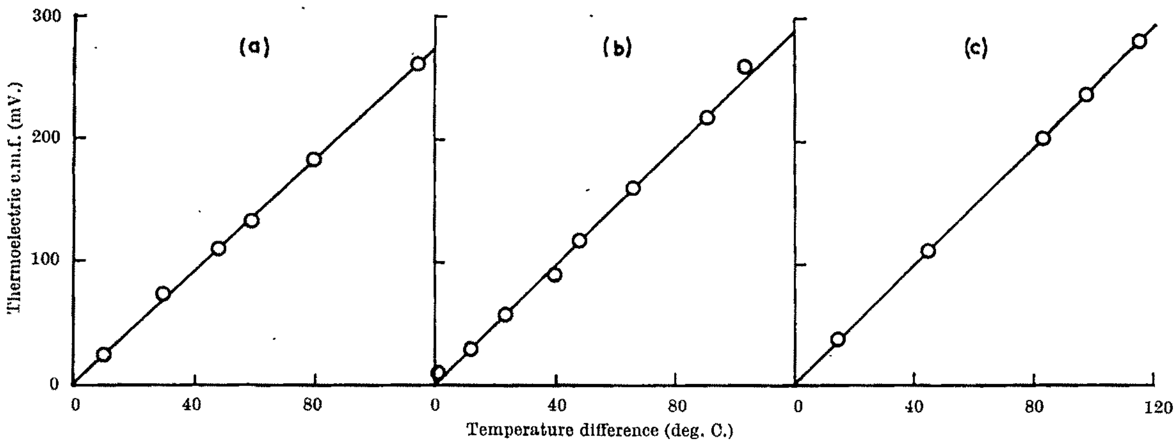

Fig. 2. Thermoelectric e.m.f. versus temperature difference characteristics for calcium oxide. *a*, $\bar{T}$ = 896° K., thermoelectric power = 2·30 mV. °C.$^{-1}$; *b*, $\bar{T}$ = 823° K., thermoelectric power = 2·41 mV. °C.$^{-1}$; *c*, $\bar{T}$ = 749° K., thermoelectric power = 2·43 mV. °C.$^{-1}$

expansion in the sandwich system ; slight changes in the area of contact of the crystallites would be expected to have a considerable effect on the component of the conduction current due to true semiconduction, but only a small effect on the thermionic emission component. The average activation energies are in reasonable agreement with the values quoted by Hopkins and Vick[2] (0·8 and 1·3 eV.) from measurements of the electrical conductivity of calcium oxide with a different sample geometry.

The experimental arrangement adopted allows, in addition, the measurement of the thermoelectric power of the oxide. If the buttons are maintained at different temperatures, a thermoelectric e.m.f. is set up between the faces of the sample which may be measured with a vibrating condenser electrometer of effectively infinite impedance. It is convenient to increase the temperature of one button and decrease that of the other so that the mean temperature $\bar{T}$ is approximately constant. It is found that the thermoelectric e.m.f. thus produced is approximately proportional to the temperature difference between the buttons (at least for temperature differences of up to about 120° C.), and the gradient of the thermoelectric e.m.f. versus temperature-difference characteristic defines the thermoelectric power of the specimen at the mean temperature $\bar{T}$. A typical series of characteristics for a particular sample of calcium oxide is given in Fig. 2. Although in many cases the thermoelectric power shows a tendency to increase with decreasing mean temperature, the experimental error involved in individual measurements of thermoelectric power is too great to allow an accurate determination of the temperature variation. Metson and Holmes[3], in a study of the thermoelectric power of the mixed oxide (BaSr)O, suggest that in the high-temperature range the thermoelectric power is in fact constant, the apparent temperature variation being due to experimental inaccuracies. On this assumption an average thermoelectric power for calcium oxide in the temperature-range 700–1,200° K. has been calculated from determinations on a number of oxide samples : the value obtained is 2·4 ± 0·2 mV. deg. C.$^{-1}$.

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Modification of Spectrophotofluorometer for Scanning Paper Strips

THE spectrophotofluorometer is a potent addition to biochemical investigations[1]. The apparatus, at present available from commercial sources, is designed for analysis of liquid solutions. By modifications of the basic design of the spectrophotofluorometer, as indicated in Fig. 1, the spectrophotofluorometric analysis can be applied conveniently to paper strips.

A mirror (*A*) deflects the pathway of the activating light which focuses at the point (*B*) on the paper strip which is mounted on the drum (*C*).

The paper strip used is obtained by high-potential paper electrophoresis. An example of such a result is shown in Fig. 2, for separation of three indolic compounds. The paper strips, resulting from the high-potential electrophoretic process, were dried and mounted on the drum (*C*) of the instrument by means of black insulation tape. There are, on the surface of the drum, six guides to accommodate six strips of paper 23 × 3/4 in. on six levels. By means of the lock mechanism of the shaft (*D*) supporting the drum, each of the six levels corresponding to the six strips can be brought into the focus of scanning without opening the lid of the drum chamber. The drum is set into rotation by a motor (*E*) at any level synchronously with the recorder.

Although the instrument can be applied to paper strips obtained by one-dimensional paper chromatography, the sharpness of separation obtained by high-potential electrophoresis makes the latter better suited to this technique.

The data so far available with reference to fluorescence of compounds of biological interest refer mainly to fluorescence of solutions. These data cannot be simply extrapolated to the fluorescence phenomena under discussion, because the compounds separated on the paper under scanning are in a solid state.

In the experiment such as Fig. 2, the best settings for fluorescence under these conditions, for most of the indolic compounds tested, are 2900 Å. for the activation wave-length, and 3400 Å. for the fluorescence. These data are not very different from those given for the fluorescence in water solution for tryptophan (*p*H 11), serotonin (*p*H 2) and tryptamine (*p*H 7) (ref. 2). It is expected, however, that data reported by other investigators, with regard to fluorescence of compounds of biological interest, in

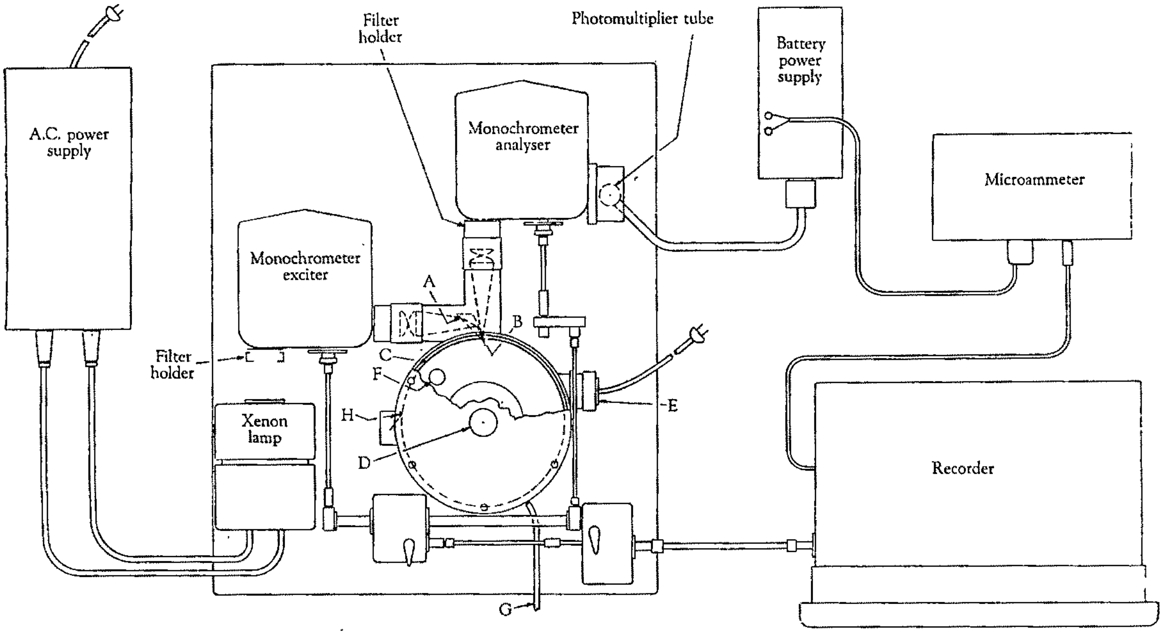

Fig. 1. Modification of spectrophotofluorometer for the spectrofluoro-phoretogram

solutions at various *p*H are not necessarily in agreement with the fluorescent data under discussion. Similarly, other salient features of fluorescence of compounds in solution such as quenching by chemical means or change induced by freezing would have to be retested. The drum in the basic part of this instrumentation serves not only for the purpose of scanning with least danger of contamination of the sample, but also to provide cooling of the paper, by introducing liquid nitrogen through one of the inlets (*F*), while the system is flushed with dried nitrogen-gas, introduced through the inlet (*G*) for prevention of fogging. The temperature of the paper surface is defined by the reading of the thermocouple making switching contact on the paper at point (*H*).

In view of the above-mentioned combination of high-potential paper electrophoresis and spectrophotofluorescent scanning technique, and because the fluorescence is of compounds in the solid state rather than in solution, the term spectrofluoro-phoretogram (SFPG) is proposed to specify the data (as in Fig. 2) obtained by scanning the paper electrophoretic pattern by means of this apparatus.

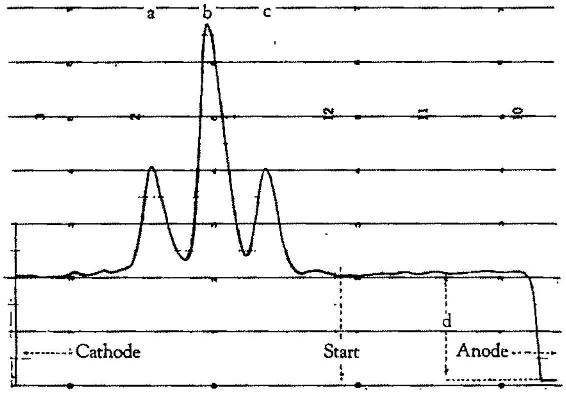

Fig. 2. Spectrofluoro-phoretogram (SFPG) of separation of L-tryptophan, serotonin and tryptamine. *a*, Tryptamine (equivalent to 4γ); *b*, serotonin (equivalent to 3γ); *c*, L-tryptophan (equivalent to 2γ); *d*, 'background fluorescence' of the paper strip. Electrophoresis, paper: Schleicher and Schuell 2043 *A*; electrolyte solution, 0·05 *N* formic acid, 0·2 *N* acetic acid; voltage, 10 kV.; time, 8 min. Activating light, 2900 Å. (filter, Corning 9863); fluorescent light, 3400 Å. (filter, *UG* 10); temperature, ambient

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Axial Migration of Particles in Poiseuille Flow

IT was reported recently[1] that rigid spheres concentrate into a thin annular region when an initially uniform suspension undergoes laminar flow through a straight circular tube at particle Reynolds numbers between 10^{-3} and 6×10^{-2}; while not completely explained, the phenomenon was attributed to the Magnus inertial effect.

We have conducted similar experiments with single spheres, rods and disks at much lower Reynolds numbers ($<10^{-6}$), and have found that migration of the particles away from the walls occurs only when the particles are deformed in the shear field. The rotation of rigid particles in spherical elliptical orbits and the deformation of fluid drops into prolate spheroids agreed well with the corresponding theories of Jeffery[2] and Taylor[3] for Couette flow.

The experiments were rigorously controlled to prevent sedimentation of the particles and to exclude convection currents. The suspending fluid flowed through a vertical precision-bore tube of radius $R =$

0·4 cm. and isolated particles were observed (without optical distortion) in the median plane of the tube normal to the viewing axis of an electrically driven travelling microscope. Reproducible flow was produced by an automatic infusion-withdrawal pump which moved the liquid back and forth through the tube.

Continuous experiments were conducted over periods up to 24 hr. at flow-rates Q varying from 3·5 to 7·0 × 10^{-2} cm.^{3} sec.$^{-1}$ using rigid polystyrene spheres (radius $b = 0{\cdot}01$ cm.) and disks ($b = 0{\cdot}04$ cm., particle axis ratio $r_p = 0{\cdot}20$) and 'Dacron' rods (length $l = 0{\cdot}04$ cm., $r_p = 30$). Even when the particle centres were within $2b$ or l of the wall, no measurable migration occurred after the particles travelled over distances between 350 and 3,500 cm.

In striking contrast, liquid drops and tiny threads undergoing shear-deformation[4,5] migrated towards the tube axis. With liquid drops the rate of migration $-(\mathrm{d}r/\mathrm{d}t)$ increased with increasing b, Q and radial distance r from the tube axis.

An analysis of the forces operating on a fluid sphere has shown that when the velocity gradient G in the suspending fluid varies from one side of a particle to the other, there is a net force driving the particle in the direction of diminishing G. In Poiseuille flow, the gradient varies linearly with radial distance r from the tube axis:

$$G = -kr \qquad (1)$$

where

$$k = 4Q/\pi R^4 \qquad (2)$$

The net radial force on a fluid drop is calculated with the aid of Taylor's theory[6] for small deformations to be:

$$F_{rr} = \pi^2 b^3 \eta_2 D f_1(p) \qquad (3a)$$

where D is the deformation given by[6]:

$$D = \frac{Gb\eta_2(19p + 16)}{\gamma(16p + 16)} \qquad (3b)$$

p being the viscosity ratio (suspended phase)/(suspending phase) $= \eta_1/\eta_2$, and γ the interfacial tension. Assuming that the drop is propelled radially with Stokes velocity (corrected for internal circulation[2]) the migration velocity becomes:

$$-\mathrm{d}r/\mathrm{d}t = \frac{\pi\eta_2}{48\gamma} f_2(p) k^2 b^3 r \qquad (4a)$$

where

$$f_2(p) = \frac{(19p + 16)(8p^2 + 39p + 30)}{3(16p + 16)(3p + 2)} \qquad (4b)$$

Integration of equation (4) yields:

$$\log_e\left(\frac{r_0}{r}\right) = \frac{\pi\eta_2}{48\gamma} f_2(p) k^2 b^3 t \qquad (5)$$

r_0 being the radial distance at time $t = 0$.

It should be noted that this theory predicts that no migration occurs with rigid spheres ($D = 0$) in Poiseuille flow and with deformable spheres in Couette flow ($k = 0$), in accord with experiment.

The variation of r with t for water drops (b = 0·02 to 0·04 cm.) in 50 poise silicone oil was measured in a range of values of k (1·8–7·1 cm.$^{-1}$ sec.$^{-1}$). When plotted according to equation (5) the experimental points fell on a single curve initially linear and then curving as the particle approached the tube axis. The mean measured/calculated slope of the linear portion was 0·3. In view of the neglect of wall proximity on the Stokes velocity and other approximations, the agreement with theory is good.

The phenomena reported here provide one possible mechanism for the development of a particle-free peripheral zone adjacent to the wall observed in the laminar flow of suspensions of deformable wood-pulp fibres[8] *in vitro* and of deformable red blood cells *in vivo*[9,10]. At the finite concentrations prevailing in these systems, however, axial migration of the peripheral particles may be expected to stop when the radial force F_{rr} is resisted by the elastic compression of the central core of particles, as actually observed in fibre suspensions.

Details of the theory and experiments will be published elsewhere.

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GEOPHYSICS

Dispersions of Multiple-Stroke Whistlers

THE dispersions of 233 multiple-stroke short whistlers recorded during the International Geophysical Year at Durban (geomagnetic co-ordinates 31° 34′ S., 93° 04′ E.) have been measured from sonagrams. Dispersions were determined graphically using the Eckersley[1] dispersion law $t^2 = D^2/f$, where t is the time-interval between the lightning flash producing the whistler and the arrival at the observer of the component of radiated energy of frequency f; D is a measure of the dispersion of the whistler. The linear relationship between $f^{-1/2}$ and t was plotted for each whistler, the dispersion being obtained from the slope.

Those whistlers with two components only were classified into three groups in which the intensity of the second component was (*a*) significantly greater than, (*b*) approximately equal to, and (*c*) significantly less than, that of the first component. In each case the mean value of the ratio D_2/D_1 of the dispersion of the second component to that of the first was calculated. The results are shown in Table 1.

Table 1. DISPERSION RATIOS OF COMPONENTS FOR WHISTLERS WITH TWO COMPONENTS ONLY

Intensity of second component	No. of records	No. with $D_2/D_1 > 1$	No. with $D_2/D_1 < 1$	Mean value of D_2/D_1
(*a*) Greater than first	43	39	4	1·10 ± 0·02
(*b*) Approximately equal to first	53	36	17	1·04 ± 0·01
(*c*) Less than first	34	24	10	1·07 ± 0·02
All groups	130	99	31	1·064 ± 0·009

Calculation of the 95 per cent confidence limits for the mean value of the dispersion ratio for all groups indicated that the probability of this ratio being less than or equal to unity is less than 1 in 10,000.

For those multiple-whistlers with three or more components the ratios of the dispersions of successive components are given in Table 2.

Table 2. DISPERSION RATIOS OF SUCCESSIVE COMPONENTS FOR WHISTLERS WITH THREE OR MORE COMPONENTS

Dispersion ratio	D_2/D_1	D_3/D_2	D_4/D_3	D_5/D_4	D_6/D_5	D_7/D_6
Mean value of ratio	1·04 ± 0·01	1·07 ± 0·01	1·05 ± 0·02	1·09 ± 0·04	1·05	1·07
No. of records	103	103	39	15	4	1

Although the number of records available for the values given in the last three columns of Table 2 is too small for these particular figures to be reliable, the overall analysis indicates a 4–6 per cent increase in the dispersion of successive components of a multiple-stroke short whistler.

It is interesting to note that this result may indicate support for the current-jet hypothesis of whistler generation proposed by Hoffman[2]. If a stream of energetic electrons passes upwards through the top of the thundercloud during each interval of time between successive strokes to ground, an accumulative process of enhancement of electron density in the whistler path may be expected, with a consequent increase in the dispersion of successive whistler components. Furthermore, if such a process were responsible for the enhancement of energy in successive whistler components, it would be expected that for those records in which the second component is the more intense the dispersion ratio would be greater than for those records in which the second component is less intense. That this is the case can be seen from Table 1. In group (*a*) the mean value of the ratio is 1·10 ± 0·02 with 90 per cent of the records giving $D_2/D_1 > 1$, while in group (*c*) a lower mean value of 1·07 ± 0·02 is obtained and only 70 per cent of the records give $D_2/D_1 > 1$.

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Palæomagnetism and the Ancient Radii of the Earth

Cox and Doell[1], applying an earlier idea of mine[2], have determined a probable value of the Permian palæo-radius of the Earth. That communication was commented upon by S. W. Carey[3]. In all probability, Carey's contention that the distance of the sampling localities has since increased due to tectonical events is correct. However, to estimate the amount of the increase in distance as suggested by Carey means substituting one hypothesis by another, since Carey's theory is itself a hypothesis which awaits detailed proof and which will perhaps be found invalid in its present formulation. Since the application of palæomagnetic measurements to the determination of palæo-radii is just a way of proving the expansion of the Earth without auxiliary assumptions, the introduction of such assumptions certainly detracts from the value of the method as a proof.

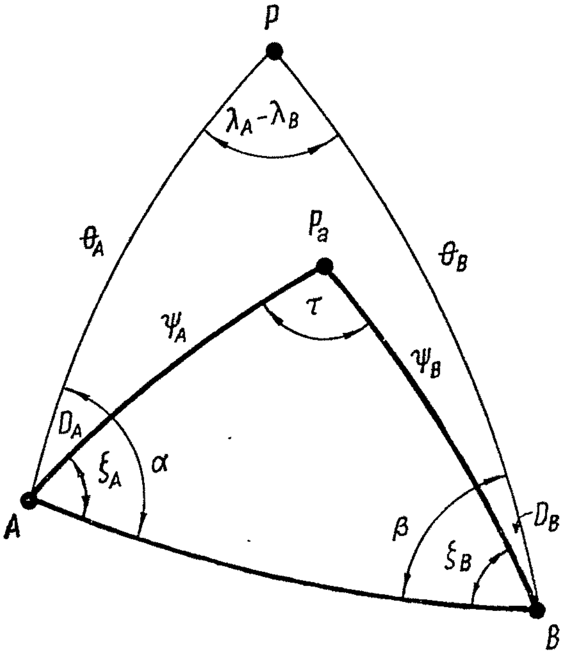

Fig. 1

Instead of the procedure suggested by Carey, I should like to propose a further refinement of the method employed by Cox and Doell as follows:

(1) In a given age, the radius was practically the same for the whole Earth: consequently, it would be possible to treat together the data concerning separate stable blocks in a given period, diminishing thereby the statistical error.

(2) Plotting the data for radii for the individual periods against time, and applying a least-squares adjustment by assuming a linear variation with time, it would become possible to use almost all palæomagnetic data in determining the trend and rate of increase of radius. This would further decrease the statistical error.

Otherwise, I am convinced that the increase of radius can be considered linear and steady to a good approximation for at least the past 3 × 10^9 years and that the rate of increase is less than 1 mm. a year. In my opinion Carey's value is exaggerated.

(3) Undoubtedly, the method of determining palæo-radii is subject to the fewest possible errors if it is applied to sampling localities along the same palæo-meridian, but a very simple generalization permits the utilization of sampling localities not on the same meridian, and thus covers the bulk of the data. The palæomagnetic measurements on a given stable block and for a given geological period yield the inclinations I_A and I_B and declination D_A and D_B for two localities A and B. The distance $AB = d$ is also known for the present. The ancient pole position P_a and the points A and B form the apices of an ancient spherical triangle; the sides $AP_a = \psi_A$ and $BP_a = \psi_B$ are known ($\text{cotg}\,\psi_A = \frac{1}{2}\tan I_A$; $\text{cotg}\,\psi_B = \frac{1}{2}\tan I_B$) and it is possible to calculate the angles situated at the apices A and B, ζ_A and ζ_B ($\zeta_A = \alpha - D_A$ and $\zeta_B = \beta - D_B$, where α is the angle subtended by the present meridian through A and the segment AB and β the angle subtended by the present

meridian through B and the segment AB). Now we have:

$$\sin \alpha : \sin (\lambda_A - \lambda_B) = \sin \theta_B : \sin \Delta$$

$$\sin \beta : \sin (\lambda_A - \lambda_B) = \sin \theta_A : \sin \Delta$$

$$\cos \Delta = \cos \theta_A \cos \theta_B + \sin \theta_A \sin \theta_B \cos (\lambda_A - \lambda_B)$$

where Δ is the present-day central angle subtended by the segment AB, θ_A and θ_B are the present colatitudes of the points A and B and λ_A and λ_B are the present longitudes.

If now the values of ψ_A, ψ_B, ζ_A, ζ_B and d are known, then from the relations:

$$\cos \psi_A \cos \psi_B + \sin \psi_A \sin \psi_B \cos \tau = \frac{b}{a} + \frac{1}{a} \cos \tau = \cos \Delta_a$$

$$\frac{\sin \zeta_A}{\sin \psi_B} \sin \Delta_a = c_1 \sin \Delta_a = \sin \tau$$

$$\frac{\sin \zeta_B}{\sin \psi_A} \sin \Delta_a = c_2 \sin \Delta_a = \sin \tau$$

we have:

$$\begin{array}{l} \cos \tau = a \cos \Delta_a - b \\ \sin \tau = c \sin \Delta_a \end{array} \quad \text{where } c = \sqrt{c_1 c_2}$$

Whence:

$$(a \cos \Delta_a - b)^2 + c^2 \sin^2 \Delta_a = 1$$

Thus we have the equation:

$$(a^2 - c^2) \cos^2 \Delta_a - 2ab \cos \Delta_a + (b^2 + c^2 - 1) = 0$$

which determines Δ_a.

The value of the palæo-radius is:

$$R_a = \frac{d}{\Delta_a}$$

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GEOLOGY

Hafnian Zircons

AMONG quadrivalent trace elements, hafnium is a constant and inseparable associate of the more common zirconium. All zirconium and zirconium-bearing minerals contain appreciable amounts of hafnium; zircon ($ZrSiO_4$), one of the most common accessory constituents in rocks, particularly those of granitic composition, is the main carrier of this element.

The highest hafnium content, as recorded recently by Levinson and Borup[1], is 22–24 per cent HfO_2. This was observed in zircons intergrown with the rare mineral thortveitite, $Sc_2Si_2O_7$, from a granite pegmatite in the Iveland area of Norway.

In connexion with geochemical studies of pegmatites in Uganda, Southern Rhodesia, Mozambique and South-West Africa, hafnium has been determined by X-ray fluorescence in zircons from various pegmatites and, for comparison, in zircons from other environments. The hafnium contents thus found have varied from 1 per cent HfO_2 in a zircon from the Sukulu carbonatite in Uganda, to 31 per cent HfO_2 in a zircon from a lithium-bearing pegmatite near Karibib, in South-West Africa.

It has been observed that zircons high in hafnium occur in certain lithium-bearing pegmatites rich in tantalum and carrying a typical suite of minerals such as tantalite, manganotantalite, microlite, cassiterite, amblygonite, manganoapatite, rose-coloured beryl, topaz, etc., which represent a late phase of pegmatite crystallization.

Coherent pairs of elements, that normally occur together, may be enriched during successive stages of crystallization, and in some cases even separate to form independent minerals. Niobium and tantalum—the odd-numbered neighbour elements of zirconium and hafnium—are in many ways geochemically similar. Thus, on one hand, zirconium and niobium, and on the other hafnium and tantalum, are found to be enriched in the same geological environments. Whereas tantalum may separate from niobium to form independent minerals, hafnium only becomes enriched relative to zirconium. According to Rankamä and Sahama[2], this may be explained partly by the abundance ratio of niobium/tantalum (11·4) being less than that for zirconium/hafnium (48·9).

Some lithium-bearing pegmatites constitute an example where almost complete separation of tantalum from niobium has occurred and tantalum minerals, for example, tantalite, manganotantalite, stibiotantalite, bismutotantalite, simpsonite, and a number of microlite varieties, have been formed[3]. If zirconium and hafnium are present in these pegmatites, the hafnium/zirconium ratio may well increase, and strongly enriched hafnian zircons, in which the weight percentage of hafnium may well exceed that of zirconium, are to be expected.

During the present work, two hafnian zircons have been examined in more detail, one from Mtoko in Southern Rhodesia, containing 21 per cent HfO_2, and the other from Karibib in South-West Africa, with 31 per cent HfO_2. In both cases the zircons are associated with the later tantalum-rich phase of mineralization. The Mtoko zircon forms small, mauve-coloured, independent crystals in the albitic zone of the pegmatite, and is associated with manganotantalite, microlite and bismutite. The zircon from Karibib occurs in larger reddish-brown masses, partly intergrown with minute manganotantalite crystals and set in a matrix of lithium-bearing mica, albite, quartz and kaolinized feldspar. Some crystals show dominant pyramid faces, with a suppressed prism.

The following properties have been determined for the hafnian zircons:

Mtoko zircon	sp.g. 4·91	R.I.ω = 1·920	ε = 1·972
Karibib zircon	sp.g. 5·2	R.I.ω = 1·92	ε = 1·97

Both zircons exhibit an intense golden-yellow fluorescence in ultra-violet light. The zircon from Karibib was found to be only weakly radioactive. A qualitative spectrographic analysis gave the following results:

Major: silicon, zirconium, hafnium.
Trace: beryllium, magnesium, aluminium, calcium, manganese, iron, tin.
Yttrium and rare-earths, if present, were below the limit of detection.

The zircon from Karibib was also chemically analysed, and the results obtained were as follows:

SiO_2	TiO_2	$\underbrace{ZrO_2 \quad HfO_2}$	Al_2O_3	Fe_2O_3	MnO	CaO	MgO	H_2O+	Total
29·20	0·0	70·20	trace	0·17	trace	0·0	0·0	0·23	99·80

The hafnium content as determined by X-ray fluorescence was found to be 31 per cent HfO_2.

X-ray powder data of the two zircons agree on the whole with the data given for zircon in the A.S.T.M. *X-ray Powder Data File.* As with many zircons, heating produces a more defined pattern for the Karibib zircon. Compared with low-hafnium zircon, *d*-values of the unheated Karibib mineral are distinctly larger, while the heated zircon has rather smaller values. (The radius of the Zr^{4+} ion is 0·82 Å. and of Hf^{4+} ion 0·78 Å.). The difference in spacing is, however, too small to be of any practical value in distinguishing hafnian from normal zircons. An interesting feature, perhaps significant for hafnian zircons, was noticed in the relative position of the three lines representing the indices (532), (424) and (523, 620). In the hafnian zircon from Karibib the centre line of this triplet is clearly displaced toward the low-angle one; in the hafnian zircon from Mtoko it lies in a median position and in normal zircons is distinctly displaced towards the high-angle line.

Some recent data on the geochemistry of hafnium in granite pegmatites have been given by Vainshtein *et al.*[4]. Their views concerning hafnium enrichment in the late pegmatite crystallates are in good agreement with those presented in this communication.

We are indebted to Dr. R. S. Bradley and Dr. D. C. Munro of the School of Chemistry in this University for provision of facilities and for helpful advice in connexion with the X-ray fluorescence analysis, and to Miss J. M. Rooke for the spectrographic analysis of zircon.

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Salinity Response of Trace Element Concentration in *Crassostrea virginica*

In a search for ecologically significant variations in elemental concentrations in marine invertebrate skeletons, the concentrations of several trace elements in shells of *Crassostrea virginica* have been investigated. Samples of 71 specimens from 11 stations along the American coast between Massachusetts and Texas were analysed spectrographically for magnesium, strontium, manganese, sodium, boron, and copper. Correlations between elemental concentrations and the temperatures and salinities at which the oysters grew were assessed statistically.

At least five shells were sampled from each station. Wedge-shaped cross-sections were cut just behind the hinge region so that all periods of shell-growth, except the earliest, were represented, since reproducibility among shells from the same station was greatest in such samples. Ideally, average annual temperatures and salinities for periods of shell-growth should have been used, but precise data were not always available, so averages that include periods of several years not coinciding with shell-growth had to be used for some stations. Salinity data and mean concentrations ($\bar{X}$) and standard errors (*S.E.*) of several elements in all shells examined from each station are presented in Table 1.

Significant correlations were found between some elemental concentrations and salinity, although no correlations with temperature were apparent and neither strontium, magnesium, boron, nor copper was significantly related statistically to either temperature or salinity. For the results summarized here, a correlation coefficient (r) of 0·219 is significant at the 5 per cent confidence level. Significant correlations between elemental concentrations and salinity were found for manganese ($r = -0{\cdot}453$) and sodium ($r = +0{\cdot}356$). If individual strontium ($r = -0{\cdot}054$) and magnesium ($r = -0{\cdot}214$) concentrations are combined and then correlated with salinity, the coefficient is a significant $-0{\cdot}253$.

A multiple correlation of magnesium, magnesium plus strontium, manganese, and sodium with salinity has a coefficient of 0·533, higher than for any element alone. Taking into consideration the analytical accuracy and range of variation encountered in measured elemental concentrations among members of the same population, it appears that multiple regressions based on these four elements without transformation of raw data allow prediction, from concentrations in a single shell, of average salinity of waters in which shell-growth occurred, with a standard error of 5·3 parts per thousand. The accuracy of prediction may be enhanced by increasing the number of stations from which raw data are assembled and by increasing sample size. Furthermore, the multiple correlation coefficient is improved and standard error reduced when calculations are based on an assumption that the diadochic elemental correlations are logarithmic. Evidently the simple correlations are not linear. To certify their forms and assess fully their significance requires knowledge of elemental concentrations in the sea-water of shell-growth, which is not available.

Elements correlated negatively with salinity are those that are presumably diadochic with calcium in the calcite structure. Discrimination of *C. virginica* against these diadochic elements thus decreases in

Table 1. Mean Concentrations ($\bar{X}$) and Standard Errors among Individuals (*S.E.*) of Selected Trace Elements in Shells of *Crassostrea virginica*, together with Mean Annual Temperatures and Salinities of Waters in which the Oysters grew

Locality	No. of shells	Mean annual temp. °C.	Mean annual Salinity ‰	Magnesium		Strontium		Manganese		Sodium	
				$\bar{X}$ p.p.m.	*S.E.*	$\bar{X}$ p.p.m.	*S.E.*	$\bar{X}$ p.p.m.	*S.E.*	$\bar{X}$ p.p.m.	*S.E.*
1	10	12·1	29·4	2062	386	1412	222	36·0	7·7	3225	1050
2	5	14·1	13·2	2186	365	1648	225	115·0	21·4	2064	245
3	16	16·1	19·2	2133	446	1472	140	61·3	10·7	2816	840
4	5	19·3	30·4	2070	340	1262	518	26·9	4·1	3170	355
5	5	20·1	?	2744	372	1142	158	75·9	44·5	2600	317
6	5	25·0	25·0	1972	429	1486	215	21·3	5·1	2570	630
7	5	21·0	30·0	1964	494	1572	23	44·6	23·3	3264	1616
8	5	21·5	13·7	2388	402	1184	57	54·2	12·3	2090	186
9	5	22·5	17·4	1964	431	1554	299	227·6	34·6	2150	317
10	5	21·9	13·3	2218	583	1425	36	67·7	54·4	2840	400
11	5	22·5	18·2	2158	459	1640	225	38·8	9·7	3190	520

Localities are: 1, West Chatham, Mass.; 2, Solomons, Md.; 3, Gloucester Point, Va.; 4, Beaufort, N.C.; 5, Sapelo Is., Ga.; 6, St. Petersburg, Fla.; 7, Alligator Harbor, Fla.; 8, St. George Is., Fla.; 9, Ocean Springs, Miss.; 10, Barataria Bay, La.; 11, Port Aransas Tex.

waters of low salinity. Perhaps the lowered concentration of the preferred calcium ions stimulates acceptance of less desirable ions for shell construction. On the other hand, sodium is probably interstitial, and thus reflects salinity directly.

As characteristic differences in tolerance for trace element concentrations are found among even closely related taxa[1,2], the responses of other pelecypods are still conjectural. However, trace elements in fossil shells of *Crassostrea virginica* may be studied with the aim of establishing palæosalinities. Presumably, the accuracy of palæotemperature determinations by the oxygen isotope method may be enhanced by palæosalinity corrections.

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Geological Age of the Sandringham Sands

THE Sandringham Sands, up to 100 ft. thick, form the basal member of the Cretaceous System in Norfolk and rest unconformably on the Jurassic (Kimmeridge Clay); their position in the Cretaceous time-sequence and their stratigraphical relations with other early Cretaceous deposits have hitherto remained in doubt owing to lack of palæontological data. Excavations near Abbey Station, West Dereham, Norfolk (Nat. Grid Ref. 53/65549969), made in connexion with the Fenland Flood Relief Scheme, have provided new sections in the Sandringham Sands from which officers of the Geological Survey and Dr. G. P. Larwood of the Sedgwick Museum, Cambridge, have collected a large suite of fossils indicative of a Berriasian (Infra-Valanginian) fauna new to Britain.

The fossils occur as clustered moulds in nodular masses of hard, grey-brown, glauconitic sandstone with carbonized plant debris, about 30 ft. above the base of the formation. Twenty-eight genera of Mollusca have been identified, mostly lamellibranchs, *Astarte* (*Neocrassina*), *Pholadomya*, *Panopea*, *Hartwellia*, *Isocyprina*, *Thracia*, *Myophorella* and *Isodonta* predominating. Cephalopods are represented by well-preserved examples of the ammonite *Hectoroceras*, described by Spath[1] in 1947 from S.W. Jameson Land, East Greenland, and by numerous hollow moulds of belemnite guards, including those of *Acroteuthis*. Some of the lamellibranch and belemnite species occur also in the Spilsby Sandstone (Berriasian) and superjacent Neocomian strata in Lincolnshire; others, such as the species of *Hartwellia*, *Isocyprina* and *Isodonta*, have their closest parallels in the Upper Jurassic (there is no previous record of *Isodonta* in the British Cretaceous, for example). Many of the shells were ravaged by the boring polyzoan *Graysonia*, only recognized recently in the Cretaceous faunas of Britain[2].

The fossiliferous sandstone is followed within a few feet by the Lower Greensand (Aptian–Lower Albian), this determination being suggested by a rolled specimen of the ammonite *Deshayesites* found among the excavated material. This means that any equivalent of the Snettisham Clay (Barremian) of north Norfolk or the Valanginian–Hauterivian sequence of Lincolnshire is either absent or is present only in greatly attenuated form.

It is now clear that the Sandringham Sands are wholly or in part of Berriasian age and that in the West Dereham area the hiatus at their top is probably as great or greater than that at their base. It is not yet possible, however, to say precisely where this fossil band in the Sandringham Sands fits in with the fragmentary Berriasian successions in Lincolnshire and Yorkshire. In the Spilsby Sandstone there are three distinct ammonite-levels, all characterized by forms of *Subcraspedites*; the lowest, with the sub-genus *Paracraspedites*, is thought to be more or less contemporaneous with the basal Berriasian of East Greenland[3]. *Subcraspedites* is found both above and below *Hectoroceras* in East Greenland, so that the most likely position of the Norfolk *Hectoroceras* fauna is a horizon within or just above that of the Spilsby Sandstone.

Dr. Larwood is preparing a preliminary stratigraphical account of the section. The Geological Survey material referred to above is being further examined.

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AGRICULTURE

Evaluation of Effective Rainfall and Irrigation from Ground-water Measurements

A FRACTION of the total rainfall that reaches the ground water-table is called the effective rainfall[1]. Where there is irrigation, a fraction of the irrigation water also has been found to contribute to the rise of the water-table[2]. Effective values of rainfall and irrigation are usually determined using lysimeters[3]. The lysimeters, however, need correction for their size and the indications obtained from them are often high. A general figure found at Amsterdam dune water works, where there was no run-off, indicated that 61 per cent of a total rainfall of 659 mm. per annum was effective[1]. An actual determination of the rise in water-table over large areas has also been carried out by means of open well water measurements[4,5]. The effective values of rainfall and irrigation, however, do not seem to have been arrived at. An analysis of the changes in the ground-water level has therefore been taken up in this investigation, and the effective values of rainfall and irrigation were evaluated from the (fortnightly) measurements of the water-levels recorded for the past 20 yr. in thirteen wells randomly distributed over 565 acres of irrigated farm area of the Indian Agricultural Research Institute, New Delhi.

Fig. 1 gives the mean water-table levels on July 1 of each year, from 1940 to 1950, just before the beginning of the monsoons. This period is selected to be suitable as the water would reach a stable level just before the monsoons start[6]. The trends in the rise of

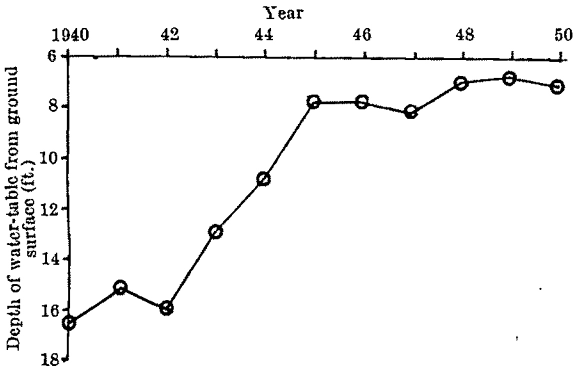

Fig. 1. Average water-table readings on July 1 each year

the water-table, however, remain the same irrespective of the period selected. As there is no conspicuous change in the level of the water-table after 1948, presentation of further data was not found necessary. The results indicate that during the years 1940–42 the increase in the ground-water level is small. During 1942–45, however, the rise in ground water is quite steep. This steep rise may be due to the fact that the drains then existing for the run-off provided on the farm area since 1936 were closed in 1942 with the view of conserving the rain water. In fact, the run-off became negligible in that period. The almost linear rise in the ground-water level permitted the evaluation of the effective values of rainfall and irrigation.

Table 1. WATER-TABLE RISE IN MONSOON PERIOD AT THE FARM AREA OF THE INDIAN AGRICULTURAL RESEARCH INSTITUTE, JULY 1–DECEMBER 31

Year	Rainfall R (in.)	Irrigation I (in.)	Well-water levels (ft.) on July 1	Well-water levels (ft.) on Dec. 31	Rise in ground-water level G (in.)
1942	21·24	15·97	15·97	12·80	38·04
1943	14·09	18·27	13·06	10·91	25·80
1944	22·82	22·86	10·93	6·53	52·80

Table 1 gives the rise in water-level in inches during the moonsoon period (July 1–Dec. 31), during which some of the rainfall or irrigation seeps down to the ground-water level. The other period, Jan. 1–June 30, is mostly a dry period at Delhi with scanty rainfall that just wets the top layers, but is not sufficient to saturate the top capillary zone and percolate down to the ground-water level.

The effective rainfall, aR, the effective irrigation bI, and the rise in the ground-water level, G, are related by the equation: $aR + bI = P.G$, where R and I are the values of the total rainfall recorded and the total irrigation given during the monsoon season, and P the fractional pore space at the ground-water level. A solution of the three resulting equations arrived at by the principle of least squares is:

$$a = 1{\cdot}69\,P \text{ and } b = 0{\cdot}36\,P$$

The first 4·5 ft. depth of the soil is sandy loam with about 50 per cent porosity, while the layers below this are characterized by the presence of *Kankar* (calcium carbonate), the concretions becoming more and more calcareous downwards[7]. Such a compact soil usually has a porosity varying from 25 to 30 per cent. Using these values for P, the values of effective rainfall and effective irrigation turn out to be 42 per cent–51 per cent and 9 per cent–11 per cent, respectively. The values of the effective rainfall seem to agree fairly well with the observations made earlier in refs. 8–10 on Indian sandy loam soils. The effective percolation from rainfall seems to be much more than that from irrigation. This is a tribute to the irrigation management, since the design avoids excessive percolation.

In balancing the ground-water rise, the lateral movements of ground water, if any, have not been accounted for. The effective values are thus the minimum values possible. Work is in progress on the effect of lateral movement on the ground-water balance.

We thank Dr. B. P. Pal for his interest in the progress of this investigation.

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CHEMISTRY

Odour and Molecular Vibration

THE low-frequency vibrations of organic molecules are largely unexplored, so that it is difficult to test directly the hypothesis that odours may be correlated with them[1]. An indirect test was therefore devised.

The low-frequency Raman lines of nitrobenzene and methyl salicylate are reported[2] to be:

Nitrobenzene	183			408 cm.$^{-1}$
Methyl salicylate	185	263	356	423 cm.$^{-1}$

If these values are correct and complete, then, on the above hypothesis, their odours should be basically similar but with the odour of methyl salicylate altered from almond-like to wintergreen by the additional frequencies not possessed by nitrobenzene. It follows that a small amount of methyl salicylate added to nitrobenzene should be perceived by the nose more easily than a small amount of nitrobenzene added to methyl salicylate, provided due allowance is made for the different absolute thresholds of the two substances.

The olfactory threshold concentration of each substance when dissolved in 'Nujol' was determined by a modification of the American Public Health Association method[3]. A dilute solution of each substance in 'Nujol' was prepared, serial dilutions were made, and 10 ml. of each solution were placed in a series of *B. O. D.* bottles. (These are 250-ml. bottles with ground stoppers and a funnel-like enlargement of the neck. They were washed and baked overnight at 105° C. before use.) Other bottles contained 10 ml. additions of pure 'Nujol'. The bottles were numbered but otherwise unmarked. Each bottle containing a solution was placed with two blanks, and the observ-

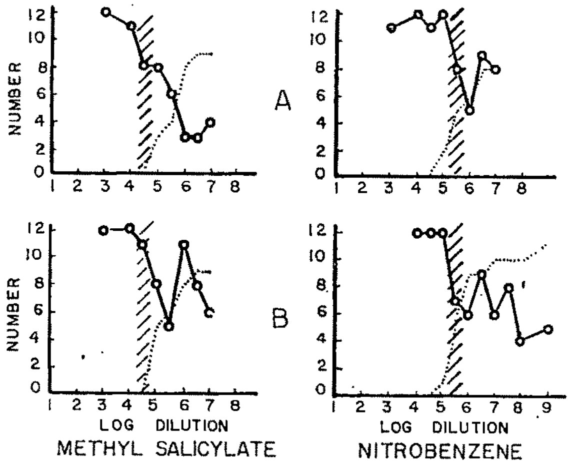

Fig. 1

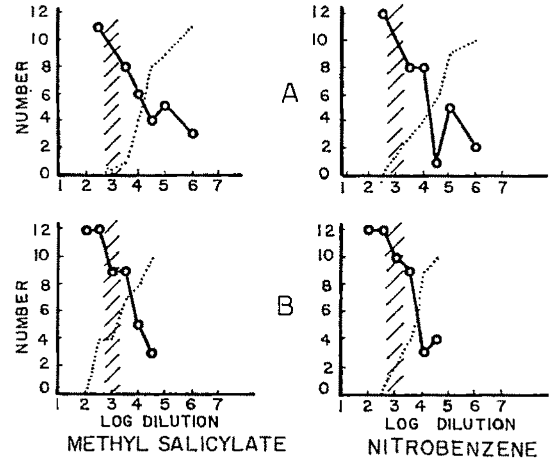

Fig. 2

ers were asked to smell all three and try to identify the 'one that was different'. The same twelve observers were used in all the experiments (except the last, Fig. 2*B*, when one new subject was used), which were all carried out in the same way and in the same room, and on four different days spread over a period of 10 weeks. The observers were given 45 sec. to smell each set of three bottles, 15 sec. to record the result, and then rested 60 sec. before smelling the next three. The strongest solutions were smelled first followed by the weaker ones in order, and all the solutions were made very dilute to minimize olfactory fatigue. The observers were told to guess if necessary, and to mark the guessed results.

The results of threshold determinations made on different days are shown in Fig. 1, *A* and *B*. The solid line shows the number of correct identifications of the 'odorous' bottle, and the dotted line the number of admitted guesses. The threshold concentration of methyl salicylate in 'Nujol' (indicated by the shaded area) is about 1 in 30,000, and that of nitrobenzene is about 1 in 300,000.

For the next experiment, a stock solution containing nitrobenzene in 'Nujol' at a concentration of 1 in 10,000 (= 30 × threshold) was made up and used in the pairs of blank bottles. The third bottle in each set contained the same base solution with a small addition of methyl salicylate. In a similar way, a base solution of methyl salicylate was made up containing 1 part in 1,000 (= 30 × threshold) and given small additions of nitrobenzene.

The observers then tried to determine the threshold concentration of one odorant in the presence of a constant amount of the other, with the results shown in Fig. 2, *A* and *B*. The threshold concentration for the perception of methyl salicylate in the presence of nitrobenzene was about 1 in 1,000, or 30 × the threshold for methyl salicylate by itself. For nitrobenzene to be smelled against a background of methyl salicylate, the threshold concentration was again about 1 in 1,000, but this is 300 × the threshold concentration for nitrobenzene alone.

Thus the wintergreen odour of methyl salicylate conceals the almond odour of nitrobenzene 10 times more effectively than the almond odour conceals the wintergreen odour. The effect is not large; but it is distinct, and it is in the direction predicted by the vibrational theory of odour.

I wish to thank Prof. E. S. W. Belyea and his students in the Department of Psychology of the University of British Columbia for carrying out the smelling tests.

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The Relation between Theoretical and Experimental Diamagnetic Susceptibilities for Aromatic Hydrocarbons

IN calculations of the π-electron diamagnetic susceptibility of aromatic hydrocarbons by the method due to London[1], it is assumed that the calculated susceptibility χ^L is equal to the experimental anisotropy $\Delta\chi$. χ^L is, in fact, the susceptibility, due to delocalization of the π-electrons, in a direction perpendicular to the plane of the ring, which will be called the z-direction. In the semi-classical treatment due to Pauling[2] the same assumption was made, but Pauling went on to add to his calculated susceptibility the value obtained from Pascal constants[3] for the susceptibility in the plane, and he thus obtained the total susceptibility in the z-direction, χ_z. He compared his χ_z with the experimental values obtained from measurements of crystal susceptibilities. Later workers[4] have followed London rather than Pauling and sought to calculate $\Delta\chi$, not χ^L.

The identification of χ^L with $\Delta\chi$ has been criticized recently (refs. 4*a*, 4*b*), and Hoarau (ref. 4*b*) has suggested that the total anisotropy $\Delta\chi$ should be written:

$$\Delta\chi = \Delta\chi^\sigma + \Delta\chi^p + \chi^L \qquad (1)$$

where $\Delta\chi^\sigma$ and $\Delta\chi^p$ are the anisotropies due to the σ-bonds and the isolated $p\pi$-electrons, respectively. Support for Hoarau's view may be found in the work of Itoh, Ohno and Yoshizumi[5], who, in a calculation by the method of antisymmetrized molecular orbitals, obtained a value for χ^L equal to 49·6 per cent of the observed anisotropy for benzene, although Lykos and Parr[6] have suggested that most of the difference can be attributed to the approximation used in evaluating the resonance integral. A recent calculation I have carried out[7] using the simplified self-consistent molecular orbital method[8] gave a value of χ^L of $-33 \cdot 26$ (all susceptibility values quoted are $\times 10^{-6}$ c.g.s.e.m.u./mole) for benzene compared to the observed anisotropy[11] of $-59 \cdot 7$. A contribution to the π-electron susceptibility in the z-direction, χ_z^π, cf $-1 \cdot 35$ per carbon atom was also obtained. This is due to the effect of the field on the isolated $p\pi$-electrons. Thus, for an aromatic hydrocarbon containing n carbon atoms, χ_z^π can be written :

$$\chi_z^\pi = -33 \cdot 26\rho - 1 \cdot 35n \qquad (2)$$

where ρ is the ratio of χ^L for the given hydrocarbon to χ^L for benzene. Values for ρ obtained by London[1] and by McWeeny (ref. 4c) may be used in equation (2), which provides a simple way of approximating to a full self-consistent molecular orbital calculation.

It is well known that diamagnetic susceptibilities can be written as the sum of a semi-classical term and a paramagnetic or high-frequency term of opposite sign[10]. The high-frequency term can be calculated for a polyatomic molecule only by making severe approximations[11], and its occurrence in $\Delta\chi$ makes the latter a difficult quantity to calculate. χ_z^π, however, does not contain a high-frequency term, and an empirical value for χ_z^π can be obtained from the experimental value for χ_z by using the equation :

$$\chi_z^\pi = \chi_z - \sum_i \chi_i \qquad (3)$$

where χ_i is the Pascal constant[3] for the ith atom, $-6 \cdot 0$ for carbon and $-2 \cdot 9$ for hydrogen. The high-frequency term occurs in Pascal's scheme for conjugated hydrocarbons as the constitutive correction for a carbon–carbon double bond[3], $+5 \cdot 5$, which is, for this reason, not included in equation (3).

Table 1. ANISOTROPY ($\Delta\chi$) AND π-ELECTRON SUSCEPTIBILITY ($\chi_z\pi$)

Substance	$-\Delta\chi$ Exp.	$-\Delta\chi$ Calc.	$-\chi_z$ Exp.	$-\chi_z^\pi$ Exp.	$-\chi_z^\pi$ Calc.
Benzene	59·7[a]	(59·7)	94·6[a]	41·2	41·4
Naphthalene	114·0[b]	131[c]	169·0[d]	85·8	86·3
Anthracene	182·6[b]	206[c]	251·8[d]	139	134
Phenanthrene	166[b]	190[c]	240[b]	127	125
Pyrene	232·9[b]	266[c]	313·5[b]	189	170
Coronene	390[e]	585[f]	480[e]	301	358
Biphenyl	118·6[b]	112[f]	182[b]	81	78·4
p-Terphenyl	178·8[b]	163[f]	271·3[d]	123	115
p-Quaterphenyl	256[b]	214[f]	372[d]	176	152

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Table 1 shows calculated and experimental values for χ_z^π obtained from equations (2) and (3) respectively. For comparison the results of calculations of the London type for the anisotropy are compared with the experimental values. The agreement between theory and experiment for χ_z^π is clearly better than for $\Delta\chi$, thus supporting the views expressed here.

A more satisfactory treatment of anisotropies would be possible if experimental values for the contribution of the high-frequency term were known for some conjugated hydrocarbons. This contribution can be obtained from the rotational magnetic moment[12], and an interesting comparison of theoretical and experimental susceptibilities has been made for certain systems[13] the rotational magnetic moments of which are known. At present, however, for conjugated hydrocarbons the comparison of χ_z^π suggested here would seem to be more satisfactory than an attempt to calculate anisotropies.

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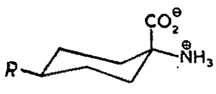
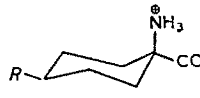
Alkylcyclohexanones in the Strecker and Bucherer Hydantoin Syntheses

THE reaction of 2- and 4-alkylcyclohexanones in the Strecker and Bucherer hydantoin reactions leads to geometrically isomeric alkylcyclohexane-1-amino-1-carboxylic acids. Paper chromatographic analysis shows that the crude product of the Strecker reaction consists of one isomer only, while the main product from the Bucherer reaction has the alternative configuration. Assuming the configuration of the 4-alkyl derivatives to be indicated by the sluggish hydrolysis of the 1-amino-4-tert-butylcyclohexylcyanide hydrochloride[1] in the Strecker synthesis, then the Bucherer products must have the opposite configuration.



Strecker product



Bucherer product

The evidence for the difference in identity is briefly : (i) The 4-methyl-, 4-ethyl-, 4-iso-propyl-, 4-tert.-butylcyclohexane-1-amino-1-carboxylic acids produced by the Strecker synthesis crystallize anhydrous from aqueous acetic acid, whereas the corresponding Bucherer products crystallize as hemi-hydrates from the same solvents. (ii) The melting points of the amino-acids and their derivatives are different :

Table 1

Cyclohexane carboxylic acid	Strecker product (melting point, ° C.)	Bucherer product (melting point, ° C.)
1-Amino-4-methyl-	356–360 (subl.)	305–310 (subl.)
1-Acetylamino-4-methyl- *	220–222	223–224·5
1-Benzoylamino-4-methyl-	209–210	234–236
4-Methyl-1-spiro-5′-hydantoin	215–216	281–282
1-Amino-2-methyl-	355–360	298–300
1-Acetylamino-2-methyl-	220–221	181–181·5
1-Benzoylamino-2-methyl-	174–175	196 (ref. 2)

* Mixed melting point, 170–185°

(iii) The infra-red spectra (in potassium chloride disks) are markedly different, particularly in the 6μ region, where the Strecker products alone give particularly well-resolved (amino-acid) bands. (iv) Different R_F values on Whatman No. 3*MM* paper using normal butanol–acetic acid–water as eluant.

These results indicate that the hydantoin synthesis does not proceed through the same intermediate amino-nitrile involved in the Strecker synthesis, as suggested by Bucherer[3].

Further work is in progress to confirm the configurations of these amino-acids and to elucidate the mechanisms of the reactions involved in their synthesis.

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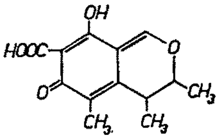
Chemical Structure of Citrinin

In our search for new antibiotics from fungi we have isolated a substance, *S*-52, a metabolic product of an unidentified *Penicillium*. From the results of paper-chromatographic investigations and from its other properties we believed the substance to be identical with citrinin. This has proved correct, since the infra-red spectra of the pure substance *S*-52 and of an authentic sample of citrinin (kindly provided by Dr. P. W. Brian, Akers Research Laboratories, Welwyn, Herts) were identical.

The measurement of the infra-red absorption spectra was carried out on a double-beam spectrophotometer *UR*-10 (*IR*-10), Zeiss, Jena. Sodium chloride cells 1·030 mm. thick were used and the concentration of citrinin was 4·5 gm./litre carbon tetrachloride.

Brown *et al.*[1] and Cramm[2] have proposed a chemical structure for citrinin according to the chemical reactions of this substance as follows:



(I)

The infra-red spectrum of citrinin (Fig. 1) indicates that the structure of citrinin differs from that proposed by these authors. Should citrinin have a free carboxylic group, this would be shown by an intense absorption band in the region above 1,700 cm.$^{-1}$, but this absorption band is absent.

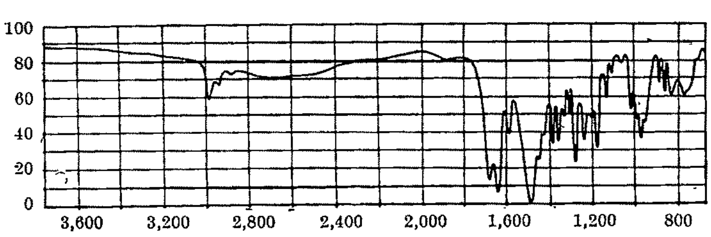
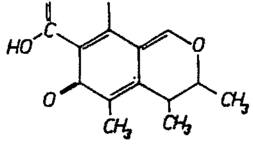


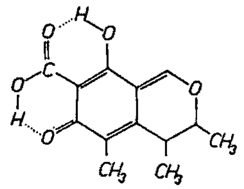
Fig. 1. Infra-red spectrum of citrinin. Ordinates, percentage absorption ; abscissæ, wave-lengths

The absorption band in the region of 1,685 cm.$^{-1}$ belongs to the stretching vibrations of the C=O bond in the carboxylic group forming a strong chelate bond with the hydroxylic group situated in the ortho position[3,4]:



(II)

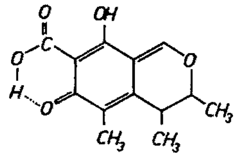
This is indicated also by the small intensity of the absorption band in the region of 3,700 cm.$^{-1}$. This is similar to the infra-red absorption spectrum of salicylic acid[3], where the absorption band in the region of 1,685 cm.$^{-1}$ belongs to the stretching vibrations of the C=O bond, forming a chelate bond. Salicylic acid can form only one chelate ring, but in the case of citrinin two can be formed:



(III)

The formation of one chelate ring as indicated by formula (II) would result in a weakening of the —OH bond in the carboxylic group and in this case there would be a relatively intensive absorption band in the region of 950 cm.$^{-1}$ belonging to the bending vibrations of the —OH group in the carboxylic group.

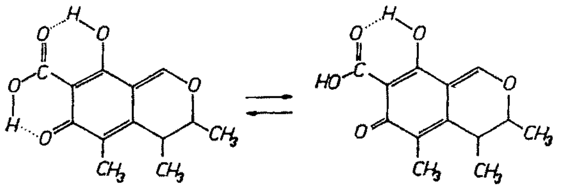
Should only one chelate ring, as indicated by formula (IV), be formed, the dilution of the electrons on the carbon atom of the carboxylic group would be compensated by the π-electrons of the double bond of the ring.



(IV)

In this case the —OH group on the ring would show itself by its +C-effect. By this the acidity of the hydrogen atom in the hydroxylic group would, of course, be greatly enhanced, and on the infra-red absorption spectrum a relatively intense absorption band would arise in the region of 3,600 cm.$^{-1}$.

As a relatively small absorption band is found in the region of 950 cm.$^{-1}$, and on the other hand a relatively broad absorption band belonging to the stretching vibrations characteristic for an intramolecular hydrogen bond appears in the region of 3,200 cm.$^{-1}$, we presume that citrinin is represented by formulæ (II) and (III) which are in equilibrium (V):



(V)

Table 1. CHARACTERISTIC INFRA-RED FREQUENCIES OF CITRININ

Frequency (cm.$^{-1}$)	Group	Nature of vibration
3,200		ring vibration (chinoid system)
2,935, 2,870	CH_3—	stretching C—H
1,685	C=O	stretching C=O
1,640	C=C	stretching C=C
1,600	C—H	stretching C—H (unsaturated)
1,480	C—H	bending C—H (asymmetrical)
1,380	C—H	bending C—H (symmetrical)
1,310	C—O	stretching C—O
1,180	=C—O—C	stretching =C—O—C
1,130	—O—	ring vibration
950	—OH	bending (in —COOH group)

The characteristic vibrations of the individual groups of atoms in the molecule of citrinin are presented in Table 1.

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BIOCHEMISTRY

Imidazole Acrylic Acid Excretion in Kwashiorkor

CHILDREN who are suffering from severe kwashiorkor (protein-calorie malnutrition) lose a large proportion of their tissue solids[1]. Earlier work[2] has shown that in spite of a very low protein intake a relatively large amount of nitrogen is still excreted in the urine. The amount increases with the severity of the illness, which strongly indicates an endogenous origin. A considerable part of the nitrogen cannot be accounted for as urea, ammonia, free amino-acids, peptides, creatine, creatinine or uric acid. Evidence was obtained for the presence of nitrogenous material which absorbed ultra-violet light in the region 268–274 mμ. The fractionation of the material and the isolation of one of its components form the basis of this communication.

Initial separation was achieved by paper chromatography. 50–100 μl. of urine were applied to Whatman No. 1 paper and the chromatogram was run in *n*-butanol/formic acid/water (77 : 10 : 13). After drying, the chromatogram was examined in ultraviolet light by the method of Markham and Smith[3], and ten abnormal compounds were detected which appear in the urine of severe cases of kwashiorkor and disappear on successful treatment. The compound with the largest absorption, temporarily called *F*, has been isolated by the following method and identified.

Two litres of urine were shaken for 30 min. with 100 gm. of activated charcoal. After washing with 2 l. of distilled water, the absorbed material was eluted twice with 2 l. of 50 per cent v/v acetic acid. The eluate was concentrated by distillation under reduced pressure at 60° C. and finally in a vacuum desiccator. The resultant dark brown tar was redissolved in ammonium formate at *p*H 11 and aliquots were applied to a 'Dowex-1' formate column. The column was first eluted with 0·01 *M* ammonium formate at *p*H 6 followed by 0·01 *M* formic acid which gave a good separation of the compound *F*. The fractions were combined, concentrated by distillation under reduced pressure and taken to dryness *in vacuo*. The material still contained some tarry impurity and was therefore passed again through the 'Dowex-1' column. The product was analysed by paper chromatography, which showed that it contained in addition to the major component a small amount of a further compound. The material was dissolved in hot acetic acid and insoluble substances removed by filtration. The filtrate was evaporated to dryness and redissolved in 4 ml. 20 per cent v/v acetic acid. On addition of diethyl ether, almost colourless needle-shaped crystals were obtained. Recrystallization from water gave a chromatographically pure product.

The crystals contained carbon, 52·3; hydrogen, 4·42; nitrogen, 20·2 per cent, indicating the formula $(C_3H_3ON)_x$. Unfortunately the material was too insoluble in camphor for a molecular weight determination. The ultra-violet absorption spectra (absorption maxima 268 mμ in 0·1 *N* hydrochloric acid and 287 mμ in 0·1 *N* sodium hydroxide) had suggested that compound *F* might be a substituted xanthine, but the minimum molecular formula for a purine on the pattern (C_3H_3ON) would be $C_{12}H_{12}O_4N_4$ and the molar extinction coefficient for this compound in hydrochloric acid would be $3{\cdot}8 \times 10^4$, which is very high. A compound that was known to have a high molar extinction coefficient was imidazole-acrylic acid (urocanic acid), and its formula would fit the analysis of compound *F* if the molecular weight corresponded to twice the empirical formula.

The ultra-violet spectra of the isolated material were identical with those of a sample of imidazole-acrylic acid obtained from Roche Products, Ltd. (Table 1). The material melted at 224° C. and the reference sample at 225° C.; the mixed melting point was 224·5° C. The infra-red spectra of both substances in potassium chloride powder were identical, and they had the same R_F values when chromatographed

Table 1. ULTRA-VIOLET ABSORPTION OF COMPOUND *F* AND IMIDAZOLE-ACRYLIC ACID

Solvent	Compound *F*		Imidazole–Acrylic acid	
	Absorption maximum (mμ)	$E^{0{\cdot}001\%}_{1\text{ cm.}}$	Absorption maximum (mμ)	$E^{0{\cdot}001\%}_{1\text{ cm.}}$
0·1 *N* hydrochloric acid	268	1·49	268·5	1·49
Water (*p*H 6·6)	265	1·26	265	1·24
Water (*p*H 8·1)	278	1·40	278	1·38
0·1 *N* sodium hydroxide	286·5	1·18	287	1·17
N sodium hydroxide	307	1·51	307	1·50

on paper in four different solvents: (1) *n*-butanol saturated with water, containing 1 per cent ammonia (s.g. 0·880), R_F 0·06; (2) *n*-butanol/acetic acid/water (63 : 10 : 27), R_F 0·47; (3) *n*-butanol/formic acid/water (77 : 10 : 13), R_F 0·50; (4) *iso*propanol-hydrochloric acid[4], R_F 0·64.

Although imidazole-acrylic acid has been tentatively identified in trace amounts in human urine[5], it has not previously been isolated from urine. In 12 severe cases of kwashiorkor, the average amount excreted was 28 mgm./12 hr. (range 10–80 mgm.). Only very small amounts have been found in the urine of early cases, and none in cases that have been successfully treated for a week or ten days.

Imidazole-acrylic acid is a known metabolite of histidine, and, in rats treated with ethionine[6], the acid has been found in the urine after intraperitoneal injection of L-histidine-2-^{14}C. The origin in the children with kwashiorkor has not yet been determined. It may be histidine that is released by tissue breakdown: if the breakdown is excessive, the enzyme system normally responsible for metabolism beyond the stage of the acid may be overwhelmed.

The possibility of using the presence of the acid as an aid to the diagnosis of kwashiorkor, and other details of clinical significance, will be described elsewhere.

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Micellar Solubilization of Fatty Acids and Monoglycerides by Bile Salt Solutions

Bile salts were recognized as detergents some years ago[1], but few investigations have been done on their detergent properties under physiological conditions. Aqueous solutions of detergents are known to form molecular aggregates called micelles[2]. These form above a critical micellar concentration (CMC) and can solubilize water-insoluble materials. Using synthetically prepared[3], chromatographically pure[4] bile salts, I have investigated the micellar solubilization of pure fatty acids and monoglycerides in bile salt solutions, under conditions simulating those present in human small intestinal content during fat absorption[5].

For lipids with melting point above 37°, an excess of radioactive material was incubated in bile salt solution with shaking, and the amount of solubilized lipid determined after filtration. For lipids, such as α-mono-olein, which form liquid crystalline suspensions with bile salts when present in excess, the maximum amount of lipid which could be solubilized was determined turbidometrically[6]. All incubations were done at 37°, for 96 hr., with bile salt solutions 0·15 *M* (sodium ions), at a *p*H of 6·3, and at physiological concentrations[7]. The resulting micellar solutions were stable and transparent, without turbidity or opalescence.

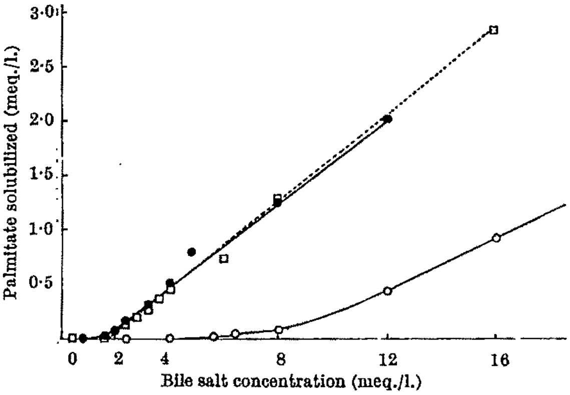

Fig. 1. Solubilization of palmitate by conjugated bile salts; *p*H 6·3, 37° C., 0·15 *M* sodium ions. □, taurodeoxycholate; ●, glycodeoxycholate; ○, taurocholate

Under these experimental conditions and in the absence of bile salts, no ordinary carboxylate soap is sufficiently soluble to form a micellar solution. The unconjugated bile salt deoxycholate is quite insoluble at this *p*H and sodium concentration, and cholate forms unstable solutions from which cholic acid precipitates. Thus stable micellar solutions under conditions normally present in small intestinal content seem possible only with conjugated bile salts (and possibly lysolecithin). Conjugation of deoxycholate and cholate with glycine or taurine lowers the *pK* and increases the solubility under these experimental conditions.

Palmitate solubilization begins at a well-defined bile salt concentration, the critical micellar concentration, and the amount solubilized rises linearly with increasing bile salt concentration (Fig. 1). The critical micellar concentration is about 7 meq./l. for the trihydroxy-bile salt, taurocholate, and about 1·6 meq./l. for the dihydroxy-salts, taurodeoxycholate and glycodeoxycholate. For a fixed bile salt concentration, the amount of fatty acid solubilized is directly proportional to the sodium ion concentration.

α-mono-olein solubilization begins at a lower critical micellar concentration than palmitate (Fig. 2) and the solubilization curves rise much more steeply. The critical micellar concentration for α-mono-olein solubilization is about 5 meq./l. for taurocholate and 0·8 meq./l. for glycodeoxycholate and taurodeoxycholate. α-Mono-olein and α-mono-linolein form myelin figures in bile salt solutions, which diffuse into the bile salt solution, rapidly forming a transparent micellar solution rich in lipid. α-Mono-palmitin, however, does not form myelin figures, and the critical micellar concentration and amount solubilized are almost identical to those of palmitate under these conditions.

A bile salt solution containing previously solubilized α-mono-olein dissolves far more palmitate than in the absence of mono-olein, and solubilization begins at a much lower bile salt concentration.

These results seem relevant to the observation that β-mono-glycerides and fatty acids are the normal end-products of pancreatic lipolysis during fat digestion[8], and that the β fatty acid of most dietary triglycerides is unsaturated[9]. Micellar solubilization by conjugated bile salts of unsaturated mono-glycerides and fatty acids may be the usual pathway of the products of pancreatic lypolysis, and it is possible that lipids may

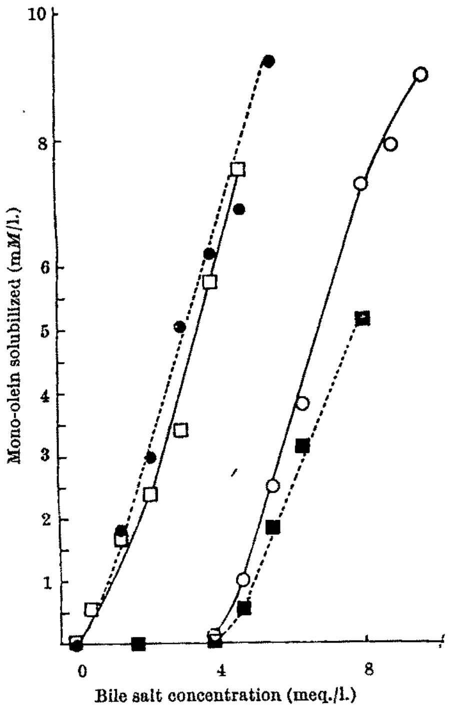

Fig. 2. α-Mono-olein solubilization by bile salts; pH 6·3, 37° C., 0·15 M sodium ions. ●, glycodeoxycholate; ○, cholate; □, taurodeoxycholate; ■, taurocholate

be absorbed in this form. It is of interest that the critical micellar concentrations of the bile salt solutions in these simple *in vitro* systems correspond closely to the lowest bile salt concentrations enhancing triglyceride synthesis in the work of Dawson and Isselbacher, who used preparations of intact small intestine[10].

I wish to thank Prof. Bengt Borgström for his interest and invaluable advice. This work was done with financial help from a National Foundation Postdoctoral Fellowship, and the Scientific Council of the Swedish Dairies' Association.

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Origin of the Potential Difference in the Intestinal Epithelium of the Turtle

WHILE we were looking for a suitable preparation to investigate the absorption of amino-acids in the digestive tract, we were impressed by the fact that the potential difference across the epithelium of the small intestine of various species is consistently lower (around 1 mV.) than the potential difference existing across the gastric mucosa or the colon (about 30 mV.). In order to throw some light on this observation we have first measured the flux of sodium across the isolated epithelium of the small intestine and the colon of the turtle *Testudo hermanni* J. F. Gmelin. We have chosen this species because it is quite easy to strip the muscle layers off the epithelium. The method used is described elsewhere[1]. Both sides of the isolated epithelium are bathed with saline having the following composition : sodium chloride 113 mM ; potassium chloride 1·9 mM ; calcium chloride 0·45 mM ; phosphate buffer pH 7·0, 1·5 mM. Table 1 gives the results obtained. It can be seen that there is an active transport of sodium from the mucosal to the serosal side of the preparation in both small intestine and colon. The fact that the values of the short-circuit current are smaller than the values of the net flux means that a cation must be transported actively from the serosal to the mucosal side, or that an anion must be transported actively in the opposite direction. It is also interesting to note that the values of the flux are higher in the small intestine than in the colon and that dinitrophenol inhibits, at least partially, both influx and outflux in the small intestine. This could indicate that part of the outflux of sodium across the small intestine is due to active transport. Let us examine now the effect of a modification in sodium and potassium concentrations on the potential difference across both intestine and colon (Table 2). In these experiments chloride is generally replaced by an equivalent amount of the non-penetrating sulphate anion. Na/10 means a saline containing 10 times less sodium than the physiological saline. Sodium is then replaced by an iso-osmotic amount of sucrose. K × 10 means a saline containing 10 times more potassium than the physiological saline. It can be seen that :

Table 1. INFLUX AND OUTFLUX OF SODIUM ACROSS THE ISOLATED EPITHELIUM OF THE SMALL INTESTINE AND THE COLON IN THE TURTLE *Testudo hermanni* J. F. GMELIN

The epithelium is bathed with a physiological saline on both sides. Results obtained when 2,4-dinitrophenol (DNP) is applied are also given. The fluxes, the net flux and the short-circuit current are expressed in m.coulomb cm.$^{-2}$.H^{-1}. *C*, control. Dinitrophenol concentration, 0·1 mM. Experimental periods, 1 hr.

	Influx	Outflux	Current	Net flux
	Small intestine			
C	492	401·4	41·6	90·6
C	540·4	437·1	40·1	103·3
Dinitrophenol	492	329	32·2	163
	419	314	28·5	105
	Colon			
C	174	42·4	104	131·6
C	121·6	34·7	61	86·9
Dinitrophenol	42·4	28·9	33	13·5
	50·2	50·2	13	0

(1) The replacement of chloride by sulphate is without any effect on the potential difference across the small intestine, contrary to the situation in the colon where the potential difference increases. A decrease in sodium concentration in the mucosal solution results in the inversion of the potential difference across the small intestine. In the colon we observe a decrease in potential difference (experiment *B*).

(2) If one then increases the potassium concentration, the potential difference decreases in the small intestine, while it stays constant in the colon (experiment *H*).

Table 2. EFFECT OF SODIUM AND POTASSIUM CONCENTRATIONS ON THE POTENTIAL DIFFERENCE ACROSS THE ISOLATED EPITHELIUM OF THE COLON AND THE SMALL INTESTINE IN THE TURTLE *Testudo hermanni* J. F. GMELIN
The sign refers to the serosal side

Experiment	Small intestine		Potential difference (mV.)	Colon		Potential difference (mV.)
	Mucosal	Serosal		Mucosal	Serosal	
B	RCl	RCl	1	RCl	RCl	20
	RSO_4	RSO_4	1	RSO_4	RSO_4	45
	RSO_4–Na/10	RSO_4	−26	RSO_4–Na/10	RSO_4	25
C	RSO_4	RSO_4	1	RSO_4	RSO_4	30
	RSO_4	RSO_4–Na/10	30	RSO_4	RSO_4–Na/10	60
H	RSO_4	RSO_4	1	RSO_4	RSO_4	57
	RSO_4–Na/10	RSO_4	−26	RSO_4–Na/10	RSO_4	48
	RSO_4–Na/10–K×10	RSO_4	−12	RSO_4–Na/10–K×10	RSO_4	48
I	RSO_4	RSO_4	1·5	RSO_4	RSO_4	13
	RSO_4	RSO_4–Na/10	20	RSO_4	RSO_4–Na/10	21
	RSO_4	RSO_4–Na/10–K×10	12	RSO_4	RSO_4–Na/10–K×10	19

(3) In sulphate saline, a decrease in sodium concentration in the serosal solution increases the potential difference across both small intestine and colon (experiment *C*). If the potassium concentration is then increased, the potential difference decreases in both small intestine and colon (experiment *I*).

The observations reported in Table 2 suggest the following conclusions. As the replacement of chloride by sulphate in the solutions bathing both sides of the colon produces an increase in potential difference, it is clear that this epithelium is relatively impermeable to sulphate. The epithelium of the small intestine is equally permeable to chloride and sulphate, since the replacement of chloride by sulphate has no effect on the potential difference. This result could also be explained if one postulates that the epithelium, although impermeable to sulphate, is permeable to potassium and sodium. This possibility is most likely to be correct as the other results demonstrate that the epithelium is permeable to sodium and potassium. Moreover, using sulphur-35 as tracer, we have been able to show that the permeability coefficient for sulphate in frog skin, turtle colon and intestine are of the same order of magnitude (unpublished results). Nevertheless, as we have found that the replacement of chloride by sulphate does not affect the magnitude of the potential difference appreciably when the sodium concentration is modified, this would suggest that the passive permeability of the small intestine to chloride is low.

The small spontaneous potential difference existing across the small intestine is related to the concentrations of sodium and potassium in the mucosal and serosal solutions: it is thus clear that both mucosal and serosal sides of the epithelium are permeable to these ions. On the contrary, in the colon the mucosal side is permeable to sodium, but impermeable to potassium, while the serosal side is permeable to both ions.

It is obvious from the results reported here that the distribution of the permeability characters in the small intestine is different from that in the colon. The organization and the distribution of the permeability characters is therefore an important aspect of cell differentiation. A purely speculative scheme has been proposed to explain the possible evolution of the permeability characters in the epithelial tissues[2]. According to this hypothesis, the primitive stage would be characterized by a mechanism of active transport located at both inner and outer borders of the cell. The cell would then evolve toward an asymmetry by losing the active transport mechanism at one of the borders. The results reported here give some experimental support to this conception.

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Constituents of *Lagerstroemia Flos-reginae* Retz.

FOR many years people of the Philippines have used certain parts of a tree (*Lagerstroemia Flos-reginae* Retz.) as an aid in the lowering of blood sugar[1,2]. The drug, commonly called 'banaba', is usually administered in the form of an aqueous 20 per cent decoction prepared from the leaves. While the drug has been employed for many years and pharmacological studies have been made using extracts of the drug[3,4], very little information has appeared in the literature concerning its constituents.

Dried leaves, obtained from an authentic source, were used in this investigation. To ensure that our material possessed physiological activity, 20 per cent decoctions were prepared. These, with and without tannins, were administered orally via stomach tube to rabbits. The dose was 10 ml. of 20 per cent decoction per kgm. of body-weight. Following administration of the drug, blood sugar determinations were made at 2-, 4-, and 6-hr. intervals using the Nelson–Somogyi procedure[5]. Results revealed that the leaves possessed hypoglycæmic properties as the average blood sugar was lowered 25 mgm. per cent after 4 hr., which was the peak time of activity. The results were essentially the same regardless of the presence or absence of tannins.

Determinations for the presence of therapeutically active compounds commonly found in plants were carried out. Alkaloid analysis was performed using Wall's method[6,7]. Flavonol determinations were carried out using Bryant's method[8], rutin being used as a control. Analysis for sterols was carried out with Liebermann–Burchard and Salkowski tests. Several methods of analysis for glycosides were employed, including (*a*) acid hydrolysis of an 80 per cent alcohol extract and subsequent analysis for reducing sugars;

(*b*) hydrolysis of an aqueous extract with β-glucosidase; (*c*) the use of ion exchange according to the method of Khym and Zill[9]. Results of the preceding tests were negative for the presence of alkaloids, glycosides, sterols and flavonols. The presence of tannins in banaba as indicated by Garcia was substantiated, and we have done some work on the nature of the tannins present.

It was suggested by Garcia[1] that perhaps some type of 'insulin-like' material might be responsible for the hypoglycæmic activity. Therefore we checked carefully for the presence of amino-acids in our extracts. Aqueous extracts were purified by Plaisted's method[10], which involves a desalting technique using ion exchange. Portions of the purified extracts were then spotted on Whatman No. 1 filter paper for chromatographic analysis. Four solvent systems proved to be the most efficient. These were: (1) *n*-butanol/glacial acetic acid/water (12 : 3 : 5); (2) *n*-butanol/pyridine/water (1 : 1 : 1); (3) *tert.* butanol/methylethylketone/diethylamine/water (2 : 1 : 2 : 2); (4) phenol/water (4 : 1). The presence of amino-acids was detected with ninhydrin. Amino-acids found were alanine, *iso*leucine, α-aminobutyric acid and methionine, the latter present in much higher amounts than the others. Purified extracts were also subjected to paper chromatographic analysis using the method of Moffat and Lytle[11]. Results of the latter procedure confirmed the presence of the above-named amino-acids.

From the results obtained it appears that constituents responsible for hypoglycæmic activity are not from those classes of secondary constituents commonly found in plants.

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A New Photo-reaction between some Furocoumarins and Flavin Mononucleotide

THE photosensitizing properties on the skin of some furocoumarins have been studied extensively by us since 1953[1] and later by other workers[2]. While the relationships of chemical structure to photodynamic effects are well known, the mechanism of action of these substances is at present not clear, in spite of the fact that xanthotoxin is used therapeutically for leucodermia. Recently, Pathak and Fellman[3] pointed out interesting relationships between activating and fluorescent wave-lengths and the photodynamic properties of many furocoumarins. Furthermore, we have been able to show[4] that furocoumarins do not operate by a photo-oxidative process on a protein substrate as do other known photodynamic substances (hæmatoporphyrin, hypericin, erythrosin, rose bengal, benzpyrene, etc.[5]).

We report here the results of research work which started from the observation, about a year ago, that some furocoumarins react with flavin mononucleotide on ultra-violet irradiation. A preliminary report of this work was given on May 22, 1960, at the meeting of the Istituto Veneto di Scienze, Lettere ed Arti, Venice.

Aqueous ethanolic solutions of equimolecular mixtures of flavin mononucleotide and of a furocoumarin (psoralen, xanthotoxin or bergapten) were irradiated by a Philips lamp (*HPW* 125) of principal wave-length 3655 Å. By paper chromatography with *n*-butanol/acetic acid/water (4 : 1 : 5) as eluant, new spots are detectable, which do not appear when flavin mononucleotide and furocoumarins are irradiated separately. There are spots of yellow colour and yellow fluorescence at low R_F values (0·15–0·22), and spots with violet or blue-violet fluorescence at high R_F values (0·85–0·90). The intensity of these new spots increases up to a maximum with the time of irradiation.

The ethereal extract containing the derivatives with violet and blue-violet fluorescence, which are formed on irradiation of a mixture of flavin mononucleotide and bergapten, was chromatographed on a column of silica gel, using water as the stationary phase and benzene or benzene–ethyl ether mixtures as the moving phase. So far three new compounds have been isolated in small quantity: substance *a*, $C_{14}H_{14}O_6$, melting point 153°, with violet fluorescence; substance *b*, $C_{12}H_{10}O_6$, melting point 178°, with violet fluorescence; substance *c*, $C_{12}H_8O_5$, melting point 208°, with grey-blue fluorescence. Compound *a* is an ethyl ester giving, by saponification, an acid identical to compound *b*. All these compounds appear to have coumarinic structure, showing that the furanic ring of the bergapten undergoes modifications. Further work is in progress for the elucidation of these substances and of those formed in the photoreaction between flavin mononucleotide and psoralen or xanthotoxin.

It seems that this photo-reaction does not take place appreciably when flavin adenine-dinucleotide is used. In fact, the irradiation of solutions of the latter containing equimolecular quantities of psoralen, xanthotoxin or bergapten did not give significant variations in the activity of flavin adenine-dinucleotide as coenzyme for D-amino-acid-oxidase, when determined by Warburg and Christian's method[6].

It has not been possible, hitherto, to say if the *in vitro* reaction between flavin mononucleotide and some furocoumarins could explain the mechanism of the photodynamic activity on the human and guinea pig skin by furocoumarins. However, the following results have to be considered carefully:

(1) We found a strict parallelism between chemical reactivity and biological action, that is, only the photodynamically active furocoumarins (for example, psoralen, xanthotoxin, bergapten, allobergapten, angelicin, 4′-methyl-psoralen, 5-*iso*propyloxy-psoralen, 3-methyl-xanthotoxin, 3,4′-dimethyl-xanthotoxin, 4,5′-dimethyl-xanthotoxin, 4,4′-dimethyl-psoralen) gave new compounds when irradiated with solutions of flavin mononucleotide, as observed by paper chromatography. No new spot is detectable when photodynamically inactive derivatives were

used (isopimpinellin, xanthotoxol, bergaptol, 4′,5′-dihydropsoralen, 4′-phenyl-4-methyl-psoralen, 3,4-dihydropsoralen, 4′,5′-dihydrobergapten, 4-methyl-5′-phenylxanthotoxin, thioxanthotoxin, erniarin, citropten, seselin, ethyl ester of psoralen-5-oxyacetic acid, etc.).

(2) There are also indications that flavin mononucleotide could give some protection *in vivo*: a batch of guinea pigs (average weight 450 gm.) was treated with bergapten (200γ/4 cm.^{2} of area); an Osram *HWA* 500-W. lamp was used as a source of radiation, with a total exposure of 60 min., and the distance between the lamp and the irradiated skin was kept constant at 30 cm. A very large dose of flavin mononucleotide (50 mgm. immediately after irradiation and 4 doses of 20 mgm. each at 12-hr. intervals) was injected subcutaneously at a distance from the place of application of bergapten and the irradiated zone. In a few cases erythema did not appear, in some animals a lighter erythema occurred, and in others erythema was delayed, compared with a control group of guinea pigs, irradiated in the same conditions and not injected with flavin mononucleotide. We emphasize that the amount of flavin mononucleotide necessary to give some protection was accompanied by a toxic effect.

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Gastric Esterases of Pigs

THERE has been considerable controversy as to the existence of a gastric lipase since Volhard[1] discovered the breakdown of fat in the stomach. Many authors have ascribed this phenomenon to regurgitated pancreatic or duodenal activities, but others believe that the stomach produces a factor which is active in the normal breakdown of fats.

During investigations of the esterolytic activities of tissues of the alimentary tract of pigs[2], using a *p*H-stat method, it was shown that the fundic mucosa had no ability to hydrolyse triolein, which is a typical lipase substrate. This confirms the findings of Cherry and Crandall[3] and of Schonheyder and Volqvartz[4]. It thus seems likely that the reported gastric hydrolyses of fats are not due to an enzyme produced in the fundic mucosa.

Preliminary investigations revealed that the fundic mucosa had low activity towards simple esters, such as 'Tween 20', ethyl acetate and triacetin. The *p*H optima of the hydrolyses of these three compounds were 8·8, 6·5 and 7·3, respectively. Such high *p*H optima suggest that these enzymes are of no practical use in the normal digestive processes of the animal, since the normal *p*H of the stomach content is much below this figure.

Detailed investigations of the behaviour of these systems in the presence of inhibitors were prohibited by the very low activities present. It was not found possible to use homogenates of the fundic mucosa containing more than 200 mgm. wet tissue/ml. because of the high viscosity of the extracts, and triacetin, which was hydrolysed at the greatest rate by this tissue, only produced 5 micro-moles of acid/min./gm. wet tissue.

However, we were able to show that the hydrolyses of all three substrates were inhibited by NN′ di-*iso*-propyl phosphodiamidic fluoride (mipafox), and that the hydrolyses of ethyl acetate and triacetin were also inhibited by *p*-chloromercuribenzoate (*p*-C.M.B.) (see Table 1).

Table 1. INHIBITION OF HYDROLYSIS OF TRIACETIN (PER CENT)

Inhibitor	Concentration	'Tween 20'	Inhibition ethyl acetate	Triacetin
Mipafox	$2 \times 10^{-4} M$	100	—	—
	$1{\cdot}5 \times 10^{-4} M$	—	74	49
p-C.M.B.	$2 \times 10^{-4} M$	0	—	—
	$1{\cdot}5 \times 10^{-4} M$	—	5	22

The inhibition of the hydrolysis of triacetin by mipafox and *p*-C.M.B. was additive, suggesting action on two active centres, and possibly indicating the participation of two enzymes.

Calcium ions activate the hydrolysis of 'Tween 20', but inhibit the hydrolysis of ethyl acetate and triacetin. This inhibition can be lessened by adding ethylenediamine tetraacetic acid, which removes calcium ions.

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Effect of Water-soluble Polymers on Nitrate Reductase Activity

SEVERAL years ago Whitaker[1] observed that the presence of small quantities of bovine serum albumin, β-lactoglobulin, and other proteins enhanced the activity of a cellulase preparation obtained from culture filtrates of *Myrothecium verrucaria* when insoluble substrates were used. Tracey[2] found a similar effect in the stimulation by cow serum of chitin hydrolysis by chitinase. More recently, activation by polymers has been observed in homogeneous aqueous enzyme systems. The action of liver diaphorase has been reported[3] to be enhanced by bovine serum albumin and polyvinylpyrrolidone while polysaccharides, particularly starch, have been shown[4] to increase the activity of horse-radish peroxidase. These findings suggest that the *in vitro* enhancement of enzymic activity by non-catalytic polymeric materials may be a phenomenon of broad biological significance.

We discovered a new example of this 'polymer effect' while studying the metabolism of inorganic nitrogen. It was observed that the enzymic activity of nitrate reductase preparations obtained from the mould *Neurospora crassa* (5297*A*) and purified by ammonium sulphate fractionation and column chromatography on hydroxyl apatite[5] is stimulated appreciably by several polymeric materials, notably by methyl cellulose and polyvinylpyrrolidone. We also tested the effect of several chelating agents, sugars, amino-acids, and other low-molecular-weight additives on the activity of the chromatographed enzyme. Reagent-grade materials were used wherever possible. Nitrate reductase activity was determined by colorimetric analysis of the rate of enzymic nitrite formation at 25° C. under zero-order reaction conditions. All additive materials were tested for interference with this analysis[7] and appropriate corrections made where necessary in calculating the nitrate reductase activities listed in Table 1.

Table 1. EFFECT OF ADDITIVES ON THE ACTIVITY OF PURIFIED NITRATE REDUCTASE

Additive	Concentration of enzyme protein (μgm./ml.)	Relative enzyme activity
None	122	100
Egg albumin	61	94
Dextran, clinical	61	94
Gelatin	61	140
Gum acacia	61	108
Methyl cellulose (100 cp.)	61	164
Polyvinylpyrrolidone (*K*-30)	61	149
Soluble starch	61	125
DL-Alanine	122	93
Creatine	122	89
L-Cysteine	122	80
Adenosinetriphosphate	122	47
L-Leucine	122	74
Ethylene dinitrilotetraacetic acid	122	105
Sucrose	122	90
D-Glucose	122	89
Glycerol	122	85
Sodium deoxycholate	122	116
Tris(hydroxymethyl)aminomethane	122	80
Reduced glutathione	122	110
Diglycylglycine	122	98

Assay mixtures: 0·50 ml. additive in 0·1 *M* phosphate, final *p*H 7·0 ; 0·08 ml. 1·25 × 10^{-4} *M* flavin adenine dinucleotide ; 0·02 ml. 1·0 *M* potassium nitrate ; nitrate reductase (specific activity 300–1,000) dissolved in 0·32 ml. neutral phosphate, 0·1 *M*. Assay reaction initiated by adding 0·08 ml. 2 × 10^{-3} *M* reduced triphosphopyridine nucleotide.

Concentrations of additives (polymers : 5 mgm./ml., other additives: 10^{-2} *M*) were chosen arbitrarily. Subsequent observations indicated that for methyl cellulose, at least, maximal enhancement of nitrate reductase activity is obtained by considerably lower polymer concentrations.

Among the polymers tested, egg albumin and dextran are conspicuous in their failure to enhance the reaction-rate of nitrate reductase. Thus it appears that a macromolecule must be endowed with specific physico-chemical characteristics in order to exercise a 'polymer effect' on a given enzyme system.

With the exception of sodium deoxycholate, none of the low-molecular-weight substances examined produced a significant enhancement of enzymic activity. At the concentrations used, most of these materials were in fact inhibitory. The 'polymer effect' is exerted by the polymer molecule and not by its monomers. This is seen by comparing the action of glucose, alanine, cysteine and leucine with that of starch and gelatine, respectively.

Since neither ethylenediamine tetraacetic acid nor *tris*(hydroxymethyl)aminomethane at concentrations of 10^{-2} *M* enhances nitrate reductase activity, chelation of inhibitory trace metals possibly present in the assay mixtures does not provide a basis for the 'polymer effect'. A kinetic explanation (Kamen, M. D., personal communication) has been suggested based on the premise that certain polymer materials may be capable of reversing a possible product-inhibition of the enzyme by physicochemical interaction with the nitrite ions formed. Kinetic studies[5] have shown, however, that at concentration-levels usually reached during assay, nitrite ion has no effect on the zero-order reaction-rate of the enzyme.

The well-known property of dissimilar polymeric materials to form binary aqueous systems[8] has been considered as a possible basis for the 'polymer effect'. The interfaces of such systems may exert an orienting effect on the enzyme molecule which could result in a more efficient catalytic system. Mixtures of methyl cellulose (5 mgm./ml.) and semi-purified nitrate reductase protein (8 mgm./ml.) slowly separate on standing into two aqueous phases. This phase separation is not observed when bovine serum albumin is substituted for the enzyme. At concentration-levels (50–200 μgm. protein/ml.) prevailing during enzyme assay such a phase separation was not found spectrophotometrically nor was it expected on the basis of Albertsson's results[9] on the composition of aqueous binary polymer systems.

Whitaker[2] noted that with less-pure preparations of cellulase the stimulation given by extraneous proteins is reduced. He attributed this observation to the presence of non-enzyme protein in the enzyme preparation. We encountered the 'polymer effect' on nitrate reductase activity only after the enzyme had undergone considerable purification.

These observations suggest to us that purification removes auxiliary substances normally associated *in vivo* with these enzyme systems, which provide a physicochemical environment permitting maximal catalytic activity. Such a physicochemical environment is provided apparently *in vitro* by certain polymer materials.

It may be argued that the presence of macromolecules such as methyl cellulose helps to prevent surface denaturation of the enzyme. Protective effects of this kind are well known, as exemplified by the protection of glutathione reductase activity by bovine serum albumin[10]. We observed, however, that the percentage increase in apparent nitrate reductase activity due to the presence of a fixed amount of methyl cellulose remains constant over a considerable range of enzyme-levels, down to protein concentrations of less than 4 μgm./ml. Furthermore, the presence of methyl cellulose has no effect on the gradual loss of enzyme activity observed when dilute purified nitrate reductase preparations are exposed to room temperature. Thus the 'polymer effect', as demonstrated by the example of methyl cellulose, promotes, but does not protect, enzymic activity.

In view of this evidence, we are inclined to believe that many macromolecules of varied structure possess physicochemical characteristics which render them capable of enhancing the *in vitro* catalytic activity of a number of enzyme systems. While the mechanism of this enhancement is still the subject of speculation[4], 'polymer effects' on enzymic efficiency should no longer be regarded as isolated phenomena. Their existence must be taken into consideration when dealing with purified enzyme systems.

Further work is in progress, and a detailed report will be published later.

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Activation of Hurain by Bivalent Iron

Hurain[1], the active principle of the sap of jabillo trees (*Hura crepitans*) which is responsible for its proteolytic action, apparently represents a proteinase with special chemical properties differing from those so far known for this class of enzymes. During purification and assay of the enzyme, it was found that the enzymatic activity of hurain is greatly affected by metal ions.

Experimental results, to be described in this preliminary communication, have been performed with crude enzyme obtained as a dry, almost white powder from the tree sap by cutting the bark and centrifuging, dialysing and freeze-drying the pooled samples. Proteolytic activity was determined by present techniques[2-5]. The enzyme preparation shows certain activity on hæmoglobin and egg albumin (Fig. 1), gelatine and Witte's peptone[1], without further additions; maximal activity is reached, however, only when bivalent iron ions are added (Table 1).

The crude hurain preparation was found to act also on a synthetic substrate. Hurain slowly hydrolyses benzoyl-L-arginine-methylester, and the velocity of hydrolysis is increased by the addition of Fe^{2+} (Fig. 2). It appears that hurain does not act on the corresponding amide. The effect of bivalent iron appears to be specific, since Ca^{2+}, Mg^{2+}, Mn^{2+} and Fe^{3+} had no influence on hurain activity; Zn^{2+}, Cd^{2+} and Ni^{2+} inhibit the enzyme action. The mechanism of activation by bivalent iron is not produced by reduction of S–S to SH-groups of the protein. Hurain is not affected by cysteine or other reducing agents[1], and *p*-chloromercuribenzoate.

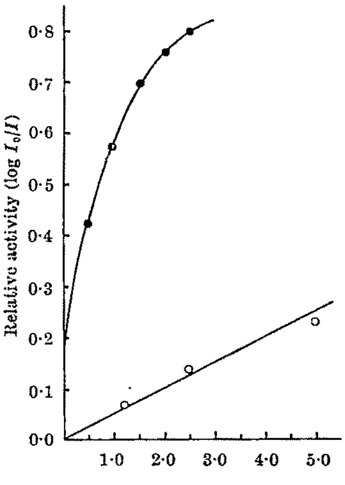

Fig. 1. Relative activity of crude hurain on hæmoglobin —●— and egg albumin —○— without further additions. Method, ref. 2

Table 1. Reversible Activation and Inhibition of Hurain Action by Fe^{2+}, Zn^{2+} and EDTA. Method, Ref. 3

Enzyme (mgm.)	Fe^{2+} (μmoles)	Zn^{2+} (μmoles)	EDTA (μmoles)	Relative activity, $\log \frac{I_0}{I}$, 280 mμ
1·0	—	—	10·0	0·267
	1·0	—	—	0·678
	1·0	—	10·0	0·237
0·25	—	—	—	0·180
	—	10·0	—	0·044
	—	10·0	10·0	0·108
0·50	10·0	—	—	0·720
	10·0	10·0	—	0·573
	10·0	20·0	—	0·351

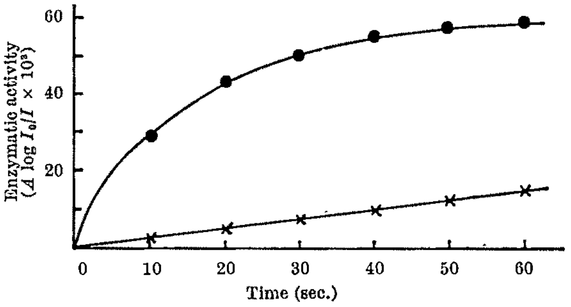

Fig. 2. Activity of crude hurain on benzoyl-L-arginine-methylester without —×— and with —●— addition of Fe^{2+}. Method, ref. 4

Apparently, active hurain represents a reversibly dissociating iron complex, in which Fe^{2+} can be displaced by inhibiting ions as Zn^{2+} or by complexing agents such as ethylenediamine tetraacetic acid (EDTA) (Table 1). The bivalent iron is firmly bound by the protein moiety, at least more firmly than the inhibiting ions. An excess of Zn^{2+} is required to displace the iron from its site of binding. Other evidence for the strong binding of Fe^{2+} is shown by the fact that after 48 hr. of dialysis against EDTA the preparation has 2·2 mgm. per cent iron, that is, about 10 per cent of its original iron content (23·5 mgm. per cent), calculated for dry weight. The amount of iron present in the preparation after dialysis of the crude extract is sufficient to activate partly the hurain.

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Terminology of Protein Structure

I WISH to suggest a revision in the practice of discussing proteins in terms of primary, secondary, and tertiary structures. This terminology, introduced by Linderstrøm-Lang in his Lane Memorial Lectures[1], served well in the few years since it was invented, and has thoroughly permeated the literature. Developments since that time have lessened its usefulness. Arguments to demonstrate this, and suggestions for a more useful general terminology, are the substance of this communication.

One difficulty connected with the terminology is that there is major and widespread disagreement on definitions. For example, in refs 2–4 disulphide bridges are called primary structures, while in refs. 5–7 they are known as tertiary. Disagreement can also be anticipated with other covalent chain-branches and cross-links. Further, there is confusion about secondary structures: some writers accept only helices as secondary structures[6,7,9], others go a step further and include pleated-sheet models[2,4] and some few[5] maintain that a peptide chain segment or loop may have a meaningful non-random structure in the absence of peptide hydrogen bonding; for example, for steric reasons. Furthermore, it is not generally agreed whether tertiary structure is to mean "the way in which primary and secondary structures are packed together in the molecule"[2] and the resultant molecular shape[2,5,7,8]; or whether tertiary structure should direct attention to the multiple forces maintaining the particular folding of α-helices or other chain conformations in a protein[3,9].

The designations, primary, secondary, and tertiary, tend to connote relative importance which can be misleading. I do not disagree with the primacy of covalent bonds in proteins, but there is no compelling evidence that peptide hydrogen bonds contribute more strongly than side-chain interactions to conformational stability; in fact, there are numerous suggestions to the contrary[2,5,8,10].

Finally, there is a sequential connotation in the primary, secondary, tertiary terminology. While present hypotheses of protein biosynthesis support the notion that formation of peptide bonds in an ordered sequence precedes the change to the three-dimensional structure of the protein, there is little knowledge of processes involved in this transformation. The same issue arises in the reversible denaturation of proteins. It is uncertain whether side-chain interactions lead or follow formation of peptide hydrogen-bonds, or whether, as is my view, there may be an advantage in considering the transformation to be a concerted, co-operative process.

To summarize, considerable ambiguity exists at present in the application of the primary, secondary, tertiary terminology. Further objections to this terminology are that it tends to connote relative importance and a sequence of formation of structural elements. Although it is conceivable that general agreement on definitions might be achieved, the other objections remain. I therefore recommend that use of this terminology be discontinued entirely.

In fact, a general move in this direction has already been noted: a recent symposium on protein structure and function[11] ran its full three-day course with scarcely a reference to secondary and tertiary structure. Apparently several other investigators concerned with protein structure have recognized shortcomings in this terminology. If my present arguments do not succeed in persuading writers to abandon the primary, secondary, and tertiary terminology, I hope that it will at least lead some to a greater awareness of its limitations.

I suggest that protein structure can be discussed generally in terms of amino-acid sequences of peptide chains, called chain sequence, and the ways in which peptide chains and side-chains are arranged in space, called chain conformations. (It has already been noted[12] that configuration has often been used synonymously with conformation. This is undesirable, since configuration traditionally has applied to optically asymmetric centres, and should be reserved for this use We should speak of the configuration of an amino-acid residue, and of the conformation of a peptide chain.) It appears desirable to subdivide the latter term into side-chain conformations and peptide chain or backbone conformations, without implying that these two subdivisions are mutually exclusive, and certainly without implying rank or sequence of development. Taken together, chain sequences and chain conformations are sufficient to define a structure, which is the three-dimensional location of all the atoms and covalent bonds of a molecule. The factors determining a structure, such as covalent bond lengths and angles, rotational barriers, steric repulsions, hydrogen bonds, electrostatic forces, 'hydrophobic bonds', etc., should not be confused with the structure itself. No general designation seems necessary for the arrangements of protein sub-units into aggregate molecules, as in insulin, hæmoglobin, collagen, the caseins, etc. However, if such a term must be employed, 'sub-unit array' seems preferable to 'quaternary structure'[13].

Finally, I wish to emphasize that I have intended criticism only for general ways of discussing protein structure; no criticism is meant for terms describing specific structural elements, such as α-helices, pleated-sheets, polyglycine II type structures and constrained non-hydrogen-bonded backbone loops.

Since there is nothing intrinsically proteinaceous in the designations 'chain sequence' and 'chain conformation' (main and side-chain), these terms may be applicable in structural discussions of chain polymers in general.

I am grateful for discussions of this matter with Dr. E. R. Blout, Prof. L. C. Craig, Prof. J. T. Edsall and Prof. D. Waugh.

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PHYSIOLOGY

Korotkov's Sounds : a Function of the Skeleton to Transmit 'Pulsactor' Signals

WORK upon Korotkov's sounds led me[1] to the conclusion that the arterial system could be regarded as an energy transmission line, into which the heart launches energy at two levels, namely, waves with a frequency of 3–15 c.p.s. and waves with a frequency of 256–512 c.p.s. The theory required that the sound waves, resulting from forces of cavitation, be transmitted throughout the arteries, with a velocity much higher than that of the waves of low frequency, to explain the development of arterial stationary waves.

Sound waves are indeed transmitted by arteries, Kerr and Harp[2] giving velocities of the order of that of the pulse-wave, 4–5 m./sec. Comparable results are found in fluid-filled elastic tubes where, however, should the wave arise from a unit impulse function, longitudinal waves may occur, transmitted with a velocity of 30 m./sec., as reported by Citters[3]. Landowne[4], moreover, has shown that externally produced impact waves are propagated along living arteries with an average velocity of 17·1 m./sec. These velocities, however, are not sufficiently high for the theory, and, certainly on transmission in fluid-filled elastic tubes, sound waves, especially of higher frequencies, undergo rapid attenuation.

Now bone is an excellent conductor of sound, the velocity of transmission is about 3,900 m./sec. and bone conduction of the heart sounds occurs *in vivo* : Turrettini[5], for example, observed that the murmur of mitral regurgitation was audible over the sacrum. McKusick[6] describes conduction of heart sounds, via the ribs rather than directly through the chest, as "circumferential transmission", when auscultation is performed over the back.

In aortic stenosis, loud systolic murmurs may be heard with the aid of a stethoscope, or microphone, applied to the olecranon : it was discovered by Levine and Likoff[7] that this sound is unaffected by occlusion of the brachial artery with a pressure cuff, so proving that the sound is conducted by bone. These workers also reported a case where a diastolic murmur, due to syphilitic aortic incompetence, was audible on auscultation over the skull.

The above observations support the following amendment to the original theory :

The sternum acts as a sounding board that resonates to the heart sounds, the body of the sternum being more favourably placed to resonate to the first sound ; the manubrium to the second. The clavicle conducts the sounds to the scapula by which they are transmitted to the bones of the arm. The ribs conduct the sounds to the vertebral column by which they are conducted upwards to the skull, which acts as a cavity resonator, and downwards to the sacrum and so to each hip bone by which they are transmitted to the bones of the leg.

Conduction at joints is by the ligaments, which are so attached that they act as matching devices. In the case of the costal cartilages, the sound may be conducted by the perichondrium. The clicking nature of the noises arising in joints suggests that sound waves of frequencies up to, say, 10,000 c.p.s. are involved : frequencies even higher should be considered.

What function could such a phenomenon subserve ? Probably the following : the skeleton acts as an aerial, broadcasting the heart sounds throughout the body, the sound being conducted, finally, from bone to soft tissue by fibrous, ligamentous and muscular attachments. Each sound acts as a signal, imparting a slight jolt to the branches of the vascular system, analogous to the effect of pulsactor circuits employed in radar and utilized to overcome initial and terminal high impedance of other circuits in which momentary flow of current is required.

A similar concept was introduced by me[8] in connexion with blood flow in the coronary arteries, applying the term 'to overcome thyxotropism'. Since the transmitted sound waves are likely to have the further effect of synchronizing pulsation of the peripheral arteries, they may be described as 'pulsactor signals'. In turn, the action of the heart, initiating these, may be termed that of a 'synchronizing signal generator'.

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An Effect of Glucose on Acetate Metabolism in Sheep

RUMINANTS derive their energy requirements essentially from lower fatty acids (acetic, propionic and butyric) and thus differ from non-ruminating animals. The concentration of glucose in the blood of sheep, which is extraordinarily low when compared with that of non-ruminants, is probably an expression of the sparsity of the exogenous glucose available in the metabolism of these animals[1,2].

It is unlikely, however, that glucose plays no important part in the metabolic pathways in the sheep. Certainly glucose has an important role[3] in the young lamb. In adult sheep glucose, though in limited supply, might well be implicated in aiding the metabolism and utilization of acetate by the tissues. It is the purpose of this communication to examine this hypothesis.

In this work estimations of the rate of disappearance of acetate after intravenous injection were used as a measure of the sheep's ability to 'utilize' acetate.

Six well-trained Merino wethers, aged 2 years, were fed in pens for 6–8 weeks on 800 gm. of chaffed wheaten hay plus 7 mgm. cobalt chloride once a week to ensure adequate vitamin B_{12} status. Four of the six animals were used in the experiments without change of diet, while two were given, 2 days before the observations were made, a ration of 500 gm. of chaffed wheaten hay plus 300 gm. of chaffed lucerne hay.

Estimations on the rate of disappearance of acetate from the blood were made by determinations of the concentration of volatile fatty acids in the blood at 30, 60 and 90 min. after an injection of 5 mM/kgm. of acetic acid neutralized to *p*H 7·0 with caustic soda and diluted to 75 ml. with distilled water. The injection was made through a polythene tube inserted and fixed into the jugular vein and usually was completed within 3–5 min.

A few days following the determination of the rate of disappearance of the acetate by this means an injection of glucose (1·0 gm./kgm.) was given, followed half an hour later by an injection of acetate, and the rate of disappearance of acetate was then again determined. The injection of glucose resulted in a profound hyperglycæmia, the blood sugar-level being elevated throughout the experimental period by at least 100 mgm./100 ml. blood over the normal control-levels. After 2–3 days had elapsed control observations (without glucose injections) were again made. The results are presented in Table 1.

Table 1. CONCENTRATION OF VOLATILE FATTY ACIDS IN BLOOD (m*M*/L.) AFTER THE INJECTION OF ACETATE (5 m*M*/KGM.)

Minutes after acetate injection	Wheaten hay chaff (mean of 4 animals ± *S.E.*)			Wheaten hay chaff+ lucerne chaff. Data from 2 animals		
	Acetate only	Glucose then acetate	Acetate only	Acetate only	Glucose then acetate	Acetate only
0	0·38 ±0·13	0·50 ±0·11	0·51 ±0·08	0·45 0·35	0·53 0·51	0·32 0·70
30	7·24 ±0·11	6·02 ±0·26	6·99 ±0·24	5·65 6·30	4·28 4·91	6·25 5·96
60	3·46 ±0·10	1·54 ±0·16	3·27 ±0·34	2·03 1·06	0·73 0·62	1·96 1·88
90	1·77 ±0·19	1·29 ±0·11	1·49 ±0·17	0·95 1·07	0·58 0·60	0·87 0·80

It is apparent from Table 1 that prior administration of glucose led to a more rapid rate of removal of acetate from the blood than occurred in control experiments in which no exogenous glucose was given. The results emphasize the importance of diet in this respect, for the incorporation of lucerne chaff in the diet, although only for so short a period as 2–3 days before the experimental observations, significantly changed the rate of 'utilization' of acetate. The importance of the nature of the diet has previously been stressed[4,5]. It might thus be considered that an interrelationship exists, not between acetate and glucose itself but between acetate and the glucose precursor, propionate. When the rations are comprised largely of crushed maize an enhanced production of propionate results, and, then after being introduced intravenously, both glucose and acetate disappear from the blood more rapidly than from the blood of control animals on standard diets[4]. Recent work[5], however, does not favour the direct implication of propionate, and from the work reported here, it is suggested that glucose, as in non-ruminants[6], may be important in aiding in the 'utilization' of acetate which is so essential to the metabolism of the sheep.

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Evidence of Secretion of an Aldosterone-stimulating Substance by the Kidney

HÆMORRHAGE causes a two-fold increase in aldosterone secretion in hypophysectomized dogs[1]. We have found recently that nephrectomized hypophysectomized dogs fail to show this rise, and that saline extracts of dog kidney contain an aldosterone-stimulating substance.

Ten male mongrel dogs (average weight, 12·1 kgm.) were subjected to left nephrectomy. Five or more days later, the dogs were anæsthetized with pentobarbital and the right kidney removed (5 dogs), or an adjustable ligature placed around the renal artery and vein (5 dogs). The right lumbo-adrenal vein of each dog was then cannulated and the animals hypophysectomized via the transbuccal route. One hour later, a control adrenal venous blood sample was collected from each of the five dogs with a ligature around the vessels of the remaining kidney. The ligature was tightened and two more adrenal vein samples were collected at 20-min. intervals. In the dogs in which the remaining kidney had been removed, two specimens of adrenal venous blood were collected starting 1 hr. after hypophysectomy. All dogs were then bled by removing 15 ml./kgm. body-weight of femoral arterial blood over a 6-min. period. Adrenal venous blood samples were collected 10, 40 and 70 min. after the start of hæmorrhage. In five instances, the experiment was continued by re-infusing the shed blood over a 6-min. period and collecting adrenal venous blood 10 and 30 min. thereafter. Four of these dogs were given 1 I.U. of adrenocorticotrophic hormone intravenously and an adrenal vein sample collected starting 5 min. after the injection. Ten similarly studied hypophysectomized dogs with intact kidneys (average weight, 12·9 kgm.) served as controls.

Extracts of tissues were made by dicing normal dog kidney or spleen, mixing the tissue with 50 or 100 ml. of isotonic saline, straining through cheesecloth, and centrifuging at approximately 3,000 r.p.m. for 15 min. The supernatant was injected intravenously over a 1-min. period into hypophysectomized–nephrectomized dogs and adrenal venous blood was collected starting 5 min. after each injection. The dose of extract used in each case was equivalent to 1 kidney or ½ spleen. Control injections of an equal volume of isotonic saline were performed in 3 dogs. All hypophysectomies were found to be complete by gross examination at autopsy. Blood pressure was monitored continuously, using a Statham strain gauge manometer and Grass model 4 polygraph. The adrenal venous blood samples were centrifuged promptly, and the plasma frozen for subsequent analysis of aldosterone content by the double isotope derivative method[2] and 17-hydroxycorticoid content by the method of Silber and Porter[3].

The results are summarized in Tables 1 and 2. There was a slight, but statistically significant, fall in aldosterone secretion following ligation of the renal vessels, and the control values in the nephrectomized–hypophysectomized dogs were significantly lower than those of the hypophysectomized dogs. Following hæmorrhage, aldosterone secretion-rates rose at least 5 mμgm./min. in all 10 hypophysectomized dogs, while no rise occurred in 8 out of 10 nephrectomized–hypophysectomized dogs. The mean values for aldosterone secretion after hæmorrhage were significantly higher in the dogs with intact kidneys. Adrenal responsiveness to adrenocorticotrophic hormone was

Table 1. Changes in Aldosterone Secretion-rate following Haemorrhage

Values are means ± *S.E.*; figures in parentheses are number of dogs for each value

	Aldosterone secretion-rate (mμgm./min.)	
	Hypophysectomized dogs	Nephrectomized–Hypophysectomized dogs
Before ligating renal vessels	—	7·4 ± 0·8 (5)
Control 1	10·6 ± 3·6 (10)	2·8 ± 0·8 (10)
Control 2	9·2 ± 3·4 (10)	4·0 ± 0·7 (10)
10 min. after hæmorrhage	18·8 ± 4·2 (10)	6·7 ± 3·3 (10)
40 min. after hæmorrhage	—	4·1 ± 1·7 (10)
70 min. after hæmorrhage	15·3 ± 3·5 (10)	4·2 ± 1·5 (10)
10 min. after re-infusion	13·7 ± 4·2 (10)	5·2 ± 0·8 (5)
30 min. after re-infusion	18·3 ± 3·0 (10)	4·4 ± 0·7 (5)
After ACTH (1 I.U. intravenously)	59·8 ± 9·7 (8)	68·8 ± 10 (4)

Table 2. Changes (mean ± *S.E.*) in Adrenal Secretion and Blood Pressure after Injection of Kidney and Spleen Extracts and Saline

The aldosterone and 17-hydroxycorticoid values are the difference between the rate following injection and the control value immediately before injection. Blood pressure values represent the maximum value after injection minus the value before injection

	Kidney extracts (9 exp.)	Saline (3 exp.)	Spleen extracts (2 exp.)
Mean change in aldosterone secretion-rate (mμgm./min.)	+26·7 ± 7·2	+1·7 ± 2·4	−5·8
Mean change in 17-hydroxycorticoid secretion-rate (μgm./min.)	+2·0 ± 0·6	+0·1 ± 0·1	−0·4
Mean change in systolic blood pressure (mm. mercury)	+72·8 ± 10·8	+16·7 ± 8·8	−2·5
Mean change in diastolic blood pressure (mm. mercury)	+66·7 ± 7·4	+11·7 ± 7·3	+2·5

retained in the dogs without kidneys, as indicated by the slightly greater response to adrenocorticotrophic hormone in this group. Except after adrenocorticotrophic hormone injection, 17-hydroxycorticoid secretion was less than 3 μgm./min. in all dogs and was unaffected by hæmorrhage and retransfusion. After adrenocorticotrophic hormone, 17-hydroxycorticoid values were 8·1 ± 1·1 μgm./min. (mean ± *S.E.*) in the nephrectomized dogs and 5·9 ± 0·9 μgm./min. in the controls.

Injection of the saline extract of 1 normal dog kidney caused a large rise in aldosterone secretion in 8 out of 9 instances (Table 2), with a mean increase of 26·7 mμgm./min. 17-hydroxycorticoid values also increased slightly. Systolic and diastolic blood pressure rose markedly. Control injections of equal volumes of saline had no effect on the secretion of either hormone, although they did cause a 10–15 mm. mercury rise in blood pressure. Spleen extracts (2 dogs) had no effect on adrenal secretion or blood pressure.

Similar experiments have been carried out independently by Davis *et al.*[4], and similar results obtained. The fact that aldosterone secretion rises after hæmorrhage in hypophysectomized dogs indicates that some non-pituitary factor is involved. A renal source for this factor is suggested by the absence of the response in nephrectomized animals and the presence of an aldosterone-stimulating substance in kidney extracts.

The nature of this aldosterone-stimulating substance is uncertain. Richards and Sayers[5] found that extracts of normal rat kidneys caused adrenal ascorbic acid depletion in hypophysectomized rats and assumed that the material giving the response was adrenocorticotrophic hormone. A slight rise in 17-hydroxycorticoid secretion followed the injection of extracts of normal kidneys in the present experiments, and it is possible that adrenocorticotrophic hormone extracted from the kidneys was responsible for the observed effects on aldosterone secretion. However, doses of commercial adrenocorticotrophic hormone which produce 17-hydroxycorticoid responses of this magnitude have no effect on aldosterone secretion in the hypophysectomized dog[1].

In 1951 Deane and Masson[6] reported that renin injections led to hypertrophy of the zona glomerulosa of the adrenal. Recently, Genest *et al.*[7] and Laragh *et al.*[8] have reported that injection of synthetic angiotensin into normal humans caused an increase in aldosterone secretion. Preliminary experiments by us indicate that synthetic angiotensin increases adrenal secretion of aldosterone and 17-hydroxycorticoids when injected into hypophysectomized–nephrectomized dogs in very small doses. Since hæmorrhage causes an increased secretion of renin[9], the present results are consistent with the hypothesis that the renin–angiotensin mechanism is responsible for the increase in aldosterone secretion following hæmorrhage in hypophysectomized dogs.

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Isolation and Partial Characterization of a Growth-control Factor

A growth-promoting factor in liver extracts has been reported in work on a chemically defined medium for the axenic cultivation of the nematode, *Caenorhabditis briggsae*[1]. An active fraction has now been isolated from horse liver by salt precipitation and column chromatography. The purified material emerged from the column as a discrete protein peak. By electrophoretic analysis (through the courtesy of Dr. Arthur Karler, Karler Laboratories, Berkeley, California) on cellulose acetate strips and ultracentrifugal analysis (through the courtesy of Dr. F.

Lindgren, Donner Laboratory, University of California, Berkeley), it appeared to be essentially a single component. Twelve amino-acids have been demonstrated in a hydrolysate of the active fraction.

Freshly excised liver was cut into small pieces and autolysed for 24 hr. at 4° C. All subsequent procedures were carried out in the cold. A 1 : 1 homogenate was made in water, and after centrifugation the residue was discarded. Ammonium sulphate was added to the supernatant fluid to obtain the material which precipitated between 40 and 60 per cent saturation at *p*H 7·0. This precipitate was dissolved in distilled water and reprecipitated with ammonium sulphate between 45 and 60 per cent saturation. After centrifugation, the precipitate was redissolved and dialysed to remove ammonium sulphate. This material was then applied to a column of hydroxylapatite[2] and eluted with successive increases in concentration of phosphate buffers. The eluates contained six discrete protein peaks. These six fractions were sterilized by filtration ('Millipore', size *G.S.*) and assayed with *C. briggsae*. Only one of them was found to be biologically active.

The growth-promoting property of this protein fraction for *C. briggsae* was tested in a chemically defined basal medium[3] which is protein-free. In the basal medium, young larvæ survived but increased in size only to a limited extent. But, in medium to which the fraction had been added, maturation and reproduction occurred. The purified protein material showed the freeze-activation phenomenon described previously[4]. When freeze-activated, 39 μgm. of protein (optical density 280/260) per ml. of culture medium was found to be adequate for maturation and reproduction. A higher level (158 μgm. protein per ml.) was selected for prolonged maintenance, and seven generations were afterwards subcultured. Successive inocula were first-generation larvæ of respective preceding cultures.

None of the other fractions from the column was active in the nematode assay, either before or after freeze-activation treatment.

In other experiments, this fraction was tested as a replacement for the serum requirement of a tissue culture cell line (McCoy, originally obtained through the courtesy of Dr. C. M. Pomerat). Stock cultures of McCoy cells were maintained in a medium composed of mixture 199 (Microbiological Associates), lactalbumen hydrolysate, and calf serum[5]. Cultures were trypsinized, washed, and resuspended in a deficient medium composed of mixture 199 plus antibiotics. Aliquots were dispensed into Leighton tubes and were incubated for 4–6 hr., at which time the rounded cells had become attached to the glass. The medium was decanted and replaced according to the following schedule: series 1—complete serum medium plus adenylic acid; series 2—serum-deficient medium plus adenylic acid; and series 3—serum-deficient medium plus fraction (79 μgm. protein per ml. freeze-activated in adenylic acid) to be assayed. After 18 hr. incubation, the cell sheet of series 1 was confluent and individual cells were like epithelium. Cells in series 2 were enlarged slightly and for the most part were still round, with little evidence of differentiation or multiplication. A small number of these cells showed evidence of fibroblast-like growth. In series 3, most cells had proliferated into fibroblast-forms and some into epithelial-forms. Although the cell sheet did not attain the confluent aspect observed in the serum controls, it far surpassed the growth and differentiation obtained in the deficient medium.

The findings of these experiments suggest that this purified protein fraction may play a general regulatory part in growth and differentiation.

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Œstrogen Protection against Acute Digitalis Toxicity in Dogs

Grinnell and Smith[1] have demonstrated that castrated female dogs prophylactically treated with œstradiol-17β are much more resistant to a toxic intravenous dose of digoxin than are untreated castrated controls.

In an extended search for a medicinal agent which would duplicate the protective effect of the ovarian hormone without œstrogenicity, a large list of steroids fabricated on the naturally occurring œstratriene nucleus have been studied. Among such steroids our best compound so far is 1,3,5(10),16-œstratetraen-3-ol methyl ether. This highly active steroid was prepared both by the methylation of 1,3,5(10),16-œstratetraen-3-ol and by the pyrolysis[2] of the *p*-toluenesulphonate of 3-methoxy-1,3,5(10)-œstratrien-16β-ol[3]. It has a melting point of 68° and an optical rotation of $[\alpha]_D^{24} = +115° \pm 2$ ($c = 0{\cdot}615$ in chloroform). Apparently a crystalline modification melting at 71–72° exists[3].

After many endocrine studies we do not yet know the physiological mechanism(s) involved in steroidal protection against digitalis toxicity. Adult, female, mongrel dogs weighing 7–14 kgm. were ovariectomized by a technique which included hysterectomy and then permitted a minimal period of 4 weeks for post-operative recovery. The test substance was dissolved in sesame oil and administered intramuscularly at a dose-level of 0·5 mgm./kgm. daily for 7 days, with vaginal smears being taken prior to and at intervals during the administration. Food was withheld from all animals for a period of 18–24 hr. before the acute experiment, but water was allowed *ad libitum*. On the eighth day of the experiment anæsthesia with pentobarbital sodium (30 mgm./kgm.) was induced and a cathode-ray electrocardiograph was installed for continuous observation of the electrocardiogram, lead aV_F being used regularly. Permanent tracings of this lead were made at frequent intervals with a Sanborn twin-viso electrocardiograph. After a 30-min. control period during which the normal electrocardiographic pattern was recorded, digoxin (0·15 mgm./kgm.) was administered intravenously. The electrocardiogram was observed continuously and the time of appearance of the arrhythmia was recorded (the term 'arrhythmia' refers to a complete atrioventricular block with idioventricular tachycardia).

Table 1

Steroid used in prophylactic treatment	Time of onset of arrhythmia (min.)	State of œstrus
1,3,5(10),16-œstratetraen-3-ol methyl ether	> 60	Anœstrus
	> 60	,,
	38	,,
	> 60	,,
	30	,,
None	21	Anœstrus
	19	,,
	24 (av. 20)	,,
	18	,,
	18	,,

If this arrhythmia did not occur within a time-limit of 60 min., protection against the digoxin intoxication was arbitrarily considered to be 100 per cent, as survival has always been indefinite with this degree of protection.

In Table 1 are recorded the results with 5 castrated female dogs prophylactically given 1,3,5,(10),16-œstratetraen-3-ol methyl ether against the results with 5 similar animals untreated. Not only was this steroid non-œstrogenic in our experiments, but it also demonstrated a markedly superior protective action. Curiously, the free phenol—1,3,5(10),16-œstratetraen-3-ol[4]—is not as potent as its 3'-methyl ether; and the free phenol, furthermore, produces œstrus in our routine experiments.

We have never observed augmentation of digoxin toxicity by an œstrogen or œstrogen-like steroid. Such steroids, if protectively inactive, demonstrate onset of arrhythmia at time-intervals identical to those of controls within experimental error.

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Hyaluronic Acid in the Uterine Wall of the Guinea Pig during the Sexual Cycle

The amount and function of acid mucopolysaccharides in the mammalian uterus are not yet known. A metachromatic staining material has been described in the endometrial stroma of the human uterus[1–3] during the proliferative phase of the sexual cycle, and recently the presence of a periodic acid–Schiff positive material in the uterus of swine during the sexual cycle has been established[4]. Nothing definite has been found in the literature concerning the possible importance of hyaluronic acid in the physiology and pathology of the uterus. Investigations concerning the acid mucopolysaccharides of the uterus have been undertaken, since alteration in the quantity or quality of the ground substance may prove to be important in the pathogenesis of uterine infections and possibly in other disorders.

Forty mixed coloured virgin female guinea pigs, 3–5 months of age, were used in this investigation. They were kept at a relatively constant temperature, between 68° and 72° F., and fed with a standard ration; fresh water was given *ad libitum*. The sexual cycle was followed by daily examination of vaginal smears, stained by Papanicolaou's method. Using this method, it was possible to kill animals in a given phase of the sexual cycle, that is, pre-œstrus, œstrus, post-œstrus, and di-œstrus. A small block of tissue was removed from each uterus approximately 1 cm./ distal to the bifurcation, and this was fixed in 10 per cent formol-alcohol. The remainder of the uterus was put in acetone immediately, and stored at -20° C. for future chemical examination. Paraffin sections were prepared from the block of tissue and stained as follows: Hale colloidal iron method for mucopolysaccharides as modified by Rinehart and Abul-Haj[5]; period acid–Schiff method of McManus; and 0·5 per cent aqueous toluidine blue.

Hyaluronic acid was extracted from the remainder of the uterus by the method described by Hayashi *et al.*[6], with the slight modification that an additional filtration was done at the end of the dialysis procedure. The extract, proved to be free of protein and sulphur, was dissolved in 1–2 ml. of 0·1 *M* veronal-acetate buffer at *p*H 6. One drop of the solution of each extract was spread over an area 0·5 cm. in diameter on a glass slide. The dried smears were then stained with toluidine blue[6], at *p*H 6·9; 4·1; 2·5 and 1·0. Some of the dried smears were treated with hyaluronidase for 1, 2 and 3 hr. at 37° C. before staining, using a concentration of 100 VR units per ml. The enzyme was dissolved in 0·1 *M* acetic buffer *p*H 4·7, containing 0·15 *M* sodium chloride. Controls were incubated at the same time with the buffer solution only. Dried smears of chemically pure hyaluronic acid, chondroitin sulphate and heparin were also used as controls.

The smears of the uterine extracts showed a significant metachromasia at *p*H 6·9 and 4·1 with diminished metachromasia at *p*H 2·5 and none at *p*H 1·0. Treatment of the smears with the hyaluronidase for 60–90 min. caused a complete loss of metachromasia. The smears of known chondroitin sulphate and heparin showed distinct metachromasia in all ranges of *p*H tested, including *p*H 1·0, while the known smear of hyaluronic acid gave results similar to the experimental extracts of the uterus. Hayashi *et al.*[6] reported that metachromatic staining with toluidine blue at *p*H 1·0 is highly specific in differentiating non-sulphate acid mucopolysaccharides from the sulphated form. On this basis, our results suggest that the acid mucopolysaccharide from the uterus is hyaluronic acid. An attempt was made to extract chondroitin sulphate from the uteri of other guinea pigs, using the method described by Hayashi[6], with negative results.

Histochemical examination of the uterine sections showed the presence of an acid mucopolysaccharide which was distinctly blue (with the Hale method of staining) and metachromatic with toluidine blue, but only slightly positive with the periodic acid–Schiff method. This material was seen in both the endometrial epithelium and stroma. The histochemical observations varied during the different phases of the sexual cycle. In particular, the amounts of demonstrable acid mucopolysaccharide were notably increased during œstrus and, at this time, the periodic acid–Schiff staining reaction became more strongly positive.

The results reported suggest that hyaluronic acid is the major mucopolysaccharide in the uterine wall of the guinea pig, and that either the amount or quality of this ground substance material varies with the phase of the sexual cycle, being maximal, as judged by histochemical methods, during œstrus. Quantitative and qualitative investigations are now being completed on the uterine extract and will be published.

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Electrical Excitability of Isolated Frog Skin

In the course of investigating the electrical properties of frog skin with short current pulses, it was discovered that this preparation is capable of exhibiting electrical excitability. The present communication is concerned with a description of this phenomenon.

The abdominal skin of the frog *Rana pipiens* was removed and immediately mounted between two cylindrical 'Lucite' compartments, 6·5 cm. long, 1·15 cm.^{2} cross-sectional area. There were holes at the far end of each compartment for the silver/silver chloride stimulating electrodes, and at each end adjacent to the skin was a small hole for the bridges (polyethylene tubes containing saturated potassium chloride in agar solution) going to the recording calomel electrodes. Each compartment was filled with Gray–Ringer solution (71·75 m*M* NaCl; 5·00 m*M* KCl; 2·50 m*M* $CaCl_2$; 1·60 m*M* $MgCl_2$; 20·00 m*M* $NaHCO_3$; 1·03 m*M* Na_2HPO_4; 0·12 m*M* NaH_2PO_4; 0·26 m*M* glucose. Solution is saturated with a 97 per cent O_2, 3 per cent CO_2 gas mixture), and within 1 hr. a steady state value of the resting potential across the skin obtained (25–60 mV., inside surface positive). Current through the skin and voltage across it were recorded on a Tektronix 502 dual beam oscilloscope (input impedance 10 MΩ); the square waves of current were obtained from an AEL laboratory stimulator through a 100-kΩ series resistance.

Fig. 1 shows a typical set of responses of the skin to square waves of current of 35 m.sec. duration. At low values of inward (depolarizing) and outward (hyperpolarizing) current (by an inward current is meant a current produced with the cathode in the solution bathing the inner surface of the skin and the anode in the solution bathing the outer surface; the reverse is the case for an outward current) (Fig. 1*a*) the voltage responses are almost symmetrical and phenomenologically identical to the behaviour of a network consisting of a resistance and capacitance in parallel (RC network). The observed resistance was from 0·5–1·0 kΩ/cm.^{2}; capacitance from 2–5 μF./cm.^{2}. As the magnitude of the current pulse is increased, the potential changes begin to show non-linearity and rectification (Fig. 1*b*). At still higher current density the system is very non-linear, and for an outward current pulse, overshoot occurs (Fig. 1*c*). As the magnitude of the current pulse is continually increased, a value is eventually reached at which the skin is near threshold. At this point, if the magnitude of the current pulse is increased slightly, the skin responds with an action potential for inward current pulses, while continuing to give an overshoot for outward currents (Fig. 1*d*). (The overshoot response for outward current pulses as seen in Fig. 1*c* seems to be a 'poor' action potential, for in some skins an action potential with a sharp threshold and a form essentially the mirror image of the type shown in Fig. 1*d* occurs.)

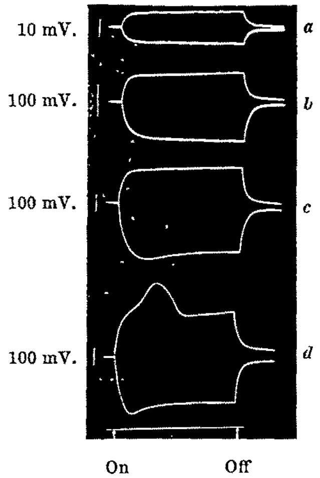

Fig. 1. The change in potential across isolated frog skin produced by 35-m.sec. square waves of current. Upward response is result of inward current; downward response is result of outward current. Currents are 20 μamp., 210 μamp., 500 μamp. and 550 μamp. for *a*, *b*, *c* and *d* respectively. Resting potential is 30 mV., inside positive. Gray–Ringer solution on both sides

Following an action potential, the skin becomes refractory for several seconds, not exhibiting an active response to the same stimulus that previously elicited it. If during this period the skin is stimulated at the rate of 10/sec. or faster, it will continue to remain refractory and show only an RC-type of response; stimulation at the rate of 1/sec. or less, on the other hand, has no appreciable effect on the duration of the refractory period.

In Fig. 2 is seen a striking example of the recovery of excitability following a full response. In this experiment a small short (2·5 m.sec.) pulse was superimposed on a 40 m.sec., just sub-threshold pulse. The interval between successive records was three seconds. Curves *a*, *b*, and *c* show a characteristic sub-threshold 'local response'; curve *d* is the full action potential. A pulse 3 sec. after this would give curve *a* again, etc.

I wish to point out several important features of these action potentials: (1) the response is large, in some skins being as great as 300 mV. (by the magnitude of the response I mean the difference between the peak height and the maximum sub-threshold level); (2) the long refractory period, which is of several seconds duration; (3) the sharp threshold, which is a general characteristic of more familiar excitable systems. Finally, I wish to contrast this response of frog skin to the changes of potential observed on stimulation of the nerves to the skin[1].

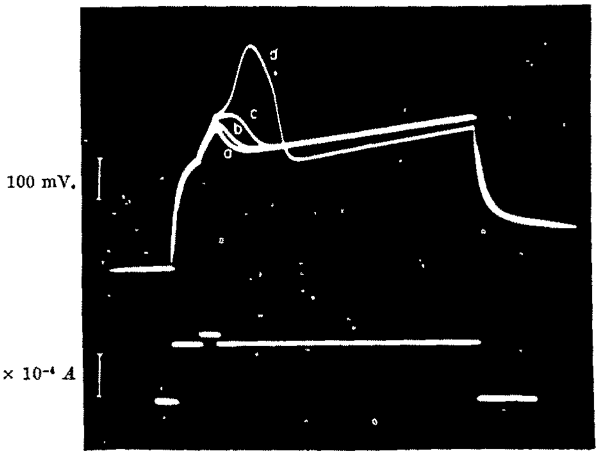

Fig. 2. The change in potential across isolated frog skin produced by a 40-m.sec. just sub-threshold inward square wave of current with a superimposed 2·5-m.sec. small inward square wave. Successive responses *a*, *b*, *c* and *d* are 3 sec. apart. Resting potential is 60 mV., inside positive. Gray–Ringer solution on both sides

I have repeated some of this earlier work and have confirmed that the response in this case is very slow, being of several seconds duration and quite different from the observations described. Other workers have attributed these slow changes to the skin glands.

With regard to the site in the skin of the electrical activity, it seems fairly clear that the magnitude of the effect and particularly the dependence of the direction of the response on the polarity of the stimulus precludes consideration of all unoriented structures, that is, smooth muscle and nerve twigs. This leaves us only with the epithelial cells and the glands. It is interesting that we have found that the toad bladder, the electrical and permeability properties of which are known to be in many ways quite similar to those of frog skin, exhibits essentially the same electrical excitability as described for the skin. In the bladder, the epithelial layer consists of a single sheet of cells with no glands. (About 5 per cent of these cells secrete mucus (Peachey, L. D., personal communication).) It seems, therefore, that the action potentials evoked in frog skin and toad bladder come from the germinal layer of epithelial cells. It is to these cells that is generally attributed the resting potential and 'transport properties' of these organs. A more detailed report on the phenomenon discussed here will appear elsewhere.

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HÆMATOLOGY

Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency in New Guinea and New Britain

COMPARATIVE investigations carried out on erythrocyte glucose-6-phosphate dehydrogenase deficiency in Negro and Caucasian subjects have revealed quantitative differences between these two groups[1]. It is suggested that different genetic mechanisms account for the deficiency of glucose-6-phosphate dehydrogenase in Negroes and Caucasians. This led to a search for other populations which might exhibit the trait, and which might therefore help to elucidate some of the questions concerning gene action in man.

Employing the rapid screening test described by Motulsky[2] a survey has been carried out on 155 subjects in the Gazelle Peninsula, New Britain, and 534 subjects in the Eastern Highlands, New Guinea. Any samples showing significant hæmolysis in the supernatant sera were discarded. Red cells from blood clots, both in field samples and in laboratory controls stored under conditions identical to those of transportation, have proved to be as satisfactory as those from similarly treated heparinized blood samples, provided the hæmoglobin value in all cases is adjusted to a constant standard value. Further controls were used in the form of repeated blood samples from a single normal European in New Guinea. The results are shown in Table 1.

Table 1

Area	Sex	No. examined	No. deficient
New Britain (Gazelle Peninsula)	Male	94	16
	Female	61	0
	Total	155	16
New Guinea (Eastern Highlands)	Male	383	3
	Female	151	0
	Total	534	3

Several females in the New Britain series had enzyme-levels in an intermediate range, possibly indicative of a heterozygous situation. For the first time the trait has been found to exist in New Guinea and New Britain, but with greatly differing incidence, 0·56 and 10·3 per cent, respectively, in these two areas. The Gazelle Peninsula in New Britain is highly malarious, while the several groups tested from the Eastern Highlands of New Guinea inhabit low- or non-malarial areas. These results thus add evidence supporting Motulsky's contention[2,3] that there may be a selective advantage in the development of this trait, in that enzyme-deficient persons may be more resistant to falciparum malaria. Populations previously reported to have a high incidence of glucose-6-phosphate dehydrogenase deficiency also exhibit the sickle-cell trait, but sickle-cell hæmoglobin has not been found in New Guinea[4] or New Britain[5]. In 13 of the 19 deficient cases reported here, there was no apparent segregation according to haptoglobin type, although the series is as yet too small for definite conclusions.

No cases of primaquine sensitivity have yet been reported in New Guinea (Schofield, F. D., personal communication). However, primaquine is given only to highland peoples when they return from the coast to their own malaria-free or comparatively non-malarious home areas. It is not used in treating native people in coastal regions, as the chances of re-infection with malaria are so high.

The finding of another distinct population with deficiency of erythrocyte glucose-6-phosphate dehydrogenase, as revealed by this preliminary survey, provides material for further evaluation of the gene-enzyme interaction concerned. Further investigations are in progress to determine more precisely the relation between malarial distribution and incidence of the trait, and to examine possible protection of the enzyme deficiency against malarial infection. A study is also being made of factors such as heat lability, pH optimum, and of the effect of storage on the enzyme in deficient subjects, for comparison with those of the enzyme in affected Negroes and Caucasians.

Acknowledgment is made of the co-operation of Dr. D. C. Gajdusek, of the National Institutes of Health, Bethesda, Maryland, and Mr. R. T. Simmons, of the Commonwealth Serum Laboratories, Melbourne, in obtaining blood samples, and of Dr. C. Curtain, of the Baker Institute, for the haptoglobin investigations.

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Isolation of Haptoglobin by Chromatography on Diethylaminoethyl-Cellulose (DEAE)

HAPTOGLOBIN was isolated first by Jayle *et al.*[1] using a technique which combined precipitation by ammonium sulphate with preparative starch-gel electrophoresis. More recently we found that haptoglobin could be obtained as a by-product during the routine fractionation of human plasma[2,3]. This technique uses rivanol as a precipitating agent for the subfractionation of fraction IV, obtained at pH 5·8, and 35 per cent alcohol concentration, the final purification being achieved by chromatography on DEAE.

This technique gives pure haptoglobin suitable for metabolic research studies with and without addition of hæmoglobin[4]. However, geneticists interested in the variation of haptoglobin groups often have only small samples of plasma at their disposal and they only need minute amounts of the isolated protein as test substances for electrophoretic work.

In our routine technique, the final purification of haptoglobin is obtained by adsorption of haptoglobin at pH 5 on DEAE using 0·03 M acetate buffer, whereas siderophilin, the main contaminant, is not adsorbed. Haptoglobin can be adsorbed under the same conditions from whole plasma, but is then accompanied by other proteins: cæruloplasmin, a second α_2-glycoprotein, cholinesterase and some α_1-globulins. Smithies[5] already showed that the selective adsorption of haptoglobin needs a rather low pH. However, we noticed that haptoglobin at about 80 per cent purity can be obtained with a slightly modified elution technique.

Plasma is diluted to give a freezing point of −0·15°. The pH is then adjusted to 5 with acetic acid 0·03 M; DEAE-cellulose is equilibrated against acetate buffer 0·03 M, pH 5, a minimum of 25 gm. of dry cellulose being necessary for 1 l. of plasma. The adsorbent is poured into a column (the diameter being proportional to the volume handled) and the diluted plasma is passed by gravity flow. The column is then washed with acetate buffer, 0·03 M, until no more proteins appear in the effluent. Haptoglobin can then be eluted with 0·1 M acetate buffer, pH 5. The solution should contain 2–4 gm./l. of proteins; the total yield of haptoglobin based on the hæmoglobin-binding capacity is about 50–60 per cent.

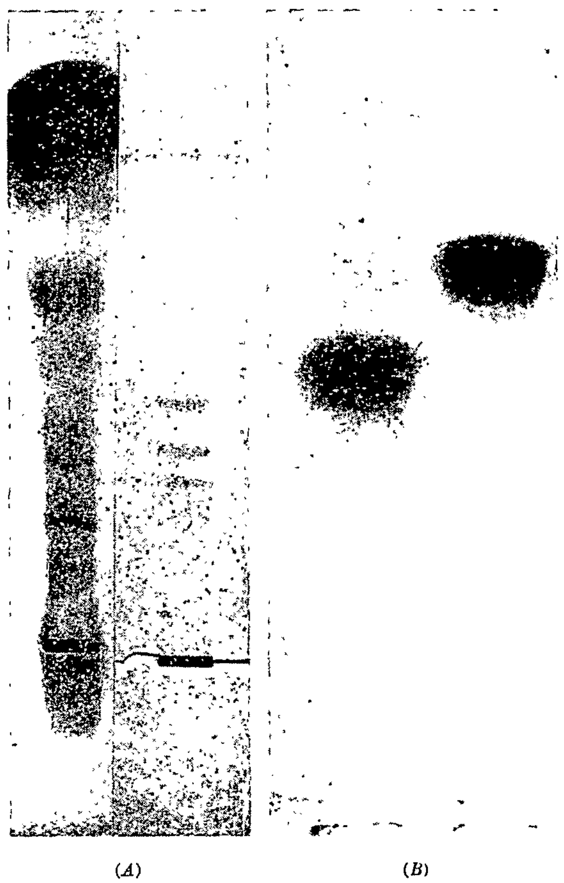

Fig. 1. *A*, Haptoglobin 2 : 1 separated by batch-adsorption from a small plasma sample. Eluate slightly concentrated. *B*, Haptoglobin 1 : 1 with and without added hæmoglobin. Sample obtained by column chromatography of 250 ml. of plasma

Cæruloplasmin together with some α_1-globulin can be eluted at 0·2 M acetate buffer concentration and at the same pH. A second, not yet identified, α_2-glycoprotein is eluted at 0·5 M acetate buffer.

Satisfying results have been obtained with small samples (2–4 ml.) as well as with volumes of 1 l. of plasma and more. Batch-adsorption can be used instead of the column adsorption. Since no dialysis is necessary, it is possible to achieve the isolation of haptoglobin a few hours after the collection of the blood sample.

The purity and the yield of the separated product seems to give data corresponding to those published by Smithies[5] for haptoglobin obtained by chromatography on 'Dowex 2 $X-10$', pH 4·2.

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PATHOLOGY

Increased Sensitivity to Bacterial Endotoxin of F_1 Hybrid Mice undergoing Graft-versus-Host Reaction

THE development of graft-versus-host reaction in adult F_1 hybrid mice, following the injection of parental strain spleen cells, is accompanied by a considerable increase in the phagocytic activity of the reticulo-endothelial system[1]. Two weeks after injection of the spleen cells, the recipients were found to have phagocytic indices (calculated from the rate of clearance of colloidal carbon from the circulation) up to 10 times greater than normal. During investigations on the immunological reactivity of such animals to heterologous antigens[2], a few observations suggested that the mice were abnormally susceptible to the toxicity of relatively small doses of killed *Salmonella typhi*. Recent work has shown that stimulation of the reticulo-endothelial system in mice either by *Mycobacterium tuberculosis* (*BCG*) infection[3-5] or by repeated injections of the yeast cell-wall preparation 'Zymosan'[6] coincides with a profound lowering in resistance to the lethal effect of endotoxins from Gram-negative bacteria. In view of this association, F_1 hybrid mice undergoing graft-versus-host reaction were tested similarly for resistance to purified bacterial endotoxin, and an analogous effect has been demonstrated.

Male F_1 (*C57BL* × *CBA*) mice, weighing 20–26 gm., were injected intravenously with 75–85 million spleen cells prepared from adult male *C57BL* donors as described previously[1]. Various dosages of *Shigella dysenteriæ* type 1 endotoxin, freshly suspended in saline, were injected intravenously 14 days after the spleen cells. Previous work had shown that at this time interval stimulation of the reticulo-endothelial system was usually maximal in the strain combination used[1], although the mice appear well and indistinguishable from normal animals. The results given in Table 1 demonstrate that F_1 (*C57BL* × *CBA*) mice previously injected with *C57BL* spleen cells are 25 times more susceptible to the lethal effect of endotoxin than untreated control mice. The calculated LD_{50}'s are 11 μgm./20 gm. for treated mice and 272 μgm./20 gm. for control mice, with confidence limits at 5 per cent probability-level of 8–15 and 197–375 μgm./20 gm. respectively. All deaths occurred within 48 hr., while those mice previously injected with spleen cells which survived dosages of 2–20 μgm. endotoxin remained severely shocked for 24–48 hr. and then recovered. The subsequent onset and incidence of fatal graft-versus-host disease during the following six weeks did not seem to be influenced by the endotoxin injection at day 14.

Table 1. THE EFFECT OF GRAFT-VERSUS-HOST REACTION INDUCED BY INJECTION OF PARENTAL STRAIN SPLEEN CELLS ON THE SUSCEPTIBILITY OF F_1 (*C57BL* × *CBA*) MICE TO BACTERIAL ENDOTOXIN

Dose of endotoxin (μgm. per 20 gm.)	F_1 (*C57BL* × *CBA*) mice	
	Controls	Animals given *C57BL* spleen cells 14 days previously
500	8/8*	—
200	0/8	—
100	1/8	—
50	0/8	8/8
20	0/8	7/10
10	—	8/14
5	—	1/14
2	—	0/4

$* \frac{\text{No. of mice dying}}{\text{No. of mice injected}}$

The present experiment provides a further instance, in addition to *BCG* infection and the effect of 'Zymosan' injection, where stimulation of the phagocytic activity of the reticulo-endothelial system has been accompanied by greatly increased sensitivity to endotoxin. The role of the reticulo-endothelial system in this state of hypersusceptibility should, however, remain *sub judice*, for there is much evidence (see refs. quoted in 5–7) supporting also a direct relationship between acquired resistance to endotoxin and an increase in the level of reticulo-endothelial activity. This phenomenon is the opposite of the one at present under consideration, although in both cases the phagocytic activity of the reticulo-endothelial system is raised.

The immunological basis for the graft-versus-host reaction has been clearly established by work on 'runt disease' induced in the young animal and of 'secondary disease' in radiation chimæras. But the immediate causes of the wasting and death which frequently attend it are at present unknown. Decreased resistance to bacterial endotoxin absorbed from the gut may be a contributory factor towards its pathogenesis.

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Immunological Differences of Deoxyribonucleoproteins from White Blood Cells of Normal and Leukæmic Human Beings

EXPERIMENTS were performed to determine: (*a*) whether deoxyribonucleoproteins from normal and leukæmic leucocytes react immunologically with their related control sera, or whether they cross-react with them; (*b*) whether they are capable of inducing formation of antibodies in rabbits; (*c*) whether normal deoxyribonucleoproteins cross-react with sera of rabbits immunized with leukæmic deoxyribonucleoproteins and vice versa.

Isolation of leucocytes was achieved by the method of Beck and Valentine[1]. Alternatively, after sedimentation of red blood cells, citrated human plasma was centrifuged at 1,000 r.p.m. for 4 min. to recover the leucocytes. Extraction of deoxyribonucleoproteins was performed with 0·01 *M* glycine overnight after washing leucocytes with 0·01 *M* sodium citrate and 0·01 *M* glycine solution. Purification of the extract was achieved by centrifugation at 24,000*g* for 25 min. Isolation and extractions were made at 4° C. Details of this new method and biophysical and biochemical work on deoxyribonucleoproteins will be presented elsewhere. Antibodies were prepared by injecting intravenously a total amount of 25–30 mgm. of deoxyribonucleoproteins into rabbits three times at weekly intervals. Rabbits were bled a week after the last injection. Titre of antibodies and optimal concentrations of deoxyribonucleoproteins were determined in test-tubes at room temperature. Human and rabbit sera prepared from venous blood were dialysed against 0·1 *M* glycine before use. In precipitin reactions a modified agar-gel diffusion technique of Outchterlony[2,3] was used. Agar (1 per cent), after washing with distilled water, was melted in 0·1 *M* glycine. For control purposes, it was melted in 0·01 *M* phosphate buffer and 0·1 *M* glycine at *p*H 6·8. Incubation of agar plates made at room temperature and at 4° C. yielded the same results. Each precipitin test was repeated 10–15 times.

The experiments used nine rabbits and the blood of seven normal and fourteen leukæmic persons.

Control tests of non-immunized rabbit sera with normal or leukæmic sera and deoxyribonucleoproteins of normal and leukæmic humans were always immunologically negative. No immune reaction was detected between control sera of normal and leukæmic individuals, between control sera of normal human beings and normal and leukæmic deoxyribonucleoproteins, between control sera of leukæmic humans and normal and leukæmic deoxyribonucleoproteins and between normal and leukæmic deoxyribonucleoproteins themselves. These results exclude the existence of a detectable naturally occurring immune limiting system which controls the growth of leucocytes in the blood both of normal and of leukæmic human beings.

Both normal and leukæmic deoxyribonucleoproteins were found to induce formation of high-titre antibodies in rabbits. They always gave a single precipitin line when fresh preparations were employed. Sera of rabbits immunized with normal deoxyribonucleoproteins did not cross-react with leukæmic deoxyribonucleoproteins, and vice versa (Table 1). These results show that the antibodies produced in rabbits are specific for the normal and leukæmic deoxyribonucleoproteins.

In a single instance a preparation of deoxyribonucleoproteins from a person with chronic lymphatic leukæmia gave a positive reaction with rabbit sera immunized with normal deoxyribonucleoproteins and a negative one with rabbit sera immunized with leukæmic deoxyribonucleoproteins. However, two other preparations from the same person, six months earlier, conformed to the general findings.

Table 1. CROSS-REACTIONS OF DEOXYRIBONUCLEOPROTEINS FROM NUCLEI OF LEUCOCYTES OF NORMAL (NDNP) AND LEUKÆMIC (LDNP) HUMAN BEINGS WITH THEIR RELATED ANTIBODIES (NAb, LAb) (NORMAL, LEUKÆMIC)

Antigen	Antibodies						White count
	NAb_4	NAb_5	NAb_6	LAb_7	LAb_8	LAb_9	
N_4DNP*	+			−			6,800†
N_5DNP		+			−		6,500
N_6DNP		+	+		−	−	6,500
N_7DNP			+			−	8,200
L_4DNP	−			+			150,000
L_5DNP	−			+			75,000
L_6DNP		−			+		342,000
L_7DNP		−			+		202,000
L_8DNP		−			+		83,000
L_9DNP		−			+		151,000
$L_{10}DNP$		−	−		+	+	46,000
$L_{10}DNP$			−			+	
$L_{10}DNP$			+			−	
$L_{11}DNP$			−			+	200,000
$L_{11}DNP$			−			+	
$L_{12}DNP$			−			‡	5,000

L_4, L_5, L_7, L_9, L_{10}, L_{11}, Chronic lymphatic leukæmia; L_6, chronic myelotic leukæmia; L_8, L_{12}, acute myeloblastic leucoma.

* Numbers indicate different individuals.

† Individuals considered normal on the basis of serological tests by Central Blood Bank of Pittsburgh.

‡ Insufficient quantity of $L_{12}DNP$.

The property of deoxyribonucleoproteins from nuclei of leucocytes of normal and leukæmic human beings of inducing formation of specific antibodies in rabbits is not restricted to these deoxyribonucleoproteins alone, but it is a property of deoxyribonucleoproteins from widely different sources (sea urchin, calf thymus, tumours). These results will be presented elsewhere.

The specificity of antibodies to normal and leukæmic deoxyribonucleoproteins indicates the existence of a structural difference between the deoxyribonucleoproteins. This specificity, at the same time, argues for the nativity of these preparations; and it implies a genetic differentiation of the nuclear deoxyribonucleoproteins in the growth of white blood cells.

It is hoped that these findings may stimulate the genetic approach to the study of leukæmia and cancer.

I wish to thank the Department of Biophysics for the use of its facilities and Dr. Frederick I. Tsuji for his help.

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Correlation between Ulcerative Colitis and *Rh* Blood Groups

Winstone, Henderson and Brooke[1] did not find ulcerative colitis to be correlated with the *ABO* blood groups, secretor status, or the rhesus factor. Their *Rh* classification was, however, restricted to rhesus-positive and rhesus-negative, presumably based on the use of an anti-*D* serum only. When we recently examined 185 patients in whom ulcerative colitis had led to colectomy, we also did not find any particular relation to *D* status, but did find, considerably to our surprise, a definite correlation with the homozygous *CC* rhesus type. Using as controls Wiener's series of 1,071 New York Whites[2], we obtained the results shown in Table 1.

Table 1

	Rhesus type			Total
	CC	*Cc*	*cc*	
Patients with colectomy	51	97	37	185
Normals	209	502	360	1,071

From these figures a value of χ-square with two degrees of freedom of 15·214 was calculated; this value is very definitely significant (P about 5×10^{-4}). So far as we could judge, the group of patients we tested did not differ in ethnic composition in any notable way from our controls. There is, however, evidence of heterogeneity in our test material ($\chi^2_{(10)}$ = 19·115, $0·05 > P > 0·02$). No correlation was found with *ABO*, *MN*, or secretor status.

We plan to extend these investigations and publish a more complete account elsewhere.

We are very grateful to the members of QT, Inc. (The Ileostomy Association of Boston), and the Ileostomy Associations of New York and Chicago for their generous and invaluable co-operation.

This work was supported by research grants (*H*-1076 (C8)), *RG*-4704 (*C*4), and *RG*-6939 (*C*1) from the National Institutes of Health and a research grant (*NSF G*-15082) from the National Science Foundation. One of us (M. H.) is a trainee in human genetics under training grant *HTS*-5388 from the National Heart Institute.

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BIOLOGY

Molecular Biology or Ultrastructural Biology?

IN a recent communication[1], Prof. C. H. Waddington protests against the increasing use of the name 'molecular biology' for a large and ever-growing field to which, he argues, it does not apply, and suggests confining it to more restricted topics for which in any event a better name would be 'ultrastructural biology'. Since, as I believe, I was responsible for first propagating the name 'molecular biology', and its widespread adoption seems to date particularly from my 1950 Harvey Lecture "Adventures in Molecular Biology"[2], it may be worth while stating again what I personally had in mind. In that lecture I said that molecular biology, as I envisaged it, "implies not so much a technique as an approach, an approach from the viewpoint of the so-called basic sciences with the leading idea of searching below the large-scale manifestations of classical biology for the corresponding molecular plan. It is concerned particularly with the *forms* of biological molecules, and with the evolution, exploitation and ramification of those forms in the ascent to higher and higher levels of organisation. Molecular biology is predominantly three-dimensional and structural—which does not mean, however, that it is merely a refinement of morphology. It must at the same time inquire into genesis and function".

I was inspired, obviously, by our early X-ray and accessory adventures at Leeds in demonstrating, for example, the folding and unfolding of protein chains and the nature of the long-range elasticity of mammalian hairs; the classification of the fibrous proteins into two principal configurational families—the *k-m-e-f* group and the collagen group; the similarity between the keratins of birds and reptiles which distinguishes them from the mammals; the apparent common configurational plan underlying the elastic and contractile properties of hair, epidermis, muscle and (more recently) bacterial flagella; the interpretation of protein denaturation and the relation between the fibrous proteins and the globular proteins (as we called them); the detailed exploration of the cellulose fabric of the cell wall of the alga *Valonia ventricosa*; first studies of the structure of the nucleic acids and nucleo-proteins, etc. Of later years, other examples of how we have continued along the same general line of thought are Rudall's work on the molecular structure of types of cuticles and of the various kinds of silks and other cocoon proteins[3], and Pautard's investigations into the problem of biological calcification, from ciliates to cetaceans, with his latest X-ray demonstration that the organic matrix of tooth enamel is not a collagen (as it is with dentine and bone) but is more like a member of the *k-m-e-f* group[4].

I trust that Prof. Waddington will not mind my recalling in this way our original concept of molecular biology, and will agree that we have kept fairly faithfully to it. It is impossible, though, to embark seriously on work of this kind without being interested in, and becoming more and more involved in, numerous associated studies, with the result that, as has happened, there soon comes a time when there seems to be no end to the business. Molecular biology has now inevitably spread to all aspects of biology looked at from fundamental molecular viewpoints—and thus includes 'molecular genetics', for example, if I may dare suggest it; and it is difficult to maintain that such an eventual extrapolation is unwarranted, for it is simply saying that that is the coming biology.

After all this it might well be asked, Why then is our laboratory at Leeds called the Department of Biomolecular Structure? The answer is that, when in 1945 I was appointed professor, the university committee considering me, and from which I was naturally excluded, preferred the name 'biomolecular structure' to 'molecular biology', which was what I myself wanted. Presumably, a majority of the members of that committee held opinions similar to those expressed by Prof. Waddington, and I offer this argument in his support; though I will confess that a probably more candid, and conceivably better justified, assessment that leaked out to me was that "he may know something about molecules but he knows precious little about biology". The answer to that one of course, in the tiresomely good old words, is that it all depends on what you mean by biology.

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OF all the recently developed types of analytical biology, the field of which Prof. Astbury was one of the main initiators has probably the best claim to the title 'molecular biology'. But even granting this, it is as well to remember that it was just because Astbury went on from the study of well-defined molecules to investigate entities such as hairs, which can at best by courtesy be called molecular, that he was able to open the doors through which so many people are now passing. If we want a title for the whole field of which Astbury's work forms a part, but which includes also the study of genes, microsomes, endoplasmic reticulum, antibodies and so on, by techniques only a few of which are definitely chemical, then I submit that we require a name which is more inclusive than 'molecular biology'.

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Feather Lipids

The plumage of fourteen widely differing species of birds excepting that of a ratite, the emu (*Dromaeus novae-hollandiae*), contained approximately 2 per cent of lipids which were readily extractable with ether or chloroform. The primitive plumage of the flightless emu yielded only 0·4 per cent lipids.

These feather lipids were found to contain cholestanol, cholestanylstearate and other steroids. Cholestanol and its esters contaminated by cholesterol were found in all fourteen species examined in amounts of about 7–15 per cent of the total feather lipids expressed as cholestanol[1]. Non-steroid esters, the alcoholic components of which varied with species, were also present. For the duck these consisted mainly of octadecanol and eicosanol. However, the greater part of the feather lipids was made up of free optically inactive fatty acids, with a chain-length of 7–22 carbon atoms. The most prominent of these were palmitic and stearic acid.

The outstanding features of the chemical composition of the bird's feather lipids are their unexpectedly high cholestanol content and the large amount of free fatty acids. Usually, cholestanol is but a small fraction of the cholesterol present in vertebrate tissue and the bulk of the fatty acids is esterified.

Feather lipids are of dissimilar qualitative composition to preen or oil gland secretion, except for the octadecanol fraction of some birds. In these cases this fraction is still quantitatively different as, for example, in the duck, where octadecanol esters comprise about 90 per cent of the entire oil-gland secretion[2] and the total feather lipids only contain about 10 per cent.

The quantitative composition of feather lipids from any one bird varies according to the type of the feather and the part of the feather examined. For example, in the duck, small feathers have a relatively high octadecanol-eicosanol ester content (17 per cent) while the large tail and wing feathers contain only about 1 per cent of this fraction. The compact shafts of large feathers are characterized by a large amount of free cholestanol (16 per cent), while the barbs contain only about 4 per cent (Table 1).

Table 1. Composition of Feather Lipids (Duck)

	Cholestanol (per cent)	Cholestanyl esters (per cent)	Octadecanol fraction (per cent)	Fatty acids (per cent)
Small feathers	5	12	17	57
Large feathers	10	7	2	82
Barbs of large feathers	4	7	1	90
Shafts of large feathers	16	6	1	76

With no sebaceous glands in the bird's skin other than the preen gland, which may not be present (dove, emu, etc.[3]) the feather lipids found in the plumage are considered to be a by-product of the keratinization process apparently being formed with the feather as it develops. They are water-repellent enough to take the place of the secretion of the preen gland. Thus, water birds which had their oil gland removed surgically remained dry[4], because the feather lipids functioned as water-repellents. In the controversy about the water-repellent properties of bird-feathers the role of the feather lipids has apparently been overlooked.

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α-Conarachin

It is generally considered that the major globulins of dicotyledons and the prolamins and glutelins of monocotyledons are reserve proteins, that is, they are digested enzymatically on germination, whereupon the amino-acids and peptides are made available for protein synthesis[1]. Actually, all the protein in the storage organ of the seed eventually disappears, as does the organ itself. But the label of reserve protein has been put on those proteins, small in number, which comprise a relatively large percentage of the total protein, which change early in germination, and which possess no obvious biological activity. Reserve proteins would be expected to have a characteristic pattern of metabolism on germination; ultracentrifuge patterns of protein extracts of pea-seeds at various stages of germination showed more rapid disappearance of the globulins[2], but this technique is not sufficiently discriminating to identify specific proteins with the pattern of change.

We have been able to achieve better identification of changes on germination with specific protein fractions of the peanut (*Arachis hypogaea*) by means of chromatography on DEAE cellulose. One fraction that changes its properties early on germination was isolated and named α-conarachin. It approaches monodispersity by standards of chromatography and ultracentrifugation.

The two major groups of globulins mentioned most often in the literature on peanut proteins are arachin, precipitated at 40 per cent saturation with ammonium sulphate from a salt extract of the peanut, and conarachin, precipitated by 85 per cent saturation of the supernatant after removal of arachin[3]. Both these fractions prepared from *NC*2 variety peanuts were dissolved in phosphate buffer, *p*H 7·9, $\mu = 0{\cdot}06$, and chromatographed on DEAE cellulose ('Selectacel', Brown Co.) by the procedures of Sober *et al.*[4]. Elution was with a linear gradient of 0–0·6 *M* sodium chloride; protein analysis was by the method of Lowry *et al.*[5].

Chromatography patterns of the two fractions are shown in Fig. 1*A*; a pattern of an extract of the globulins from peanut cotyledons in the same buffer is shown in Fig. 1*B*. We have grouped the proteins into four arbitrarily selected fractions: the first is that which is not adsorbed on the cellulose; the second is eluted by salt concentrations up to 0·14 *M*; the third is eluted in the range of 0·14 to 0·23 *M*; and the fourth by concentrations of salt above 0·23 *M*. Arachin is mostly of group IV; conarachin comprises groups I, II, and III.

With the same chromatographic techniques, observations were made of changes in the proteins of peanut cotyledons after three days germination at

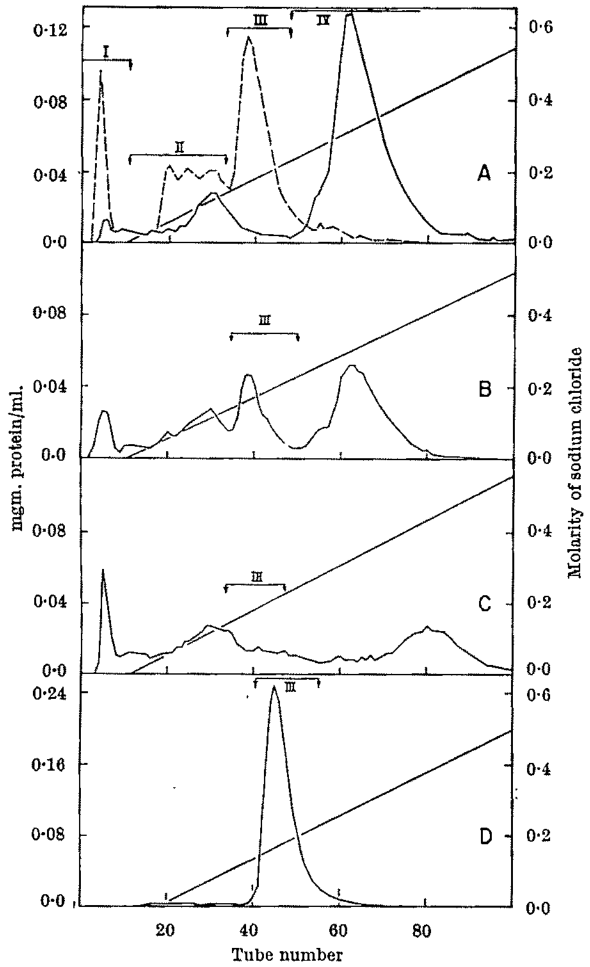

Fig. 1. Chromatography of peanut protein fractions on DEAE cellulose. 10 mgm. of each sample were put on 2 gm. of cellulose. The straight line indicates the sodium chloride gradient as measured on the eluate. *A*, Full line represents arachin fraction; broken line, conarachin fraction; *B*, extract of proteins from peanut cotyledons with buffer, *p*H 7·9, μ = 0·06; *C*, extract of proteins from cotyledons of germinated peanuts with same buffer; *D*, α-conarachin

25–28° C. Examination of the results given in Fig. 1*C* shows that the globulins of group III disappeared almost entirely from the chromatograph; there was an increase in the salt concentration at which the globulins of groups II and IV were eluted and a reduction in the relative amount of group IV; the proteins of the first group were mostly unchanged. We have no evidence yet of the nature of the changes that cause the disappearance of group III from the chromatogram; these may be selective hydrolysis of this protein or may arise from changes such as deamidation, which would result in an increase in negative charge at the *p*H of chromatography. Indeed, only 70 per cent of the proteins were recovered from the chromatogram when those of the germinated seed were examined as compared with 99–95 per cent from the resting seed. By using a higher concentration of salt and higher *p*H, other proteins were recovered from the column, but their relationship to the original protein has not been established.

The major protein of group III was purified by chromatography as follows: 250 mgm. of the conarachin fraction were dissolved in phosphate buffer at *p*H 7·9, ionic strength 0·06, dialysed for 24 hr. against the same buffer, adsorbed on a column (4 gm.) of DEAE cellulose, and chromatographed with 1,200 ml. of eluant under a linear gradient of 0–0·1 *M* added sodium chloride. This is a much gentler gradient than that used for the analytical chromatography. The eluate was discarded; a fresh gradient of 400 ml. of 0·1–0·2 *M* added sodium chloride served to elute the desired fraction. The middle portion of the eluate was saved, rechromatographed and concentrated; the yield was 30 per cent of the original conarachin fraction.

Chromatography of this fraction showed the presence of only one component (Fig. 1*D*); it contains 15·3 per cent nitrogen.

In buffer of ionic strength 0·5, *p*H 7·9, α-conarachin sediments as one component with an apparent sedimentation constant of 7·8; at $\mu = 0·06$, the sedimentation constant is 12·6; at intermediate ionic strengths, the pattern is a mixture of these two species. With the same constants as assumed for peanut protein by Johnson and Naismith[6], molecular weights of 142,000 and 295,000 were calculated for the two species. This confirms and extends the observations of Johnson and his associates that there are components of the peanut proteins which undergo association-dissociation.

Further work on the chemistry and on the metabolism of α-conarachin during germination might be expected to provide more information on the nature of reserve proteins. A full account of this and related work will be published at a later date.

We are grateful to W. K. Bailey for furnishing the sample of carefully selected peanuts.

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Use of Agar Diffusion and Fluorescent Antibody

THE agar diffusion technique is very useful and has made a significant contribution in the analysis of antigenic materials. However, the difficulty encountered when trying to observe some of the precipitation zones is known to anyone who has used this method. Successful photography of the zones can be especially difficult to achieve.

By using fluorescent antibody in the agar diffusion procedure, the precipitation zones when viewed or photographed in the near ultra-violet range appear much clearer than with non-fluorescent antibody.

Fig. 1 shows the zone pattern of the plate on the right when photographed using fluorescent antibody and specific antigen. The plate on the left contains fluorescent antibody and non-specific antigen. Fig. 2 depicts the same materials when photographed without fluorescence.

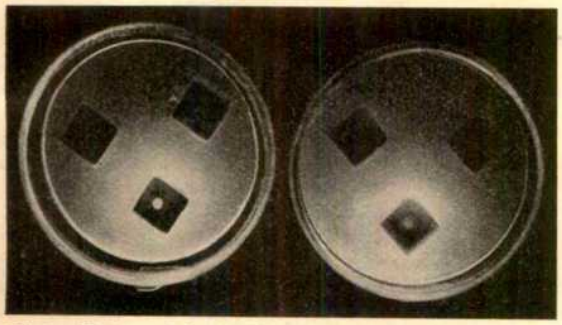

Fig. 1. Photographed using 'black light' and fluorescent antibody

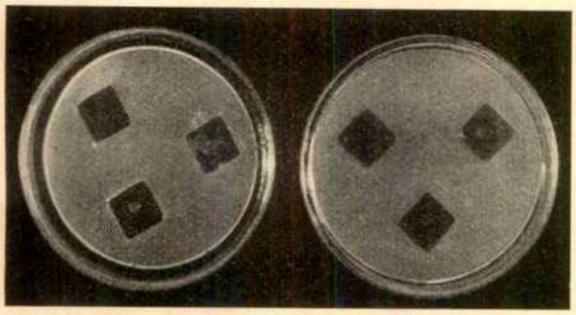

Fig. 2. Photographed using visible light without fluorescence

The method may be especially helpful if the concentrations of either of the reactants are limited.

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An 'Apical Body' in the Galea Capitis of the Normal Bull Sperm

WORK on the ultrastructure of the bull sperm[1] has been continued on semen from normal, fertile bulls of the Red Danish Milk-breed. These earlier investigations were performed on bull semen kept on ice 24–48 hr. prior to osmium fixation.

More recently we have attempted to get the semen samples centrifuged as soon after the ejaculation as possible, and two specimens have been fixed (1 per cent osmium tetroxide) within 30 min. In such freshly prepared material the following new observations on the head of the bull sperm have been made.

Due to the characteristic tennis racket-like shape of the head (dimensions 8 × 4 × 0·5μ), complete coronal sections through the full flat area of the sperm head have only rarely been encountered.

For this reason more has been learned from the study of the sagittal sections such as those shown in Fig. 1*A*. An especially well-preserved median sagittal section of this type is marked with an arrow, and the schematic drawing (Fig. 1*B*) is based on this and similar photomicrographs.

The illustrations show that the sperm head contains a dense nucleus 0·2–0·3μ in thickness (*a*–*b*) with a somewhat thicker base (*a*) and a sharp-pointed apex (*b*) which forms a front edge. The basal half of the nucleus is covered by a rather thin but relatively electron-dense post-nuclear cap (*c*).

The apical half of the nucleus is covered by a somewhat thicker (about 0·1μ) and less electron-dense cap : the galea capitis (*d*). This structure has been described earlier[2] in the detached state in a light microscope study of the bull sperm.

Along the front edge of the sperm head the galea capitis forms a characteristic thickening, which we have chosen to name the apical body (Fig. 1*B*, *e*–*f*).

Our investigations indicate that the galea capitis with its apical body and the post-nuclear cap are completely enveloped by the cell membrane of the sperm, which thus covers the entire cell without interruption from the head to the tip of the tail. Further details of this morphological feature will be published elsewhere.

The apical body consists of a more or less closed fold (*f*) and a thickening in which a narrow cylindrical body or vacuole is situated (*e*). This presents a somewhat smaller density than the rest of the galea capitis, and seems to lie parallel with the fold along the front edge of the sperm head.

In the median section of Figs. 1*A* and *B*, the fold is seen as a hook-formed element and the vacuole as an oval less-dense area with a diameter of about 0·1μ.

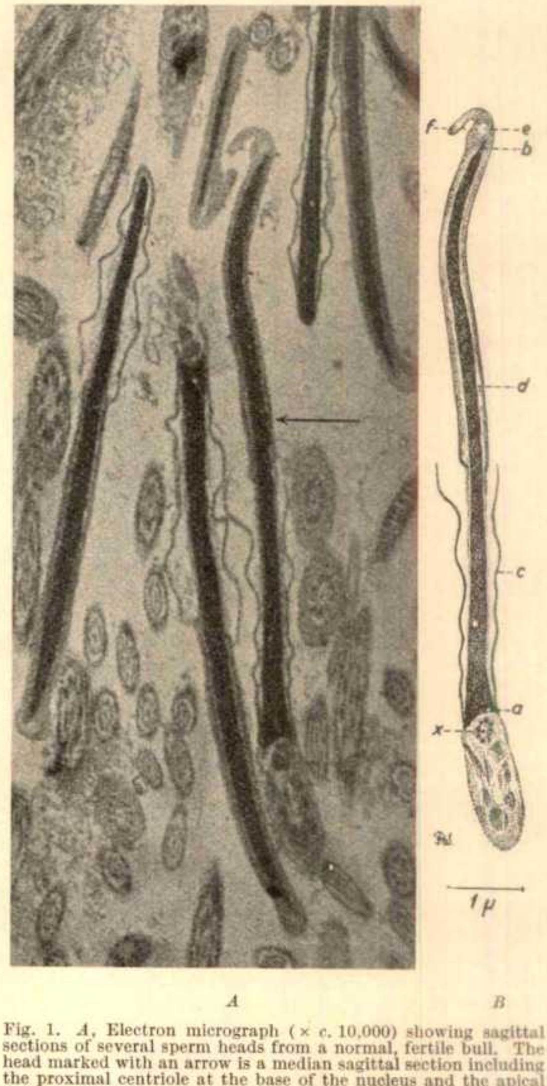

Fig. 1. *A*, Electron micrograph (× *c*. 10,000) showing sagittal sections of several sperm heads from a normal, fertile bull. The head marked with an arrow is a median sagittal section including the proximal centriole at the base of the nucleus and the apical body at the front edge of the head. In four of the other sagittal sections the apical vacuole is visible. *B*, Schematic drawing of a median sagittal section of the sperm head: *a*, base of the nucleus; *b*, apex of the nucleus; *c*, post-nuclear cap; *d*, galea capitis; *e*, apical body with the apical vacuole; *f*, fold of the apical body; *x*, proximal centriole. (Drawn by Poul H. Winther)

Often the fold is more acutely bent towards the sperm head proper than shown in Figs. 1*A* and *B*, and frequently the space between the fold and the rest of the galea has been almost closed.

At higher magnification the morphology of the apical body just described has been verified in sections of 12 individual sperm heads from 3 different bulls.

A similar structure has also been demonstrated in the galea capitis of rabbit sperm.

Most likely the apical body of the galea capitis in the bull sperm is a very delicate structure, so delicate that it can only be preserved and thus demonstrated in sperm fixed in the living state. Examination of the electron micrographs of unsectioned bull sperm published by Hancock[3] seems to present some support for this interpretation. The front edge of the head of a cell fixed in the living state (shown in plate 19*a* of Hancock's paper) contains a structure which could very well be identified as the apical body described in this communication, whereas the head of a sperm which was dead prior to preparation for electron microscopy and which is illustrated in plate 19*b* of the paper shows no evidence of this structure.

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Structural Asymmetry in Cilia and Flagella

IT has become possible recently to describe the fine structure of cilia and flagella in more detail than was feasible before, and a characteristic structural asymmetry has been noted in the flagellum of certain flagellates[1]. This communication reports a similar asymmetry in cilia and flagella of a wide range of organisms.

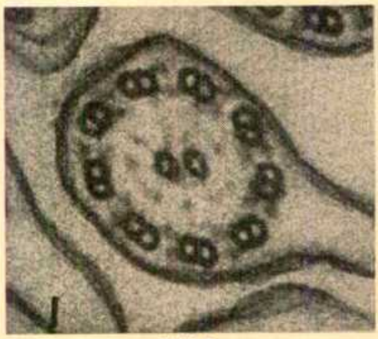
The material investigated includes the ciliate *Paramecium multimicronucleatum*, and ciliated epithelia from mussel (*Anodonta cataracta*), frog (*Rana pipiens*) and the rat. Further observations are reported on the flagellates *Holomastigotoides*, *Pseudotrichonympha*, and *Trichonympha*. For convenience, the term 'cilium' will be used here to include the flagellum of these flagellates.

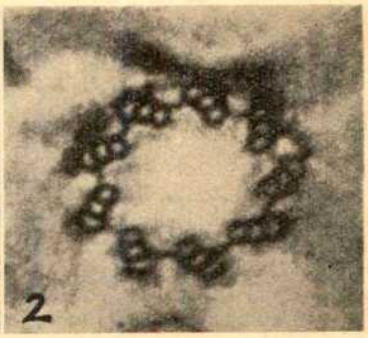
The cilia are cylindrical structures containing a complex array of fibres oriented longitudinally. Their asymmetry results from the arrangement of the nine outer fibres (Figs. 1, 3). In transverse sections of the cilium each outer fibre appears as a doublet with a figure-of-eight cross-section and bears two projections, or 'arms', that extend towards one adjacent outer fibre. These arms point in the same direction on all the fibres in a given cilium. This configuration could exist, theoretically, in two enantiomorphic forms; the clockwise form may be defined as that in which, to an observer looking outwards along the cilium from base to tip, the arms all point in a clockwise direction. In the basal body, at the cytoplasmic end of the cilium, the arrangement of the outer fibres is different, but again asymmetrical. Here the fibres are triplets; they do not bear arms, but each fibre is tilted inwards at about 40° to the overall tangent to the basal body. The relationship between basal body and cilium is such that in the clockwise form (as defined here), the configuration shown in Figs. 2 and 4 is seen by an observer looking outwards from the end of the basal body that lies deepest in the cytoplasm[1]. All these structural features are essentially the same in cilia of all the organisms examined.

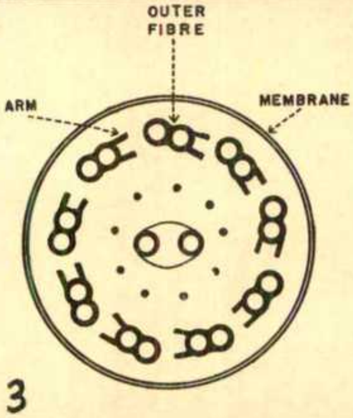
In order to determine whether a cilium has the clockwise or counter-clockwise form it is necessary to examine in the electron microscope an accurate cross-section of known absolute orientation. With *Paramecium*, random sections were cut and the orientation determined in the microscope from the known asymmetry of the kinetodesmal fibrils[2]. With the other organisms it was necessary to know the orientation in which a section was cut from the specimen, and to keep track of it through later manipulations.

The asymmetry has been observed in six individuals of *Trichonympha*, one *Pseudotrichonympha*, one *Holomastigotoides*, and six *Paramecium*; gill epithelium from four mussels; pharyngeal epithelium from one frog; tracheal epithelium from six rats. The following results were obtained:

(1) So far as could be determined, all the cilia in any one specimen had the same enantiomorphic form (an average of about fifty cilia were examined in each specimen).







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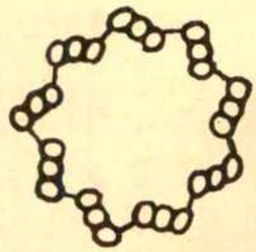

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Fig. 1. Electron micrograph showing a cross-section of a cilium in *Holomastigotoides*
Fig. 2. Electron micrograph showing a cross-section of a basal body in *Holomastigotoides*
Fig. 3. Diagrammatic reconstruction based on Fig. 1
Fig. 4. Diagrammatic reconstruction based on Fig. 2

(2) All specimens had cilia of the clockwise form. This apparent uniformity in such widely separate groups of animals suggests that all cilia, whatever their source, have the same clockwise form. However, the occasional occurrence of the counter-clockwise form cannot be excluded.

Essentially the same arrangement of doublet fibres bearing arms occurs also in many sperm-tails, for example, from the sea-urchin[3], butterfly[4], and rat (Gibbons, I. R., unpublished observations). (The frequent presence of extra components not found in cilia is irrelevant to the present consideration.) It seems probable that sperm-tails will show the same uniformity of asymmetry as cilia, but the difficulty of determining the absolute orientation of cross-sections has so far prevented examination of this point.

For a number of years it has been known that the movement of spermatozoa from many animals is asymmetrical[5,6]. The most obvious, though not the most fundamental, expression of this is seen when freely swimming sperm come into contact with an obstructing surface: they usually move around on it in counter-clockwise circles (as seen on looking towards the surface). More recently, Parducz[7] has described a characteristic counter-clockwise motion of the cilia of *Paramecium*. Such asymmetry of movement might perhaps be related to the structural asymmetry that has been described here.

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Genetically Mixed Perithecia in *Neurospora*

SANSOME[1] has produced evidence that the asci in a single perithecium of *Neurospora crassa* may originate from more than one pair of gamete nuclei. Despite Sansome's evidence, it has been accepted generally that there is only one egg and one sperm nucleus[2].

Sansome used a heterocaryon as one of the parents in her experiment, so that the genetically different sperm nuclei in each perithecium could have originated from a single multinucleate cell. If one accepts Sansome's results, the question still remains whether or not the sperm nuclei in the ascogenous hyphæ of a single perithecium can originate from two or more different conidia. An answer to this question is presented here by using an ascospore colour mutant *ts* (tan-spore)[3].

A mixed suspension was made of approximately equal numbers of conidia from a *ts* culture and from a wild-type culture, both of mating type *A*. This suspension was used to fertilize protoperithecia, which had developed from a wild-type culture of mating type *a* on Westergaard and Mitchell's minimal agar medium[4]. More than seventy-five conidia were applied per protoperithecium. Fertilization was carried out within 5 min. after the mixed suspension was prepared to prevent formation of heterocaryons by the germinating conidia. The average number of nuclei in these two kinds of conidia was determined by Huebschman's method[5] to be 2·6 and 2·7 respectively. After the perithecia had matured, 2,778 perithecia were dissected and examined under the microscope. All the perithecia examined were observed to have a single ostiole. Those with two or more ostioles were discarded.

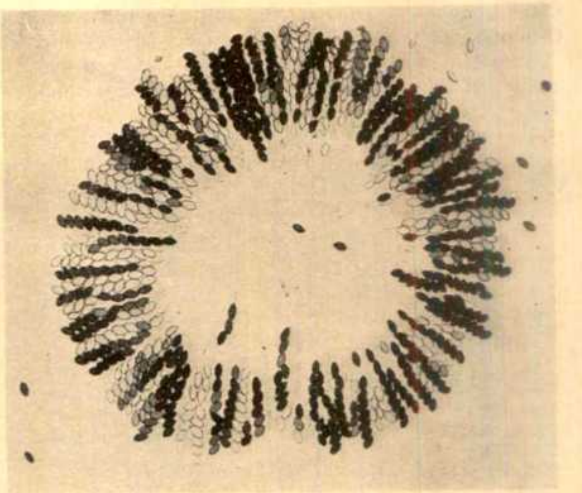

Fig. 1. Ascus cluster from a mixed-type perithecium, showing wild-type and segregating asci (the asci containing eight colourless ascospores are immature).

The great majority of these perithecia were homogeneous types, that is, they consisted entirely of asci segregating for *ts* and wild-type, or entirely of asci in which all the ascospores were wild-type. There were 1,231 perithecia homogeneous for the segregating asci, and 1,487 homogeneous for the non-segregating asci. Sixty perithecia, however, were found to be heterogeneous, containing both segregating and non-segregating asci (Fig. 1). The mixed perithecia indicate that two or more sperm nuclei from two or more conidia can be involved in the formation of a single perithecium. The frequency of heterogeneous perithecia (2 per cent) would indicate that at least 4 per cent of perithecia result from protoperithecia that have been fertilized by two or more conidia. This frequency is considerably lower than that reported by Sansome, who found four heterogeneous perithecia in a total of eight perithecia examined.

A count of the mature asci in 51 of the heterogeneous perithecia gave 933 of the non-segregating type and 692 of the segregating type. This differs significantly from a 1 : 1 ratio ($\chi^2 = 35 \cdot 7$, $P < 0 \cdot 001$), showing an advantage of the former during the time of ascus formation. The ratio of the two types of homogeneous perithecia also deviates significantly from a 1 : 1 ratio ($\chi^2 = 24 \cdot 1$. $P < 0 \cdot 001$), indicating a selective advantage of the wild-type nuclei over the *ts* nuclei for their entrance into the protoperithecium. Further analysis, at present being carried on by one of us (T. E.), shows that the ratio is affected in various ways by the stocks utilized in the crosses, indicating that a number of factors are involved in this phenomenon.

When this manuscript was in preparation, our attention was directed by Dr. D. D. Perkins to the unpublished work of Grant[6]. Grant obtained results very similar to those reported here. She obtained 2 heterogeneous perithecia from a total of 106 examined. One of these two, however, had two ostioles and was double the normal size, indicating that it was the result of the fusion of two separate trichogyne systems.

The supervision of Prof. T. Haga is greatly appreciated. Criticisms and corrections to the manuscript were supplied by Drs. D. D. Perkins and C. S. Gowans.

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Flower Initiation in *Trifolium repens* L. : a Short-Long-Day Plant

PAST investigations into the photoperiodic control of flowering have led to the conclusion that *T. repens* is a long-day plant with a critical day-length of approximately 13·5 hr.[1]. It is also known that the response to photoperiod varies with temperature[2,3], the critical day-length being much shorter at low than at high temperatures. Investigations reported here now show that *T. repens* reacts as a typical short-long-day plant.

In a series of preliminary experiments, seedlings of New Zealand Government Stock white clover, grown at average temperatures of 20° C. at night and 25° during the day, failed to flower in long days unless they had previously received short days for at least 4 wk. Thus the response of *T. repens* to day-length is similar to that of *Campanula medium*[4] in which short days are necessary for the attainment of ripeness-to-flower before long days lead to the initiation of inflorescence primordia. Within the strain of clover used, however, 5–10 per cent of the individual plants fail to respond to day-length in any way.

A series of more detailed investigations was made into the nature of this short-long-day reaction system in clonal material from the same source. Ramets of two selected clones of white clover, *A* and *C*, were grown in glass-houses in which the temperature varied between minima of 15° and 21° C. at night and maxima of 21° and 32° C. during the day. Four day-length treatments were imposed: SDs (8-hr. photoperiods; plants darkened from 4 p.m.–8 a.m.); NDs (unsupplemented natural daylight providing photoperiods which decreased from 13 to 11 hr. during the experimental period); LDs (16-hr. photoperiods consisting of natural daylight supplemented from 4 a.m. to 8 a.m. and from 4 p.m. to 8 p.m. by artificial light); and CL (continuous light consisting of natural daylight supplemented by artificial light from 4 p.m. to 8 a.m.). Supplementary illumination was provided by 200-W. 'Sieray' mercury vapour lamps ballasted with a tungsten filament which gave an intensity of approximately 500 ft.-candles at the leaf surfaces.

In the first experiment, plants were exposed to either NDs or LDs for a preliminary period of two months and then transferred into either LDs or NDs. There were thus four groups of plants, each consisting of three ramets of each clone, which received NDs followed by LDs, NDs followed by NDs, LDs followed by NDs or LDs followed by LDs. Flowering was recorded on twenty vigorous runners from each ramet. Plants transferred from NDs to LDs produced an average of 0·82 flower head per runner in clone *A* and 0·91 in clone *C* as an immediate response to such treatment. No initiation of inflorescence occurred on the plants of any other group; 9 months after their period of preliminary treatment these were still entirely vegetative.

The first morphological sign of the transition of a vegetative apex to the reproductive state in white clover is the precocious development of a bud in the axil of the youngest leaf primordium. Such a bud appears in the axil of the first leaf primordium to be initiated at the apex of a ripe-to-flower runner after its transfer from short to long photoperiods. Following its initiation, this bud develops rapidly into a lateral inflorescence and the main stem apex reverts to the vegetative condition. This pattern of development is identical to that occurring in other *Trifolium* species[5,6].

The minimum requirement for both short days and long days has been determined by more recent investigations. The response to short days was tested by growing twenty plants of clone *A* for 2 months in LDs, exposing them to 0, 3, 6 or 14 SDs, and then returning them to LDs. The results (Table 1) show that in some circumstances initiation of inflorescence can occur in LDs after a preliminary exposure to as few as three SDs.

Table 1. THE EFFECT OF TREATMENT WITH SHORT DAYS ON INFLORESCENCE INITIATION AFTERWARDS IN LONG DAYS

No. of short days	0	3	6	14
Average number of inflorescences per runner Standard error	0·000 ± 0·000	0·105 ± 0·052	0·812 ± 0·098	1·595 ± 0·085

The sensitivity to long days is even greater than to short days. The combined results of two experiments to determine the minimum number of long days necessary to bring about inflorescence initiation are shown in Table 2. In both experiments plants of clone *A* which had been grown in NDs for 3 months immediately before treatment were exposed to a period of CL and then returned to NDs. Under these conditions a single day of CL was sufficient to induce the initiation of inflorescence primordia.

Table 2. THE EFFECT OF THE DURATION OF EXPOSURE TO CONTINUOUS LIGHT ON SUBSEQUENT INFLORESCENCE INITIATION IN SHORT DAYS

No. of days continuous light	0	1	2	4	6
Total number of runners	54	100	18	18	18
Percentage of runners bearing inflorescences	0	94	100	100	100

Some individuals within a population of New Zealand Government Stock white clover are thus very sensitive short-long-day plants at the temperatures used during the present investigation. Ripeness-to-flower will only occur in short days and, once this condition is attained, a single long day is sufficient to bring about inflorescence initiation. A comparable sensitivity to long days has so far been reported to occur in only one long-day plant: *Lolium temulentum*[7].

Further investigations into the nature of the short-long-day reaction system are in progress and will be reported in detail elsewhere.

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Early Flower Initiation in the Banana

THE commercial banana is typically seedless and must be propagated vegetatively by lateral buds which appear on the true stem at the base of a large plant. Depending on climate and season, a flower bud appears when the pseudostem is 12–20 ft. tall (variety Gros Michel) and only after a succession of more than 40 leaves. Recently, however, on farms of the United Fruit Co. in Guatemala and Honduras, shoots have flowered when only 2–3 ft. tall, while still attached to the base of a 'mother' plant, and following the production of seven or fewer leaves.

These fruited suckers were noted in the field in conjunction with the application of a rapid method of banana multiplication[1], which is based on the forcing of immature lateral buds while harvesting the more matured buds as vegetative 'seed'. By this means up to 24 propagules have been obtained from a single planted bit within a year. The majority of these forced suckers have been normal and have flowered after the appropriate vegetative period. However, in at least 21 instances lateral buds have produced fruits after an abbreviated vegetative period and while still attached to the parent.

The circumstances surrounding this early flowering were investigated in Guatemala. Invariably the fruited suckers appeared as lateral shoots from the base of plants that were themselves in flower. Invariably the suckers developed from the latest formed viable buds—those appearing close to the terminal apex and passing through their early development at the time of the conversion of the apical parental bud to the flowering state. No more than one flowering sucker has ever been observed to be attached to any single parent plant and all have been noted on the variety, Cocos, a dwarfed sport of the Gros Michel.

The fruited small suckers generally were of normal appearance but dwarfed in size, as in Fig. 1. There were a number of variants; some produced a series of bladeless leaves enclosing anomalous structures that resembled lateral inflorescences yet showed no flowers; a few flowered directly without any leaves. Most significantly, however, all produced the terminal and clearly recognizable flower bud very early.

In three respects the plants involved were treated differently from similar plants grown in similar areas. All were wounded heavily and continuously in connexion with the multiplication technique. All were provided with an extremely high level of nitrogen, as urea, applied as a side dressing. Immature buds proximal to the parental apex were forced at the time of the conversion of the parental bud to the flowering state.

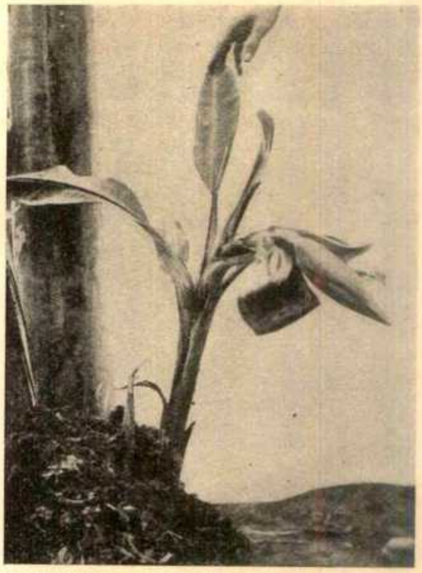

Fig. 1. Small, fruited sucker. The form is normal, however, the number of vegetative leaves preceding the inflorescence has been reduced to 7 and the number of flowers sharply decreased. Note the size relative to the hand and the parent pseudostem

It is not likely that the luxury supply of nitrogen would be concerned since classically this would favour vegetative development and suppress reproductive tendencies. The heavy wounding must not entirely be discounted since the stimulatory effect of wounding on plant and cell activity is well documented[2–4]. In addition, it has been noted that field banana fruiting abnormalities are almost invariably associated with wind damage, insect injury, machete cuts, or disease incidence. However, the rarity of such abnormalities would suggest that the response could only be elicited when the apex was in a susceptible stage.

The third possibility relates the conversion to flowering of the parental terminal bud to a simultaneous, but often less-efficient, induction by the same stimulus of a proximally located forced lateral bud. In untreated plants (not undergoing multiplication) such buds would be rudimentary and would never develop to form a shoot. In treated plants, and even so in rare instances, buds sufficiently advanced to be influenced by an overflow of the parental conversion factor are located sufficiently near to respond. These may then be imperfectly or wholly converted to the flowering status by the parental stimulus. Hence in the subsequent development of the lateral shoot, where this is permitted, the newly acquired character is manifest as either a perfect or anomalous inflorescence, with the number of preceding leaves dictated by the status of the bud at the time of its conversion.

Pertinent to this is a body of literature concerning lateral inflorescences in the banana[5]. Invariably these have appeared in conjunction with the flowering of the main axis and have involved buds very close to the terminal bud. The rarity of such occurrences in the field undoubtedly relates to the observation of Skutch[6] that buds do not form along the elongated true stem of the fruiting banana. Should buds occupy sites along the elongated axis, and should their later development occur, then the lateral inflorescences of Ghosh[5] could be explained by the overflow mechanism outlined above.

The occurrence of these early flowers is important from the point of view of the theory of flowering. For some years attempts have been made at influencing the onset of flowering in the banana, as has been done in the pineapple[7,8], but without success. The conversion of these young buds at such an early stage indicates that there is a good probability that the meristem of older plants also can be influenced if treated appropriately. The benefits of such control over the fruiting habits of a commercial crop need not be discussed.

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ENTOMOLOGY

Control of the Responsiveness of the Blowfly to Water

'Hunger' in the blowfly (*Phormia regina*, Meig.) has been the subject of several studies. The present experiments were undertaken to examine the related problem of 'thirst' or the control of water intake. Bolwig[2] showed that blood osmotic pressure was correlated with responsiveness to water applied to the tarsi of a fly. Wolbarsht[3] suggested that the response of the contact chemoreceptors to water was increased in desiccated flies. Since ingestion is primarily controlled by the taste receptors[1], such a relationship would provide for a direct and specific regulation of water ingestion.

Flies, if dehydrated enough, reflexively extend their proboscis on contact of the tarsal or labellar chemoreceptors with water. Normally, taste thresholds are expressed as the effective concentration of the test substance in solvent. In the present case, the number of flies that responded to water was the measure used. Only those flies were included that were either uniformly negative or positive on each of three tests before treatment. Dehydration was accomplished by confinement of the flies over calcium chloride in a sealed vessel. Water-negative controls were members of the same population that had been kept in the normal room atmosphere or that had been given water after desiccation. Injections were given as described by Evans and Dethier[4]. Unless stated otherwise, tests were performed within 5 min. after the injection (Table 1).

Water-positive flies were rendered negative by injections of distilled water into the hæmocœl (exp. 1–3). The effect was immediate (exp. 1–3) and persistent (exp. 4) and depended on the volume injected (exp. 3, repeated injections). Injection of small (exp. 5 and 7) or large (exp. 6) volumes of hyper-osmotic solutions into the water-negatives did not render them positive. On the other hand, these same injections did, like distilled water, make positive flies negative (exp. 8–11). Some of the treatments produced a high mortality, but the effects on the water response appeared specific since the flies would respond normally to dilute sugar solutions after the most severe treatment (exp. 3, 5, 6).

Table 1. Effect of Injections on Responses of the Blowfly to Water

Experiment No.	No. of flies	Response before treatment	Injected †	No. negative after treatment
1	35	+	2·5 μl. water	6
2	52	+	8 μl. water	44
3	27	+	2 μl. water	2
			2 μl. water	6
			2 μl. water	18*
4	26	+	3 μl. water	
			immediately	15
			at 10 min.	15
			at 60 min.	14
5	120	−	3 μl. 2 *M* glucose	98*
6	116	−	7 μl. 2 *M* glucose	111*
7	29	−	2·4 μl. 4 × saline	29
8	53	+	3 μl. 4 × saline	29
9	69	+	3 μl. 2 *M* glucose	38
10	28	+	3 μl. 2 *M* glucose in saline	14
11	25	+	6 μl. 2 *M* glucose in saline	21

* Responded afterwards to 0·1 *M* sucrose.
† Water indicates distilled water; the saline was Bodenstein's (ref. 9); 4 × indicates saline four times more concentrated.

Since, however, all the foregoing procedures that were effective blocked a response and none elicited one, it was crucial to reduce blood volume and thereby elicit responses to water. The only effective method of bleeding *Phormia* is to cut off the forelegs near the base and compress the thorax on all sides[4]. In this way up to half the blood can be expressed with little damage to structures other than the forelegs. In the present experiments all the blood was removed that could be with gentle pressure. Although the forelegs are important bearers of contact chemoreceptors, the other two pairs of legs, when stimulated, can produce proboscis extension[5]. If a uniform population of flies was desiccated until some became responsive to water, most of the remainder were made positive by bleeding (45 out of 53). The result was dramatic: as quickly as the fly could be tested after bleeding (a few seconds), a vigorous response was obtained when before there was none. Even when the bleeding was deliberately traumatic, a feeble response could be obtained as the animal died. In flies that were originally water-positive, bleeding did not alter the response (12 out of 12). The amount of blood obtainable seemed markedly less in the positive than in the negative flies; and it seemed that the few flies that did not respond after bleeding were those from which the greatest volume could be obtained. Apparently, if blood volume was above a certain level, bleeding could not reduce it sufficiently below some threshold value. Similarly, when members of a population were bled that had been given water to repletion, few were made water positive (4 out of 13).

These results do not conflict with Bolwig's[2], since blood osmotic pressure may well be a function of body water and blood volume. The results, however, demonstrate that neither osmotic concentration nor the concentration of any blood constituent determines sensitivity to water. Moreover, they do strongly suggest that blood volume via a rapid acting (neural) pathway sets the sensitivity of some element involved in the feeding response. Since some aspect of blood volume is the effective agent, the detector is most likely a mechanoreceptor. Although dehydration of the chemoreceptors as the mechanism cannot account for the present results, Wolbarsht's[3] suggestion that chemoreceptor sensitivity is the regulated factor is tenable. This might operate in two ways: either the chemoreceptors (one or both) are also affected by mechanical stimuli and some aspect of blood volume is an effective mechanical stimulus, or mechanoreceptors elsewhere influence chemoreceptor discharge. Neither of these possibilities is unprecedented: Hodgson and Barton Browne[6] have evidence that bending of a chemosensory hair does alter (not very predictably) the response of the chemosensory cells to chemicals; and there are many demonstrations of the control of sense organ discharge by afferent neurones of the central nervous system[7]. However, histological[5] and electron microscopic (Dethier, V. G., personal communication) studies have not revealed nerve terminals on the chemosensory cells. The equally likely possibility remains that the mechanoreceptor discharge sets the sensitivity of some element further along the path of the feeding response. This latter mechanism has been shown to be the case for the regulation of taste thresholds to sugars[1]: sugar solutions in the foregut via an unknown connexion (neural or hormonal) with the central nervous system determine the behavioural sensitivity of the fly to sugars. Since all evidence indicates that the ingestion of both water and sugars is evoked by discharge of the same chemosensory cell[5], only a specific regulation of sensitivity to water at the level of the sensory cell would appear at first sight to provide for an independent regulation of water and sugar ingestion; but as will be shown elsewhere, this is not necessarily so[8]. There is evidence that the two are independently controlled (Dethier, V. G., personal communication). Additional details of the present experiments and other data relevant to the regulation of water ingestion will be reported elsewhere[8].

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VIROLOGY

Multiplication *in vitro* of a Nuclear Polyhedrosis Virus in Insect Amœbocytes

Culture, *in vitro*, of nuclear polyhedrosis virus has been achieved, so far, in cells derived from what appears to be the outer epithelial sheath of the larval ovariole of Lepidoptera[1,2]. Grace[3] reported virus induction *in vitro* in cells from explants of the same organ.

A method for the preparation of primary monolayers, using the usually abundant supply of larval hæmocytes of one easily mass-reared species of Lepidoptera, *Peridroma saucia* (Hübner), is being described elsewhere[4]. Our interest in this type of cells for virological work was stimulated by the encouraging results obtained with similar cultures from vertebrate leukocytes[5]. Furthermore, it is not improbable, according to Wigglesworth[6], that the cells migrating from the explants of the larval ovariole maintained *in vitro* are hæmocytes formerly adherent to the tissues.

Glaser[7] attempted infection, *in vitro*, of hæmocytes from larvæ of *Malacosoma americanum* (Fabricius), with nuclear polyhedrosis virus. The results were negative. Formation of the typical intranuclear inclusions was observed by Glaser only when infection of the hæmocytes occurred *in vivo*, and the cells transplanted *in vitro*, 10–12 days following infection of the animal. The lack of success in Glaser's infection experiment could have been derived from a lack of virus adsorption, or more likely from inactivation of the labile Berkefeld filtrate or from its preparation at a time when filterable elements were extremely rare in the larvæ used. Glaser's hypothesis that "... the early stages of the virus require some particular organ or tissue or some particular condition" is not supported, in part at least, by our investigations, as we have achieved direct infection of amœbocytes *in vitro*, in the particular microenvironment of our cell cultures.

The hæmocytes of sixth-instar larvæ of *P. saucia* are cultured on Corning 22 × 22 mm. No. 1 cover glasses, in 60-mm. Petri dishes, with 4 ml. of medium[4] containing 15 per cent homologous insect blood plasma and 15 per cent fœtal bovine serum. After 20–24 hr. incubation at 25° C. in a gas mixture of 1 per cent oxygen, 3 per cent carbon dioxide and 96 per cent nitrogen, a continuous monolayer of single cells and polynucleated syncytia is formed (Fig. 1). The culture medium is discarded and the cells are rinsed with 3·5–4 ml. of a balanced salt solution[4]. The virus suspension is prepared as follows: Larvæ of *P. saucia* showing the definite syndrome of nuclear polyhedrosis are anæsthetized at the seventh day after peroral infection; their integument is cut laterally, along a longitudinal line, and the intestinal tract, with the Malpighian tubules, is excised and discarded. The remaining parts of the larvæ are placed immediately

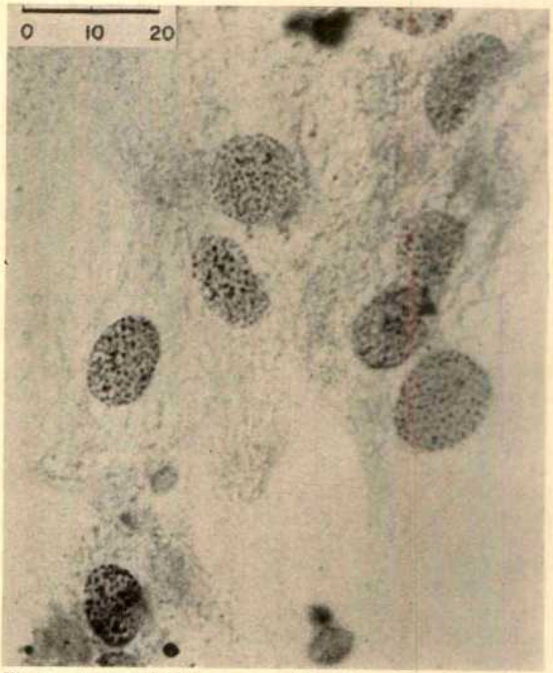

Fig. 1. Average field from a non-infected control culture of the same cell pool as in Fig. 2. Note the basophile granulations in the nuclei, which are typical for normal larval amœbocytes of *Peridroma saucia* (Hübner) *in vitro*. Culture, 6 days old. Duboscq–Brasil. Giemsa. Scale, microns

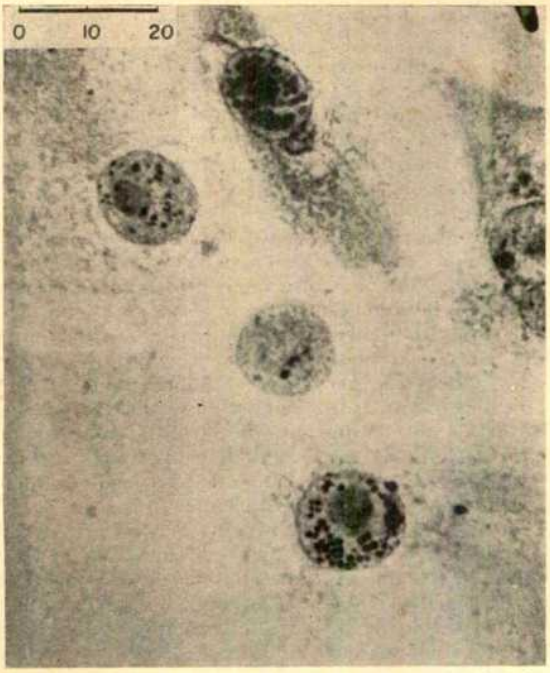

Fig. 2. A group of infected amœbocytes with nuclear polyhedra in an advanced stage of development. The central chromatin mass is still visible in two nuclei. Same cell pool as in Fig. 1. Culture, 6 days old. Infection, 5 days old. Duboscq–Brasil. Giemsa. Scale, microns. (Photography, V. G. Duran)

in a Ten Broek tissue grinder, with 2·5–5 ml. of culture medium[4] per larva. After homogenization, the fluid is centrifuged for 5 min. at maximum speed (3,200 r.p.m.) in the horizontal swinging tube head of an International clinical centrifuge, in order to eliminate the larger tissue particles and the polyhedral inclusion bodies. The supernatant is transferred to cellulose nitrate tubes and centrifuged for 10 min. at 10,000 r.p.m. in the angle rotor of a Servall *SS* 1 Superspeed centrifuge. The final supernatant is passed through a 'Millipore' microfibre glass prefilter disk and a 'Millipore' *HA* filter (pore size 450 mμ). The cultures are incubated with the virus suspension (0·5–2·0 ml.) for 3–4 hr., at 25° C. in the gas mixture described here. After this period the cultures are rinsed with balanced salt solution and supplied with 3·5–4 ml. of fresh culture medium, to which 30 per cent fœtal bovine serum has been added. On the third day after infection the medium is changed and at the fourth to sixth day after infection the cultures are fixed. The cover glasses are rinsed with balanced salt solution in a small Coplin staining dish, for about 5 min.; the balanced salt solution is replaced with Duboscq–Brasil's fluid (12 hr.), the slides are rinsed in repeated changes of 70 per cent ethanol and stained overnight in a 1 per cent solution of Giemsa's stain in *M*/112 phosphate buffer, at *p*H 7·17. Differentiation is performed in 80 per cent ethanol containing 0·5 per cent glacial acetic acid. The preparations are dehydrated in acetone–xylene mixtures and mounted in 'Caedax' Bayer.

Infection with nuclear polyhedrosis virus *in vitro* produced the typical sequence of cytological changes observed *in vivo*[8]. The cytopathogenic effect is particularly evident in the large, flattened nuclei of the amœbocytes (plasmatocytes), with a loss of basophilia, central condensation of the chromatin mass, formation of the 'anneau miroitant', and formation of the polyhedral inclusion bodies within the nucleus. In our preparations, most polyhedra were fixed and stained during their forming phase, when residues of the central chromatin mass were still visible: at this stage (Fig. 2) the inclusions are strongly eosinophile, and the infected cells can be spotted easily in the monolayer cultures by the bright red colour of the inclusion bodies, even at low magnification. These infected cells sometimes appear in groups, suggesting a possible use of these primary monolayers for virus titration *in vitro* by the plaque technique. Quantitative work on viral multiplication in our cell cultures is in progress.

This investigation was supported in part by research grant *E*–2307 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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GENETICS

Effects of Maternal Age on the Incidence of Congenital Abnormalities in Mouse and Man

The value of data from laboratory animals for the interpretation of the incidence of congenital abnormalities in human beings was first pointed out some time ago by Wright[1]. Results I have obtained from a balanced two-point backcross linkage experiment in the mouse have shown an effect of maternal age both on viability and recombination. The purpose of this communication is to present these results and discuss their implications for similar phenomena in human beings.

The factors used in the linkage experiment were *pallid* and *fidget*; they are both recessives and known to be in linkage group V. Both factors are generally associated with fairly severe reductions in viability and fertility. The results were obtained from female coupling and repulsion heterozygotes using *pallid fidget* males. A summary of the results, analysed according to the age of the maternal parent, is given in Table 1. Following Fisher[2], the regression of the proportions of *fidgets*, *pallids* and recombinants with maternal age was analysed after applying the angular transformation. A more detailed analysis of these data will be published elsewhere.

The linear regression in the proportion of *fidgets* with maternal age is highly significant and the quadratic regression is significant at the 5 per cent level. The proportion of *pallids*, on the other hand, shows no significant linear regression but a highly significant quadratic component. It seems worth noting that the deterioration in *fidget* viability apparently starts before the major decrease in litter size.

The linear regression of recombination fraction with increasing maternal age is significant at the 5 per cent level and there is no evidence for any significant departure from linearity. A significant downward

Table 1. DATA FROM FEMALE HETEROZYGOTES TABULATED ACCORDING TO THE AGE OF THE MATERNAL PARENT

Age (months)	Total number of mice	No. of litters	*Fidgets* (per cent)	*Pallids* (per cent)	Recombinants (per cent)
2	115	21	38·3	32·2	32·2
3	84	19	53·6	41·7	30·9
4	106	17	42·5	42·6	24·5
5	98	18	35·7	37·8	28·6
6	108	17	36·1	48·2	34·3
7	73	15	42·5	47·9	23·3
8	92	19	34·8	39·1	23·9
9–10	80	20	27·5	41·2	22·5
11 and over	49	17	18·4	28·6	20·4

		Fidgets	*Pallids*	Recombinants
Linear regression	χ^2_1	13·77*	0·23	4·08†
Quadratic regression§	χ^2_1	4·20†	8·10‡	0·18
Residual	χ^2_6	6·00	3·01	4·47

*, P < 0·1 per cent. † 5 per cent > P > 1 per cent. ‡ 0·5 per cent > P > 0·1 per cent. § Approximate test giving equal weight to observations from different age groups.

trend of recombination with maternal age in mice was also reported by Fisher[2]. The phenomenon is well known in *Drosophila*[3].

Mongolism in man is now known to be caused by aneuploidy for one of the smaller somatic chromosomes[4]. This is most probably the result of meiotic non-disjunction for the relevant chromosome. Mongolism is also well known to increase in incidence with maternal age[5]. Early work with *Drosophila* showed that an increase of non-disjunction was generally associated with a decrease in crossing-over[6]. Thus the increase in the incidence of mongolism in man, reflecting an increase in non-disjunction, and the decrease in the frequency of recombination in mice may well have a common origin in the effect of maternal age on the efficiency of chromosome pairing at meiosis. In rye, Rees and Naylor[7] have shown that developmental variations within an anther cause significant differences in chiasma frequency. Chiasma formation and chromosome pairing may clearly be considerably affected by developmental differences such as probably occur in the ovum of the ageing mother. In fact Polani *et al.*[8] have suggested a specific mechanism for the way in which this might happen.

The effects of maternal age and order of birth on a number of developmental abnormalities in humans have been extensively studied (see, for example, Penrose[9]). The incidence of congenital deformities of the nervous system, in particular anencephaly, spina bifida and hydrocephaly, shows, in general, a marked increase with increasing maternal age. These are abnormalities which cannot be explained by any simple genetic hypothesis and which are considerably subject to environmental influences, as is well illustrated by Edwards's remarkable results[10]. An increase in the frequency of white spotting in guinea-pigs was also found by Wright[1]. It is in marked contrast to the decrease in the number of *fidgets*, and later *pallids*, with increasing maternal age in mice. In general it seems unlikely that any congenital abnormality associated with a single gene difference should show an increase in incidence with maternal age. Such a factor is likely to be associated with fairly severe viability disturbances, although perhaps not so severe as in mice because of the lack of intra-uterine competition. However, a decrease in incidence with maternal age certainly does not necessarily imply a simple genetic basis. Thus it has been shown in guinea pigs[1] and in mice[11] that the incidence of polydactyly decreases with maternal age. In both cases the variation was probably basically non-genetical developmental variation. It is, perhaps, surprising that it did not follow the same course as the developmental congenital abnormalities in humans.

Just as studies in mice may help in interpreting human phenomena, results from work on human beings may also be of relevance to the interpretation of mouse data. For example, Karn and Penrose[12] showed that birth-weight increases with parity and that birth-weights somewhat less than the maximum were most favourable to survival. Probability of survival, therefore, showed parabolic variation with parity. It is interesting that *pallid* viability, and probably also *fidget* viability, show similar parabolic variation with maternal age. No attempt has been made to separate the effects of maternal age and parity in these results; but the two are clearly highly correlated. Certainly *fidget*, and to a lesser extent *pallid*, reduce birth weight so that an initial increase in birth weight may well account for their initial increase in viability.

An initial decrease in the incidence of spina bifida and hydrocephalus with increasing maternal age in human beings was found by Edwards[10]. This may well reflect a similar phenomenon to the initial increase in *pallid* and *fidget* viabilities in the mice.

The parallelism between effects on the incidence of congenital abnormalities in mouse and man, which is illustrated by the above data, serves to emphasize the mutual benefits to be derived from workers on either organism studying each other's results.

I am grateful to Prof. K. Mather for directing my attention to the relationship between non-disjunction and crossing-over.

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Variation in the Behaviour of Different Genotypes of *Drosophila* towards Odoriferous Vegetable Oils

Drosophila are repelled by certain odours; the repulsion from medium containing peppermint oil shown by adult flies can be converted into an attraction towards such food by exposing the individuals as larvæ or adults to the odour[1]. It might therefore be expected in a population exposed to two or more odours, for example, food plants, that part of the population could become specifically adapted to one and part to the alternative odour. Disruptive selection of this type could well give rise to polymorphism[2] or even biological races, and the work reported here was part of an attempt[3] to produce divergence within populations by such means, though Thoday[4] and others have already demonstrated experimentally the production of polymorphism as an outcome of artificial disruptive selection.

About forty etherized *D. melanogaster* were placed in a tube connecting two half-pint milk bottles. In

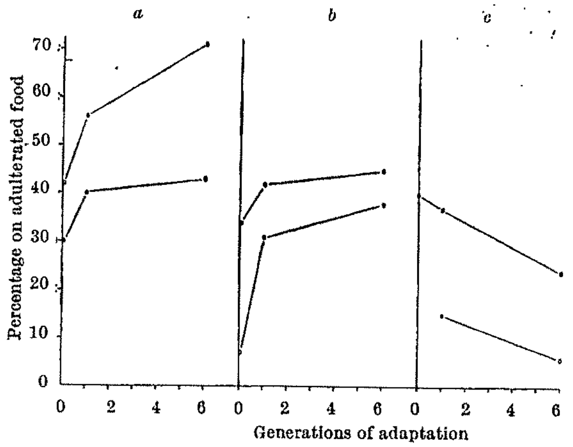

Fig. 1

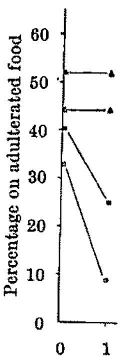

Fig. 2

Fig. 1. Response of Dronfield flies given a choice of normal food and food containing (*a*) 2 per cent peppermint oil; (*b*) 7 per cent juniper oil; (*c*) 3·5 per cent lavender oil. Solid symbols, flies; open symbols, eggs laid. Each point based on at least eight replicates

Fig. 2. Response of two mutant stocks when given a choice of normal food and food containing 3·5 per cent lavender oil. Solid symbols, flies; open symbols, eggs laid; squares, *th st cp*; triangles, *se cp e*. Each point based on at least ten replicates

one bottle was a spoon with food containing an adulterant, in the other was a spoon with normal food. The number of flies in each bottle was recorded after 1 hr. and after 24 hr., at which time the numbers of eggs laid were also scored. Flies were adapted by mass culture in bottles with adulterated food. Where more than one generation of adaptation took place, flies were transferred to new cultures at intervals of twenty days.

Fig. 1 shows the response of unadapted flies, one-generation adapted flies and six-generation adapted flies of a wild-type stock (Dronfield) presented with a choice of normal and adulterated food, for three adulterants. The black points represent the mean number of flies in the bottle with adulterated food (mean of two counts) as a percentage of the total. The circles represent the mean number of eggs laid on the adulterated food as a percentage of the total.

The response to food containing 2 per cent peppermint essence (Fig. 1*a*) is clearly one of increasing attraction with increasing adaptation. After six generations of adaptation to peppermint food, flies choose to lay eggs on this, rather than on normal, food; though as individuals they do not show so marked a preference for remaining on the adulterated food. Fig. 1*b* shows comparable data for food containing 7 per cent juniper oil, and clearly the pattern of response, both of flies and egg laying, is similar to that found with peppermint food. By contrast, flies reared on food containing 3·5 per cent lavender oil are more repelled by such food than are flies reared normally (Fig. 1*c*). The proportion of eggs laid on adulterated food by six-generation adapted flies is less than the proportion laid by one-generation adapted flies. Comparable egg-laying data for unadapted flies are unfortunately not available because of culture failures.

Two mutant genotypes $\frac{th\ st\ cp}{th\ st\ cp}$ and $\frac{se\ cp\ e}{se\ cp\ e}$ tested as unadapted and one-generation adapted flies with food containing lavender oil produced results shown in Fig. 2. The *th st cp* stock resembles the Dronfield stock in that adaptation increases the repulsion of the adulterated food for the flies. The behaviour of the *se cp e* flies is unaffected by rearing on food containing lavender.

In two strains adaptation to food containing lavender oil produces increasing repulsion from such food, and we do not know whether these behavioural changes are the result of physiological conditioning[5], natural selection, or both processes. It seems highly unlikely that only the conditioning process is involved, for considerable mortality was observed in the adapting cultures, and the stocks of *Drosophila* used are known to be genetically heterogeneous. The adaptation (*sensu* biological efficiency) of the populations to lavender food after six generations of exposure to such food must be greater than the initial level of adaptation and therefore adaptation to, and preference towards, lavender food are negatively correlated. Lavender oil essence is toxic, and a behavioural tendency to avoid it, when a choice of food with and without it is presented, probably confers selective advantage in such an environment.

It is unlikely that the distribution of lavender oil in the culture bottles was heterogeneous, for the oil in solution was mixed into the food whereas the latter was liquid. Thus the increased repulsion with adaptation demands that relative ability to survive on lavender food and relative distaste for this food are effects either of pleiotropy or of rather closely linked genes. Whatever the mechanism controlling the behaviour, it is clear that genetic variation for it exists within the species.

The only other example of a similar situation which we have been able to find is that described for *Leptinotarsa* (Colorado beetle) fed on various species of *Solanum*[6]. After feeding on potato, larvæ and male adults preferred horsenettle to this plant. After feeding on horsenettle all individuals preferred potato to this, and after feeds on bitter nightshade this was rejected for horsenettle. Clearly only a conditioning process and not natural selection is involved here.

In any biological population in which this phenomenon of adaptation producing relative distaste occurred, the chances of evolutionary advance to the formation of a biological race via disruptive selection would be minimal, but the population might retain a greater range of exploitable habitat and hence greater versatility. Nevertheless since, as our results show, genetic variance for this character is available in the species, it is possible that some populations could produce polymorphic systems based on behavioural differences.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, June 19

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Annual General Meeting.

Monday, June 19—Tuesday, June 20

Plastics Institute (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 9.30 a.m. daily—Conference on "Education and Training in the Plastics Industry".

Monday, June 19—Thursday, June 22

Laboratory Apparatus and Materials Exhibition (at the Royal Horticultural Society's New Hall, Westminster, London, S.W.1).

Tuesday, June 20

Oliver Bird Trust, and the Society for the Study of Fertility (in the Large Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Dr. A. S. Parkes, F.R.S.: "The Biological Control of Conception" (Fifth Oliver Bird Lecture).

Wednesday, June 21

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. D. Tyrrell: "Some Recent Experiments on the Common Cold Problem".*

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Scientific Papers.

Society for Analytical Chemistry, Microchemistry Group (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "Quantitative Paper Chromatography" introduced by Mr. E. C. Hunt and Dr. D. Gross.

Thursday, June 22

Birkbeck College (in the Large Physics Theatre, Malet Street, London, W.C.1), at 4.15 p.m.—Prof. H. Zocher (Ministry of Agriculture, Rio de Janeiro): "Spontaneous Orientation in Suspensions of Macromolecules and Phase Particles".*

Friday, June 23

University of London (in the Chemistry Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Riccardo Passerini (University of Catania): "Refining Processes and Petrochemical Raw Material Sources".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer (with special qualifications in pure mathematics) in Mathematics (Grade I or II)—The Registrar (Room 22, O.R.B.), The University, Reading (June 21).

Lecturer or Assistant Lecturer in the Department of Physiology—The Registrar, The University, Liverpool (June 23).

Assistant Lecturer, Grade B (with a good honours degree in chemistry or an equivalent high qualification, and preferably a higher degree) in the Department of Chemistry and Biology, to assist with teaching in Higher National Certificate and Graduate R.I.C. courses with special reference to inorganic and analytical chemistry—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff (June 24).

Scientific Officer or Senior Scientific Officer (graduate, experienced in agricultural research), to assist the Head of the Field Experiments Section—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (June 24).

Assistant Lecturer or Lecturer (with special qualifications in geometry) in Mathematics—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (June 26).

Chair of Bacteriology—The Registrar, The University, Manchester 13 (June 30).

Demonstrator in Geography—The Registrar, University College of North Staffordshire, Keele, Staffs (June 30).

Research Assistant (honours graduate (or prospective graduate this summer) in botany or agricultural botany) in Agricultural Botany in the Department of Agricultural Sciences, School of Agriculture, Sutton Bonington, to carry out ecological and genetic studies on natural hybrids between ryegrass and meadow fescue—The Registrar, The University, Nottingham (June 30).

Research Fellow (with research experience in biochemistry or chemistry and interest in physico-chemical aspects of biochemistry) in Physical Biochemistry at the John Curtin School of Medical Research, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, June 30).

Scientific Officer or Senior Scientific Officer (with research experience in enzymology and/or protein chemistry, and some knowledge of, and interest in, fundamental genetic problems), to work on biochemical aspects of genetics—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (June 30).

Assistant Lecturer, and a Lecturer in the Department of Physics—The Registrar, University College, Singleton Park, Swansea (July 1).

Lecturer or Assistant Lecturer in Physics—The Registrar, The University, Sheffield (July 1).

Lecturer (preferably with a Ph.D. or equivalent experience in metallurgy, or in pure science if interest and experience are related to metals) in Metallurgy—The Registrar, The University, Sheffield (July 1).

Chair of Chemical Pathology in the University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (July 3).

Lecturer or Assistant Lecturer (botanist, with special experience in the taxonomy, morphology or genetics of horticultural plants) in the Department of Agricultural Botany—The Registrar (Room 22, O.R.B.), The University, Reading (July 3).

Lecturer in Zoology—The Secretary, The University, Aberdeen (July 6).

Lecturer (preferably qualified to teach ancient philosophy or the philosophy of science) in Logic—The Secretary of University Court, The University, Glasgow (July 8).

Research Student (with a good honours degree including chemistry) in Chemistry, to read for a higher degree working on problems related to cationic polymerization under Dr. P. H. Plesch—The Registrar, University College of North Staffordshire, Keele, Staffs (July 8).

Senior Lecturer or Lecturer in Hæmatology in the Department of Pathology, University College of the West Indies—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (July 8).

Professor of Philosophy at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 15).

Research Student (with an honours degree or equivalent qualification) in Electrodialysis—Prof. G. M. Burnett, Department of Chemistry, The University, Aberdeen (July 15).

Lecturer in the Department of Physiology—The Secretary, The University, Aberdeen (July 18).

Lecturer in Psychology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 28).

Chair of Chemistry in the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, July 30).

Senior Lecturer in Physiology at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 30).

Lecturer (preferably with experience in teaching, research and the design and construction of hydraulics works and apparatus) in Civil Engineering (Hydraulics) at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 4).

Assistant Experimental Officer (with experience in analytical chemistry), to work on protein and carbohydrate metabolism—The Secretary, Rowett Research Institute, Bucksburn, Aberdeen.

Assistant, Grade B (male), to teach Chemistry, Physics and some Mathematics to Ordinary level G.C.E. standard—The Principal, North-West Kent College of Technology, Miskin Road, Dartford, Kent.

Assistant Lecturer (preferably with an interest in fuel technology) in the Department of Chemical Technology—The Secretary, The Royal College of Science and Technology, George Street, Glasgow.

Laboratory Technician (preferably with biochemical experience, and A.I.M.L.T. or equivalent), for the Neonatal Unit at the Hammersmith Hospital, Ducane Road, London, W.12—The Secretary, Institute of Child Health, The Hospital for Sick Children, Great Ormond Street, London, W.C.1.

Lecturer (preferably with a higher degree and research experience in an industrial environment) in Metallurgy in the Department of Materials, to lecture at postgraduate level on the fundamental metallurgical aspects of technological processes—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

Master to teach Physics—The Headmaster, Plymouth College, Plymouth, Devon.

Reader (with a higher degree in electrical engineering and adequate research experience) in Electrical Engineering; and a Principal Lecturer (with a good honours degree in electrical engineering and practical experience in the electrical industry) in Electronics—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Research Assistant (preferably with a medical qualification) in the Clinical Bacteriology Laboratory—The Administrator and Secretary, University College Hospital, Gower Street, London, W.C.1.

Research Assistant (science graduate, with an interest in either biology or chemistry), for duties concerned with the investigation of immunological problems—The Regional Director, Blood Transfusion Service, Law Hospital, Carluke, Lanarkshire.

Scientific Officer or Senior Scientific Officer (suitably qualified honours graduate) in the Bacteriology Department, for work concerned with microbiology of the rumen—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/17.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Institute of Seaweed Research, Inveresk. Annual Report for 1960. Pp. 23. (Inveresk, Midlothian: Institute for Seaweed Research, 1961.) [44

The Scottish Field Studies Association. Annual Report, 1960. The Importance of Field Studies in Modern Education. By Paul F. Holmes. Garth House as a Centre for Ecological Botany. By Fred N. and Brenda D. Haynes. Pp. 27+4 plates. (Glasgow: Scottish Field Studies Association, 1961.) 2*s*. 6*d*. [44

Opinions of British Scientists of Home Office Control of Experiments on Animals. (Report addressed to the President, Animal Welfare Institute, New York, by Major C. W. Hume, M.C., Secretary-General of U.F.A.W.). Pp. 8. (London: Universities Federation for Animal Welfare, 1961.) [44

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Department of Scientific and Industrial Research: Building Research Station. National Building Studies. Research Paper No. 32: The Forces Applied to the Floor by the Foot in Walking. 1: Walking on a Level Surface. By Dr. F. C. Harper, W. J. Warlow, and B. L. Clarke. Pp. iv+28+8 plates. (London: H.M. Stationery Office, 1961.) 3*s*. 6*d*. net. [44

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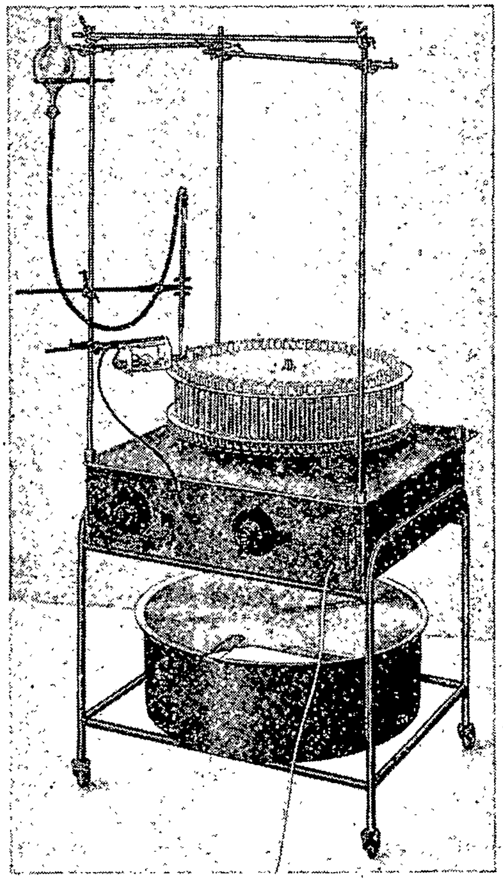

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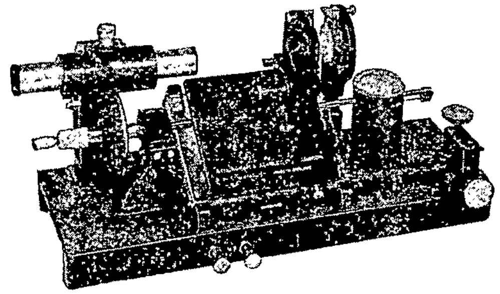

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SCIENTIFIC AND TECHNICAL DEVELOPMENT IN PAKISTAN

IN January 1960 the Government of Pakistan appointed a Scientific Commission, under the chairmanship of A. K. Khan, to consider how best scientific research in the country, at present disjointed and scattered, could be promoted and integrated; ways and means for providing adequate facilities to the research organizations of the country and of ensuring the utilization of the results of their researches for the overall development of the country; and ways and means of making scientific careers attractive and comparable in prospects, emoluments and status to other services of equal national importance. The Commission was assisted in its deliberations by Prof. A. V. Hill and Dr. L. A. Jordan, Dr. F. T. Rosser, of the National Research Council, Canada, Mr. F. E. Nicholls, of the Australian Commonwealth Scientific and Industrial Research Organization, and Dr. S. Komagata, formerly chairman of the Japanese Atomic Energy Institute. Apart from an introductory chapter reviewing the importance of science to under-developed countries and Islamic contributions to science, which gives a timely reminder of the outstanding contributions Muslims have made to science, the Commission's report* follows the pattern of numerous other reports in this field in other countries. Reviews of the development of scientific organizations and of the tasks and problems for science in Pakistan are followed by chapters on a National Science Council, the establishment of which is recommended, on the organization of defence research, the careers and status of scientists, utilization of the results of research, and on facilities and resources for research.

Generally speaking, the recommendations of the Commission are in line with the policies that have been adopted in Great Britain and in the United States, and with the recommendations of the recent report, *Strengthening Western Science*. It is recognized, for example, that in view of the rapid industrialization of Pakistan and the programme of general economic development envisaged in the Second Five-Year Plan, it is necessary to accelerate scientific research in certain fields that have lagged behind. Emphasis is placed on strengthening scientific effort in the universities and the assumption of greater financial responsibility by the Government. It is also recommended that more attention should be given to research ability and the needs of research in recruiting staff; Western experience would suggest that the Commission goes too far in recommending that the promotion of lecturers, readers and professors in the Science Department should depend on the quality of their research. It is now widely recognized that one weakness in British universities is due to the failure to pay sufficient attention to teaching and organizing ability, and to the practice of giving undue weight to the amount of published work in appointments to university posts.

The Commission recommends that universities should be primarily concerned with fundamental research, leaving applied research to the Government-sponsored research councils. It suggests, however, a mutual exchange of personnel between universities and research councils; such mobility of research workers, as well as of academic staff generally, might with advantage be more encouraged in Britain. The subjects noted as comparatively neglected in Pakistan's universities at present and requiring greater attention include the biological sciences, biophysics and biochemistry, geology, mining, astronomy, metallurgy and chemical engineering.

Of the administrative structure of Government departments, the Commission is highly critical. Apart from recommending considerable decentralization and study of the possibility of creating a separate Scientific Service, the Commission found considerable duplication of activities of the provincial and central Government departments, and that the administrative and executive heads of scientific departments were exercising control over teaching and research institutions which was detrimental to efficiency. Such teaching and research institutions should either be autonomous or linked up with universities. In particular, reconstitution of the Agricultural Research Council and the Medical Research Council is recommended; the Council of Scientific and Industrial Research should reorganize its laboratories and research division so as to conserve the limited scientific man-power available and should devote greater attention to major problems of consequence to the national economy, as well as initiate a co-ordinated programme for training research workers. The Atomic Energy Council, besides intensifying its own training programme, should establish an Institute of Nuclear Sciences and Technology. The Commission also advocates the formation by industries of co-operative research associations which should be subsidized by the Government.

The research organization visualized by the Commission, outside the universities and Government departments, comprises the five councils already mentioned, together with the Council for Works and Irrigation Research and a National Science Council with purely advisory and co-ordinating functions located in the President's Secretariat, and responsible either to the President or to a Minister designated by him. The Research Councils, which should be autonomous bodies, attached to the respective Ministries of the central Government, should establish research institutes and laboratories in different parts of the country, while defence research should be entrusted to an independent organization under the Ministry of Defence, headed by a scientific adviser, and with a Defence Science Liaison Committee with the chairman of the National Science Council as chairman to maintain close liaison with the National Science Council. The Defence Science Organization should be entitled to place contracts with any of the Research Councils for the solution of specific problems.

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* Government of Pakistan: Ministry of Industries. Report of the Scientific Commission of Pakistan. Pp. iv+102. (Karachi: Ministry of Industries, 1960.)

As regards the programme for science in Pakistan, priority is given to the food problem; but this is not conceived simply in terms of extending and intensifying agriculture. It embraces also research on the utilization of plant material and on improving the nutritional value of food crops. In the field of irrigation, problems concerned with waterlogging, subsoil structure, soil mechanics, rate of water-flow, de-silting, dredging and bed-lining of canals call for intensive research, and there are the special problems of de-salting sea-water and conservation of subsoil water resources involved in irrigating the arid zones of West Pakistan. Cheap electric power, however, is a pre-requisite for the development of agriculture and irrigation, as of industry and communications, and the Commission recommends raising the consumption per head of electricity within the next thirty years from 30 to 400 units, a figure which is still only about half the present consumption in Japan. The upgrading of mineral ores, the various aspects of fibre technology and the utilization of indigenous raw materials all offer great opportunities for scientific and technological research to contribute to the development of Pakistan. Attention is also directed to the serious problems in the field of public health, including the eradication of epidemics and control of preventable diseases, the investigation of diseases peculiar to Pakistan, to family planning and birth control, and to nutrition. These may require tackling in special institutions, and finally the Commission recommends the establishment, attached to a university, of an institution where modern statistical methods of sampling can be investigated.

The Commission recognizes that utilization of the results of research requires special attention, and in agriculture it makes a number of specific suggestions for establishing co-operation between research workers and those engaged in extension services and securing the co-operation of farmers. As regards industrial development, it recommends that processes developed by the Council of Scientific and Industrial Research which call for heavy investment should be handled by the Pakistan Industrial Development Corporation, and that where exploitation is undertaken by private industrialists, foreign exchange should be released for import of the machinery and raw material required. It also recommends the organization by the Council of Scientific and Industrial Research of an Industrial Extension Service with the view of publishing and disseminating information on the latest techniques and processes.

It is interesting to read these specific recommendations for Pakistan in the light of the papers on science and the new States which occupy the greater part of the February 1961 issue of the *Bulletin of the Atomic Scientists*, more especially those by Principal W. A. Lewis and Prof. E. Shils. Principal Lewis points out that the primary need of the new States is not the development themselves of new knowledge but the application of existing knowledge to their problems, and especially the devising of new technologies which will make man-power much more efficient without much capital—a point on which both Lord Fleck and Prof. P. M. S. Blackett have already laid some stress. Principal Lewis devotes most of his paper to this question of man-power, stressing the critical importance of secondary education and the need for political leaders to give priority to economic development and the factors which promote it. Although he writes rather pessimistically about this point in general, the fact that the Pakistan Government has already indicated its intention of implementing the recommendations of the Science Commission's report shows that in that country at least there is the will to seek new ways of progress.

On the financial aspect Principal Lewis has little to add, but stresses once more the importance of political stability, in order to attract technical assistance and to use it wisely and productively. Prof. Shils, discussing scientific development in the new States, is concerned more particularly with the conditions required to foster research and training in science and technology, and likewise stresses the importance of secondary education, as well as the major dependence of technological and scientific research in such States on Government policy and support. Of specific fields of research he only mentions the importance of the prospecting and appraisal of resources, and of work directed to the adaptation to new circumstances of industrial or agricultural processes already used in other countries. He notes, however, the impracticability of planning fundamental research, or even technological research, from a central position, and his paper largely discusses the policy and organization required to create the appropriate conditions of scientific work in laboratories and universities. So far as organization is concerned, his suggestions fully support the recommendations in the report from the Scientific Commission of Pakistan, and that is also largely true of the recommendations relating to the careers and status of scientists and those facilities and resources for research contained in the fourth and eighth chapters of the Commission's report. One comment might here be made in respect of both Principal Lewis's and Prof. Shils's observations about fundamental research: while undoubtedly it would be unwise for the new States to devote more than a minor effort to such research, some such research has its place both in attracting creative workers to a scientific career and in providing the stimulus of participating in scientific advance generally. Such factors are sometimes more important than remuneration, on which the Commission reasonably enough lays a good deal of stress. Conditions in Pakistan are, in fact, so poor that the Commission makes detailed recommendations for the creation of a Pakistan Science Service, and specific salary scales are recommended for scientists and technicians employed by the Research Councils. There are recommendations regarding the ratio of scientific to supporting staff which, while intended to increase the proportion of scientific staff, do not seem to take account of the number of supporting technicians required to make effective use of the scientists and technologists. Some attention is given to the question of status, and Government support of scientific societies is recommended; but generally the report is a good way behind the recommendations of the Barlow Report on scientific staff in Britain published in 1945.

To overcome the existing shortage of scientific and technical man-power, the Commission advocates a massive training programme to be entrusted to the National Science Council, which will be responsible for co-ordinating the requirements of the various Research Councils. The programme contemplates sending abroad at least a hundred talented science graduates each year for intensive training during the next five years, the establishment of two big training centres, one in East Pakistan and one in West Pakistan, for training technicians in glass-blowing, precision tool-making, high-vacuum maintenance and the like, while import duties on scientific instruments and apparatus, laboratory chemicals and furnishings should be waived until manufacturing capacity is developed within the country. Similarly, to facilitate the import of scientific books and technical periodicals and the equipment required to maintain scientific laboratories, the universities, selected scientific departments of Government and the Research Councils should be granted an 'individual general licence' which they could operate freely. Establishment of branches at Lahore and Dacca of the Pakistan National Scientific and Technical Documentation Centre is recommended, while the Commission also recognizes the importance of training librarians and offering them an attractive career.

The Commission has clearly taken a most comprehensive view of Pakistan's needs, and stresses some factors to which little attention has yet been given in Government quarters in Britain. It visualizes the allocation to this scientific effort of at least 2·5 per cent of the national budget. Principal Lewis estimates that most under-developed countries need to provide about 25 per cent of the national output to promote economic growth at the rate of about 4 per cent per annum, of which about half is required for capital formation and half to supply an adequate framework of public services—3 per cent for education, 2 per cent for public health, 3 per cent for communications, agriculture and geology, and about 4 per cent for general administration and welfare. Actually, in most such countries only 15 per cent of the national income is available for the public services and capital formation together; but Principal Lewis takes an optimistic view of the prospect of providing the necessary saving from taxation.

However that may be, the report of the Scientific Commission of Pakistan shows as clearly as the papers in the *Atomic Scientists Bulletin* how large a contribution science can make to the development of new States, and the opportunities for the scientist or technologist to assist the process. These papers add little to our knowledge of the conditions in which technical assistance can be most effective; but in emphasizing the conditions in which scientific work can flourish, they point to the value of the interest and support of the scientific community. When Prof. Blackett writes of the need for a greatly increased international effort and the co-operation of such organizations as the World Health Organization and the Food and Agriculture Organization, or of the primary need to understand the problems and difficulties of the emergent countries and appeals for more men and money from the more affluent countries, he is unlikely to be overlooking the way in which the scientists and technologists of Britain and other advanced countries can help to create the conditions in which such assistance is effectively used. The association with the Scientific Commission of Pakistan of Prof. A. V. Hill and Dr. L. A. Jordan from Britain, of Dr. F. T. Rosser from Canada and Mr. F. E. Nicholls from Australia, illustrates one way in which help is given. It can be given all the time, however, not only in response to such specific requests, but also in the continuing contacts between scientists and technologists in Britain with those of the under-developed countries at present in British universities and colleges of technology—now perhaps some 50,000—in the interchange of visits in many different ways, in the Overseas Scientific Service and the expanding programmes of the Commonwealth Education Conferences. These opportunities were emphasized by several speakers in a debate in the House of Lords on February 22. Only perhaps as such opportunities are used to the full can the vital task of educating the Governments of these countries to the full understanding of the conditions in which science and technology flourish be fully discharged. The report of such a Commission as the Scientific Commission of Pakistan is only a beginning. Its implementation requires public understanding as well as the co-operation of scientist, technologist and administrator in countries where such collaboration has scarcely begun; and the declaration, unanimously accepted by the Rehovoth Conference last summer, which Abba Eban describes in his article "Science and the New States" in the *Bulletin of the Atomic Scientists*, fittingly urges the scientists of advanced countries to become more pre-occupied with the problems facing newly developing communities, and to advise the Governments of new States to include the establishment of a scientific tradition and practice among their early targets.

ATOMS AND ELECTRONS

Quantum Theory of Atomic Structure

By Prof. John C. Slater. (International Series in Pure and Applied Physics.) Vol. 1. Pp. xii+502. 85*s*. 6*d*. Vol. 2. Pp. ix+439. 101*s*. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1960.)

IN 1933, only seven years after the introduction of wave mechanics, Slater and Frank published their *Introduction to Atomic Physics*. They allotted 200 pages to atomic structure. But in 1951, when wave mechanics was just 25 years old, this material had grown to a complete book, *Quantum Theory of Matter*, with 500 pages. To-day, in 1961, Prof. Slater takes the first half of this second book, and expands it into two full volumes totalling nearly 1,000 pages. This is a striking indication of the almost catastrophic way in which our knowledge of this field has developed.

But the field itself has spread. Atomic theory is no longer the province of the spectroscopist measuring line frequencies and line widths. It plays a fundamental part in the totally new field of molecular structure, and its ideas have taken root in much of nuclear structure and the most exciting of all modern branches of theoretical physics—the solid state.

The subject is ripe for a major exposition. It seems likely that most of the main results and theories are now known. The advent of electronic computers has already influenced techniques. Much remains to be studied, but the methods of doing this are now fairly clear. It is entirely appropriate that Prof. Slater should write this monumental work. For during the past fifteen years his group at the Massachusetts Institute of Technology has studied atoms, molecules and solids with great success. We are now promised further books on these other topics. If they match the present ones in excellence they will be very welcome indeed.

Here is effectively everything that matters in the theory of atomic structure. It is set out in Slater's lively style (sometimes almost too colloquial), and with very great assurance. Reading these volumes is like watching a first-class chess player march irresistibly to his object. There is complete mastery of the material, much of which is his own, and a complete certainty about its ordering.

The first volume starts with 160 pages recapitulating elementary wave mechanics. Despite the author's claim, I do not feel that this is suitable for a beginner: it is inevitably compressed. But it is excellent for a student who already has some knowledge. Then follow accounts of the hydrogen atom, self-consistent-field, angular momentum, multiplet structure, with application in the iron group, and a series of some twenty appendixes elaborating points inadequately covered before. This material is enough to understand the main lines of thought, but not sufficient to start research.

Volume 2 starts with Hartree–Fock theory, and then goes on to group theory, angular momentum operators, Racah's method for complex spectra, the Dirac theory, magnetic properties of complex atoms, transition probabilities and hyperfine structure. It contains a wonderful set of tables, and carries the subject right up to the present day. Both volumes are enriched with a series of bibliographies that greatly enhance their value.

Comparison is inevitable with Condon and Shortley's *Theory of Atomic Spectra*. In Slater there is much less direct comparison with experimental data, and more 'drive' towards the basic theory: there is almost nothing about *jj*-coupling (this is a pity) and isotope effects, or about polarizability and Stark effects. But there is the first full-scale development of Racah's work, the use of projection operators, the Fermi hole, nuclear quadruple effects, none of which is found in Condon and Shortley's book. Numerical techniques are also more advanced, and the whole outlook is more modern.

No one can expect to be entirely satisfied. But I found only one minor mistake. I could have wished for more reference to the Thomas–Fermi statistical method, and a better account of Hund's rules (Hund does not get quite his share of credit). I also wish that the Rydberg unit of energy had been replaced by the atomic unit, so that a self-consistent set of units could be used. But these are relatively trivial. Here—without any doubt at all—is a definitive account of atomic structure. It is a magnificent work, which no one but Slater could have written: and it is beautifully published.

C. A. COULSON

PROBLEMS OF ECONOMIC AND SOCIAL DEVELOPMENT

Jamaica, 1830–1930

A Study in Economic Growth. By Gisela Eisner. Pp. xxiii+399. (Manchester: The University Press, 1961.) 50*s*. net.

THE publication of this work can be welcomed without reservation by all who are interested in the problem of economic and social development. The investigations on which it is based were initiated and guided by Dr. Arthur Lewis, when professor of economics in the University of Manchester, and it thus provides evidence of the growing sense of responsibility of the new West Indian middle classes for the welfare of their communities. The project is as ably conceived as it is brilliantly executed.

Our knowledge of economic growth and social change has hitherto been severely restricted by the scarcity of accurate studies of the history of the societies which have been subject to the most rapid development over the past century. Here is one more, and a very good one, to add to our small collection. The author has extended her work over a wide field; she has thus been able to show how economic growth and organization have been interrelated with the social structure and social history of Jamaica. The result is to give the book a much wider interest than a more restricted study of economic statistics and economic trends would possess.

It would have been even more helpful if the period covered could have been extended to 1943, a year in which an elaborate census provided much material which could have been used for purposes of general comparison with the situation which prevails to-day. Even though the data were somewhat affected at that time by the impact of the Second World War, this was not so important as might be supposed; the influence on economic and social life of the post-war period of planned economic development has been made more profound. It is probable, therefore, that 1943 will in the future be regarded as the end of the old era of colonial policy, rather than the start of the new period of democratic self-government. One thus hopes that Mrs. Eisner's work will be used as the basis for further studies by Jamaican economists. It will serve this purpose admirably.

T. S. Simey

ORGANOMETALLIC CHEMISTRY

Organometallic Chemistry

Edited by H. Zeiss. (American Chemical Society Monograph Series, No. 147.) Pp. xiv+549. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1950.) 140*s*. net.

THE enormous output of work within the broad definition of organometallic chemistry fully justifies the production of this monograph.

The ten chapters, occupying 528 pages, consist of "a series of research subjects which are under active investigation—at the present time—by their respective authors". The book is intended primarily for graduates and the more advanced chemist. The subjects and authors of the chapters are: carbon-metal bonding, James W. Richardson; benzyne chemistry, Rolf Huisgen; vinyl-metallics, H. D. Kaesz and F. G. A. Stone; organoboranes, Herbert C. Brown; organo-aluminium compounds, Karl Ziegler; organo-silylmetallic chemistry, Henry Gilman and Hans J. S. Winkler; cyclopentadienyl metal compounds, P. L. Pauson; arene complexes of the transition metals, H. Zeiss; transition metal alkyls and aryls, G. E. Coates and F. Glockling; metal carbonyls and related compounds, J. Chatt, P. L. Pauson and L. M. Venanzi.

In Chapter 1 a modern qualitative treatment by molecular orbitals of the carbon–metal bond is given. An introductory molecular orbital description of the HeH^+ molecule is extended rather rapidly to some specific metal cyanide, carbonyl and cyclopentadienyl complexes. This specialization is intended to "demonstrate and catalogue the variety of interactions present in metal–carbon bonds in detail" rather than review a broad range of compounds. A final discussion of the stability of alkyl and aryl transition metal complexes is complementary to the theoretical discussion in Chapter 9. The thorough review of the very recent benzyne chemistry is decidedly more 'organo' than 'metallic' and its inclusion in this monograph emphasizes the wide scope of organometallic chemistry. A competent systematic coverage of vinyl metallic chemistry complementary to recent reviews is given. The infra-red spectra of a number of vinyl substituted compounds are reproduced and the group characteristic patterns clearly shown.

The chemistry of organoboranes has also been reviewed recently and therefore the emphasis in this work is laid on the most up-to-date advances, and on new synthetic procedures. The hydroboration reaction is discussed in considerable detail and the great familiarity of the author with this field is evident.

Nearly 400 references including a large number to patent literature demonstrate the importance and extent of organo-aluminium compounds. As would be expected, there is an authoritative coverage of the synthesis and classification of these complexes by K. Ziegler. This is followed by a discussion of their molecular structure and properties. The necessarily limited size of one chapter has resulted in rather a compromise between useful detail and generalizations.

Organosilylmetallic chemistry is a field which surely must expand rapidly in the next few years, particularly where transition metal chemistry is concerned. The few organosilyl transition metal complexes which have already been reported are, however, not mentioned in this review. Detailed preparations of the alkali-metal derivatives are given and their reactions are described. This chapter does not make very exciting reading. The division of the so closely interrelated fields of aromatic, carbonyl, and alkyl and aryl transition metal complexes into four chapters, under different authorship, has resulted in a good deal of confusion as to who should write what about which. In the attempt to avoid duplication with recent reviews and with subsequent chapters, the topic of cyclopentadienyls is given a fragmentary treatment. The aromatic ring substitution reactions are, however, summarized and some useful reaction schemes are given.

A historical approach to arene complexes is developed into a concise survey of the known examples which are given in a seven-page table. Non-benzenoid arene metal complexes are briefly considered. The acetylenic synthesis by cyclic condensation of arene compounds is discussed, as is also the mechanism of the Job–Cassels reaction.

Chapter 9 on transition metal alkyls and aryls is largely culled from the book on *Organometallic Chemistry* which is written by one of the authors of this review. Insufficient progress has been made in this field in the few months separating the appearance of these two books to warrant this duplication. The topic is well and more extensively covered in the original book.

Finally, Chapter 10 consists of a rather limited review of metal carbonyl and related compounds. The most recent and important developments are, however, considered and more than 300 references are given.

The book has an author- and subject-index and is excellently produced. On the whole a useful book but unfortunately rather expensive.

M. L. H. Green

THEORIES OF CARCINOGENESIS

Carcinogenesis

By I. Hieger. Pp. xi+138. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1961.) 35*s*.

DR. HIEGER'S principal theme is "the putting into perspective of the theories which have been advanced to interpret the process whereby normal cells and tissues are transformed into cancer". Except for one chapter in which the main types of cancer-producing agents are described, and their effects on various tissues illustrated by experimental records, the book is devoted to the consideration of the most important theories which have been advanced to explain the carcinogenic process in general. It is in the bringing together in one place of critical accounts of these various theories that the main value of the book lies.

Hieger analyses them with refreshing candour and no undue respect for persons. His criticisms merit serious attention. The evidence on which cancer theorists rely is often equivocal and always incomplete, but it must be remembered that criticism necessarily rests on a re-interpretation of the same data. For example, Hieger uses the "multicentric view" of carcinogenesis to support his attack on the somatic mutation theory. He argues that the observed multicentric genesis of tumours in a region of tissue undergoing carcinogenic stimulation requires that all or almost all the cells in the region should mutate, and that this is numerically impossible given the known mutagenic capacity of effective doses of carcinogens. But it is pre-cancerous changes which are regional, that is, appear to affect all cells in a treated region (as Hieger points out in one place, but ignores in his argument); tumours arise at relatively few points within the region. The number of cells in which the definitive neoplastic change (whatever that is) occurs may be only a minute fraction of those at risk.

One might pick out other instances of this kind, but they would not invalidate Hieger's main theme—the unsatisfactory state of cancer theory to-day. That is undeniable, and, indeed, to survey the divergence and mutual incompatibility of rival theories of carcinogenesis is a chastening experience. But I doubt if it is reasonable or wise to demand, at this stage, that they should be consistent and generally applicable. Most fruitful hypotheses have started with a strictly limited application; inconsistencies were disregarded for a time and eventually explained, and in the meantime experiment and speculation were stimulated.

A frequent complaint of Hieger's is that theories of carcinogenesis are "tautologous". For example, he quotes the following passage of Burnet's: "In many ways it (carcinogenesis) resembles the evolutionary process among free-living organisms. Change in Nature results from the appearance of mutations, from their phenotypic expression in various combinations, and from the selective action of environmental factors in ensuring the survival of the fittest. In the body the cancer cell has an undoubted short-term survival advantage over normal cells and in a sense we can look at it as representing a clone, which, by processes formally equivalent to mutation, has developed a gross advantage in proliferation within the existent internal environment of the body". Hieger's comment is: ". . . Sir Macfarlane has only said: 'An advantageous mutation confers an advantage'—a tautology which does not assist us in understanding carcinogenesis". I do not think this type of criticism is well directed. Opinions differ as to whether Burnet has drawn a valid comparison; but it must be remembered that what he and some other theorists are trying to do is to show the formal equivalence of carcinogenesis to other processes in Nature which are better understood, or for which there are, at any rate, generally accepted explanations covering wide fields of fact. They attempt this by making assumptions, in line with other evidence, from which the known facts of carcinogenesis can be deduced. The steps of such arguments are certainly tautologous, but only in the sense that all mathematics is tautology (as some in fact maintain).

There is much more to be learned from these theories than appears from a catalogue of their inadequacies, real or apparent, and I hope that the readers of this book will not be prevented by the author's scepticism from studying them.

When reading or listening to a severe critic of the theories of others it is natural to look forward to the moment when he will introduce a theory of his own, in order that we may give him a taste of his own medicine. Dr. Hieger does not fall into the trap. Since he believes that all present theories of carcinogenesis are inadequate he is surely wise in not adding to their number. He indicates, however, what he regards as the most hopeful directions for future work, some of which have received less attention than they deserve. In his last paragraph he states his opinion that "the most hopeful approaches to the solution have not been from a theoretical direction but by a more modest and empirical route". His own important contributions to the study of carcinogenesis have been made chiefly by the latter method, and many experimental pathologists, whether or not they endorse his strictures, will agree with his conclusion.

M. H. Salaman

THE PROBLEMS OF AGEING

Man against Aging

By Dr. Robert S. de Ropp. Pp. v+310. (London: Victor Gollancz, Ltd., 1961.) 21*s.* net.

One of the results of medical advances in the past hundred years has been an increasing proportion of elderly people in the population, and the public is becoming increasingly aware of and interested in the new medical and social problems which these changes are creating. *Man against Aging* is intended to provide them with the sort of information about old age which they have grown accustomed to receiving in other branches of medicine.

To get the basic information and attitude over to lay readers requires special techniques and unusual gifts from an author and perhaps modified standards of criticism from a reviewer. The start of this book is not at all encouraging, for it contains a collection of the various fanciful notions that have accumulated over the centuries, interspersed with vaguely salacious remarks and unwarranted, and irrelevant, side-cracks at the standards of biological science in communist countries. One assumes that this is intended to persuade someone who picks up the book to go on reading, and having got it all off his chest the author settles down to a very reasonably accurate assessment of the present state of our knowledge about the ageing process, our difficulties in defining it, how it affects different forms of animal life and different parts of the body, and how we are trying to analyse the process and decide whether there is a specific cause of ageing or not. After dealing with these gerontological problems the author goes on to the geriatric aspects and describes the common serious diseases of the latter part of life. Once again his reading of the specialist literature seems to have been wide and his assessment not at all extravagant. The last part of the book is entitled "The Art of Aging". This part, which boils down to a recommendation to old people to keep on working, is less successful perhaps because the social science side of gerontology is less well developed than the biological side.

Once one has discounted the jargon and lavish use of adjectives that it is apparently necessary to introduce into any popularized account—the incredibly complex, the ceaseless stream, the wealth of vital chemicals and so on—one can recognize in much of this book a successful attempt to make the reader aware of the problem and our present position of ignorance in ways which can only be helpful.

P. L. Krohn

CARL NÄGELI AND THE FINE STRUCTURE OF LIVING MATTER*

By Dr. J. S. WILKIE
Department of the History and Philosophy of Science, University College, London

THE status of Nägeli as the founder of a new branch of biological science has been very generally recognized. Among his contemporaries, we find Sachs, Hofmeister and Pfeffer speaking in terms of the highest admiration of his work in this field; and among workers in the present century Seifriz, Meyer and Mark, Preston and particularly Frey-Wyssling, seem to be in no doubt that he was the first to obtain results of value in the study of fine structure.

Prof. A. F. Huxley, in his inaugural lecture delivered at University College, London, has pointed out that the work of Brücke on the fine structure of muscle has been confirmed in important respects by recent studies. Brücke was a contemporary of Nägeli, who refers to his work on muscle, and if the title of 'founder' were to be awarded simply for brilliance or originality it would be difficult to decide to which of the two it should properly be given. By this criterion there might, indeed, be others entitled to claim the title. However, just as we rightly award to Darwin the honours due to the founder of evolutionary studies, because of the extent of his labours and the cogency of his arguments, so do we, I think, appropriately assign to Nägeli the status of founder and originator of studies in fine structure.

I have for some time been engaged in studies intended to supply answers for the following two questions: (1) What exactly was the nature of the original contribution to biology made by Nägeli ? (2) By what processes of observation, experiment and ratiocination did he produce the most original part of his work ? To such historical questions it is scarcely possible to offer final and complete answers, and certainly the answers which I shall suggest here are not intended to be more than tentative.

It seems reasonably certain that Nägeli's work in this field was original in the three following respects:

(1) He appears to have been the first to appreciate the great and universal importance of what are now called high polymers—their importance, that is, in the fine structure of living things. Pfeffer[1] goes so far as to assert that Nägeli employed the concept of higher-order molecules, that is, of polymers, at a time when it was 'unknown to chemistry' ("zu einer Zeit . . . als die Chemie meines Wissens Molecülverbindungen als nähere Körperbestandtheile noch nicht kannte"); this is not strictly correct, for the possible existence of higher-order molecules formed by the union of many similar or identical unit-molecules was suggested by Berzelius in 1827 [2]. It seems, however, only reasonable to accept the testimony of Pfeffer that the notion was not widely known or widely accepted among chemists at the time when it was successfully applied by Nägeli. It is in any event certain that Nägeli owed nothing to Graham in this respect, for Graham's suggestion that "colloid molecules may be constituted by the grouping together of a number of smaller crystalloid molecules" appeared in a paper of 1862 [3], whereas Nägeli had formulated his hypothesis fully in his monograph on starch-grains, published in 1858 [4]. It seems to me probable that the idea of polymerization was in the air, as a mere notion without much empirical substance, during the 'fifties; but, if so, it is certain that Nägeli was among the first to make use of it and to give it the empirical substance which it lacked.

(2) Nägeli seems to have been the first to appreciate the presence and importance of a variety of cohesive forces in the structure of living matter at the molecular level. That this idea was in itself very original I cannot assert, though I believe it was; but the empirical observations by which Nägeli sought to establish it, and the arguments drawn from these observations are, I think, among the most original, as well as the most lasting, of his contributions. I describe this as a 'lasting contribution' because it seems to be a vague perception of what is more precisely formulated in, for example, the theory of *Haftpunkte*[5].

(3) A final original and lasting contribution is his discovery (for here we may use this dangerous word) of microcrystals or submicroscopic regions of lattice order in starch-grains and in the walls of plant-cells, together with the extensive development of his theory of the universal presence of such structures in plant-cell walls.

For the sake of completeness it will be as well to quote here Nägeli's formulation of his set of hypotheses concerning the structure of 'organized materials', by which he meant the structured parts of organisms, as contrasted with, for example, blood-plasma or cell-sap. In this passage[6], which belongs to the middle period of his activity, he is still using an old terminology, in which it was usual to call molecules 'atoms', so that he is able to use the term 'molecule' to designate his polymer-particles. This usage will be more readily grasped if it is remembered that the 'atoms' mentioned in this passage are 'atoms of starch', or 'atoms of cellulose', or even 'atoms of albuminous material'.

"Organized materials consist of crystalline, birefringent molecules (themselves consisting of very many atoms), which lie freely, but with a definite regular arrangement, alongside one another. As a consequence of preponderating attraction, each is surrounded in the moist state by an envelope of water; in the dry state they touch one another. Hence there are in organized materials two kinds of cohesive force: one unites the atoms to form molecules, in the same manner as they unite in general to form crystals; the other unites the molecules."

In his later writings, Nägeli calls his crystalline particles *Micelle*, or *Micellen* (for, *in unkonsequenter Weise*, as Prof. Frey-Wyssling says, he used both forms of the plural), a word which has passed into English as (still considering the plural) micelles or micellæ.

Fig. 1 shows, above, one of the two figures given by Nägeli to illustrate his concept of micellar structure; and, below, a diagram published in 1929 by the late Prof. Seifriz (the illustration is made from the diagrams as reproduced in Prof. Frey-Wyssling's

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* Substance of a lecture in a series, "Case Histories in Biological Discovery", delivered during Trinity Term in the University of Oxford, and arranged by Dr. A. C. Crombie and H. B. Havré.

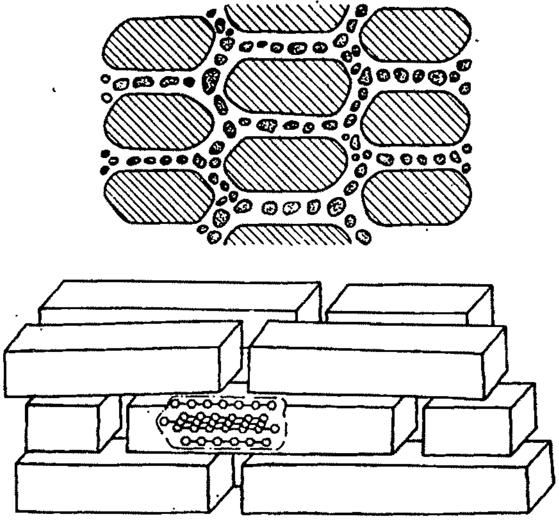

Fig. 1. Former conception of the micellar structure. Above, from Nägeli and Schwendener (1877); below, from Seifriz (1929) and K. H. Meyer (1930). (Above, reproduced by permission of Elsevier Publishing Co. from Frey-Wyssling, *Submicroscopic Morphology of Protoplasm*, Fig. 59; and below, by permission of *The American Naturalist*)

book, *Submicroscopic Morphology of Protoplasm*, of 1953, by kind permission of the author and of the Elsevier Publishing Co., of Amsterdam).

There is some superficial resemblance in the diagrams, but in fact the concepts represented are much nearer one another than are the diagrams: indeed, the concepts are virtually identical.

In the first place, Nägeli did not give a figure to illustrate his general notion, so we have to be content with one representing a particular case. Between the larger blocks, representing the micellæ, where in the general case there is simply water or some dilute aqueous solution, we see smaller blocks representing some incrusting material, such as lignin. To obtain a representation of the general case, we have to think these away ; we then have only micellæ and water, as in Seifriz's diagram. Then, Seifriz has represented the constituent molecules of the micella, which should be shown in Nägeli's figure, but are not.

Seifriz's diagram[7] was drawn as an interpretation of results obtained in X-ray analysis by K. H. Meyer and H. Mark[8], and the diagram was accepted by Meyer as a correct interpretation[9]. Thus we find micellar order conceived in exactly the same way by Nägeli and by Seifriz and Meyer. Nägeli's figure was published in 1877; but the concept it illustrates was virtually perfected in 1858, and completely formed in 1863. This is sufficiently remarkable, even though more recent work has shown that the Seifriz diagram is a most inadequate representation of what seems to be the actual structure of cellulose. But this is not the end of the matter.

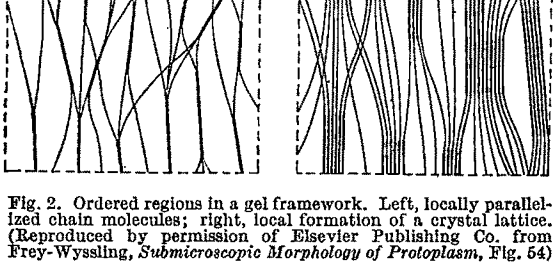

Fig. 2. Ordered regions in a gel framework. Left, locally parallelized chain molecules; right, local formation of a crystal lattice. (Reproduced by permission of Elsevier Publishing Co. from Frey-Wyssling, *Submicroscopic Morphology of Protoplasm*, Fig. 54)

Nägeli's concept is, of course, intended to have a much wider generality than Seifriz's model, which was suggested only for the fine structure of natural cellulose; but Nägeli was, of course, as a botanist, particularly interested in the structure of cellulose, and his idea of micellar order was undoubtedly intended to explain, also, the structure of this substance as it occurs in plant cell-walls; so let us consider the further development of ideas on this structure.

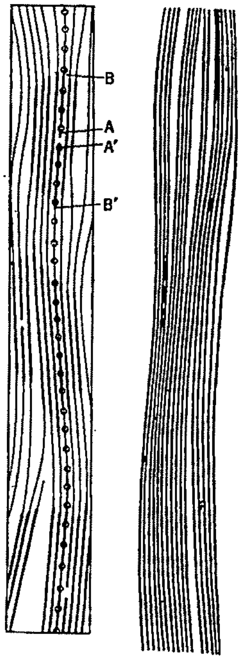

Fig. 3. Diagrammatic representation of the micellar structure of cellulose. Right, as suggested by K. H. Meyer. Left, micellar structure from primary valency chains (Kratky and Frey-Wyssling). The successive molecules, marked by circles, behave as a single molecule traversing the whole structure, since the ends, *A* and *A'*, lie within a crystalline region (thick lines). (Reproduced by permission of Interscience Publishers, Inc., of New York, from K. H. Meyer, *Natural and Synthetic High Polymers*, Figs. 87*a* and 87*b*)

Seifriz, in making his diagram, had neglected an argument already propounded by Sponsler and Dore in 1926 [10]. Sponsler supposes that the polymer chains in plant-fibres must be of very great length, for one of the most striking features of plant-fibres is their very great tensile strength. This argument from tensile strength to continuity of polymer chain soon became widely known and generally accepted as valid. Thus later workers modified the Nägeli–Seifriz diagram (at least in its application to the fine structure of fibres) in various ways, to make it more consistent with the known facts of tensile strength of plant-fibres ; though there were other reasons for modifying the original 'bricks in a wall' model, for it seemed that the length of the micellæ must be about 600 Å., the minimal length given by X-ray analysis, whereas Staudinger obtained values for the molecular weight of natural cellulose which would suggest a chain-length of 10,000 Å. It was suggested that one chain might belong to many micellæ. The model thus arrived at is illustrated in Fig. 2, also derived from a figure in Prof. Frey-Wyssling's book.

Although these drawings were prepared specially for the book published in 1953, they represent suggestions made earlier. On the left is a figure showing the fine structure proposed by Herrmann, Gerngross and Abitz[11] for gelatine, and on the right the new model for cellulose suggested by Prof. Frey-Wyssling[12].

It will be seen that this model satisfies both the requirement of micellæ (localized regions of crystal-order) and that of very long polymer chains. The chains lie parallel in some regions and are elsewhere arranged loosely. Where they are parallel they give a lattice with the optical properties of a crystal.

This notion of the fine structure of fibres was found unacceptable by K. H. Meyer[13], who suggested that the regions of lattice order were themselves continuous. "It seems more correct," he writes, "to represent the micellar structure as in Fig. 87*b* [on the right of Fig. 3 of this article], where no limit is set to the crystalline regions in the direction parallel to the fibre-axis."

The figure on the left shows a slight modification of the Frey-Wyssling model, a modification suggested by Kratky in 1938[14]. Fig. 4 shows Meyer's 'micellar ropes' in cross-section. The diagonal strokes represent the position of the 'glucose rings' (in the cellulose molecule), the regular arrangement of which gives the crystal lattice. This notion of micellar ropes is accepted by Prof. R. D. Preston, in his elegant monograph, *The Molecular Architecture of the Plant Cell Wall*, of 1952. Fig. 5 shows an electron-micrograph of a layer of the cell-wall of the alga *Cladophora rupestris* showing just such ropes as Meyer had postulated. Prof. Preston has kindly supplied me with this micrograph.

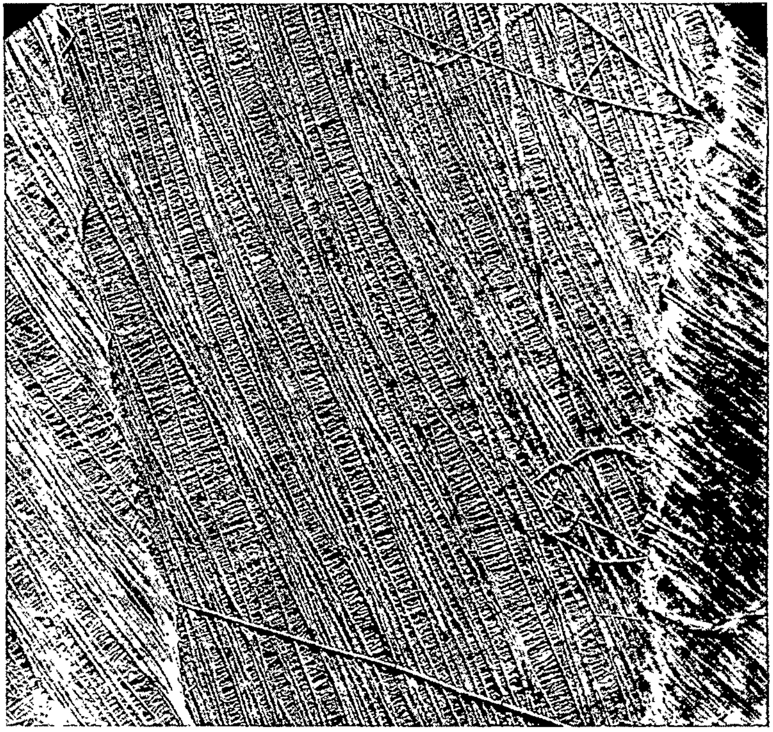

Fig. 5. Electron micrograph of inner-wall lamellæ of *Cladophora rupestris* viewed from outside. Palladium-gold shadowed. Top to bottom is direction of cell axis. (× 24,000.) (From V. Frei and R. D. Preston, *Proc. Roy. Soc.*, *B*, **154**, 70; 1961)

We seem to have come a long way from Nägeli's model. In fact, however, this development of ideas took place in Nägeli's mind between 1877 and 1879, for in 1879[15] he himself suggested that the tensile strength of plant-fibres requires a modification of the model of 1877, and pointed out that what is required is that the micellæ should "grow together" end to end, so as to form continuous strands.

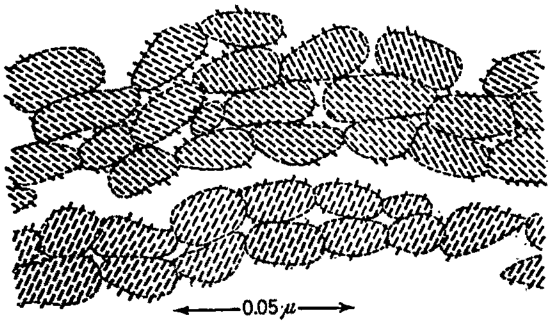

Fig. 4. Part of a schematic cross-section of two successive lamellæ of the cell-wall of a cellulose fibre. The strokes indicate the position of the glucose rings; the scale refers to the micellar ropes (the glucose rings are about four times too large). The micelles should therefore contain more glucose units. The spaces contain water, air or cementing materials. (Reproduced by permission of Interscience Publishers, Inc., of New York, from K. H. Meyer, *Natural and Synthetic High Polymers*, Fig. 90)

This must serve as an illustration of what I may call the 'predictive force' of Nägeli's ideas. It is clear that he must have obtained a true insight into the structure of the objects he was examining; for his predictions, if we may so call them, are too elaborate to be the results of mere luck. What then was his method? How did he arrive at these results?

It is often supposed that Nägeli's principal tool was the polarizing microscope; and if this had been the case it might perhaps be argued that the model of discrete crystallites surrounded by envelopes of water would be the simplest and most obvious for the observed phenomena. A starch-grain between crossed nicols shows the spherulite cross characteristic of a spherical body composed of radially arranged crystallites. Dry starch absorbs water, and in certain reagents starch-grains swell very greatly without losing their shape. Thus a system of radially placed crystallites with interspaces containing water seems, perhaps, an almost inescapable proximate hypothesis for the structure of the starch-grain. When it is added that cell-walls are also optically anisotropic and liable to swelling, the initial stage of generalization is also given.

The truth is, however, that Nägeli had completed his model for the fine structure of starch-grains before he made any extensive use of the polariscope. The model suggested in the monograph of 1858 is exactly that represented by the figure in my Fig. 1 (even the encrusting substance is here appropriate; for Nägeli knew that there are two substances in a starch-grain, though he incorrectly identified the less soluble as cellulose). Indeed, the figure (Fig. 1, here) is particularly apt to illustrate the concept of 1858, for it shows no internal order in the micellæ of starch; and in 1858 he had not yet decided that the micellæ were micro-crystals[4]. He says explicitly that the forces holding the individual molecules (then called 'atoms') together within the micellæ are "analogous to the forces of crystallization", but are not identically the same as these forces; for starch is formed in the plant from aqueous solution, but is not itself soluble in water, which it would be (he argues) if composed of crystals.

This statement is of the first importance. In the first place it shows, I think conclusively, that Nägeli was not then using the polarizing microscope. Then, it seems to me that, had he from the first believed that the compound molecules, later called micellæ, were in fact crystallites, he might have had more difficulty in conceiving that idea which is, if not identical with the notion of polymerization, at the least a close precursor. For the molecules, or 'atoms of starch' as he then called them, would simply have been united by what seemed to be the familiar forces of crystallization, and no new and unexplained forces need be postulated.

I will now attempt to give some idea of the process by which Nägeli arrived at his most important hypotheses. As I have pointed out, most of these hypotheses are already formulated in the monograph on starch-grains, of 1858. This monograph is a huge work of more than 600 quarto pages, of which probably 550 are in a type usually reserved for footnotes. Though I have read it, it cannot be expected that I should do more here than give a summary account of what seem to me to be the most significant observations and the most important arguments.

The notion of a system composed of solid particles each surrounded by an envelope of water, a system which, however, is itself solid and rigid, was partly suggested by the phenomena of imbibition and of swelling, particularly swelling without loss of shape, but also, I think, by his belief that starch-grains grow by intussusception. From a review article by N. P. Badenhuizen[16], I gather that there is little doubt that starch-grains grow by deposition on the outer surface, and not by intussusception, so that this looks like an erroneous belief leading to a truth. But I think it likely that Nägeli had first formed the much more probable hypothesis[17] that cell-walls must grow at least partly by intussusception, and that this inclined him to believe that the same could be asserted of starch-grains. He does refer in his monograph to the immense growth in length of some cylindrical cells, and argues cogently that this can scarcely be accounted for by passive stretching of an already formed wall[4]. Naturally the space allotted to the consideration of cell-walls cannot be very great in a monograph on starch-grains, and it is possible that they were of greater importance in Nägeli's theorizing than the space assigned to them would suggest.

The hypothesis of supermolecules (polymers) is of great interest, and I am far from certain that all the influences leading to it are made explicit in the monograph. I suspect that this idea was latent in the earlier work on osmotic phenomena, which was certainly well known to Nägeli, as it was to Graham (the first volume of the *Pflanzenphysiologische Untersuchungen*, dated 1855 and published with the second volume, *Die Stärkekörner*, dated 1858, is concerned exclusively with osmotic phenomena); but this is a subject which I have not yet been able to investigate.

The explicit argument seems to be:

(1) Starch-grains are composed of alternating layers of dense and loose material, the loose material being much richer in water than the dense.

(2) The whole grain is, however, certainly all starch (or at least, the cellulose which he thought to be present is evenly diffused throughout the grain, and does not account for the difference between the layers).

(3) The only explanation of the difference in water-content in the alternate layers is that the particles of starch are larger in the dense than in the loose layers; but, since both large and small particles are chemically starch, the larger must be compounded from the smaller, which, of course, cannot be less than one molecule each.

(4) Nägeli also asserts that the larger particles cannot be isodiametric, but must be elongated in the direction of the radii of the starch-grain. This is, of course, a subsidiary proposition, and the hypothesis of compound molecules does not depend on it; but it is of considerable importance in the development of the theory of fine structure.

All these conclusions, which together give an outline of the main argument, seem rather poorly supported when examined individually. Nägeli could not be very certain that the layers differed only physically, not chemically, from one another; though he gives evidence for his belief that the difference could not be due to the second substance which he thought to be cellulose: if the starch is digested out with ptyalin, a complete ghost of 'cellulose' remains.

The conclusion that the difference is physical would receive powerful support from Nägeli's mathematically developed argument based on the supposition that growth is by intussusception; but the basic supposition is erroneous.

Nägeli's arguments[4] for the existence of larger compound molecules in the dense layers of the starch grain are extremely complicated, and it is not easy to decide the degree of interdependence between the parts of the whole logical complex.

Two main arguments seem to be involved:

First there is an elaborate dynamical argument, based on the supposition that organized materials have the kind of structure illustrated in Fig. 1—a sort of 'bricks in a wall' structure. The model is itself suggested by the belief that there are only two possible fine structures: the 'bricks in a wall' on one hand, and a network structure on the other. The network-model is then eliminated by the following argument. Starch can swell to 27 times its original volume; it then contains 90–98 per cent water, and between 10 and 2 per cent starch. Some cell-walls may probably contain as much as 200 or more parts of water to one of solid. If the solid parts formed a kind of scaffolding full of holes able to contain water, it would follow that starch and cellulose must possess a high degree of extensibility, a property entirely foreign to them. Further, it would necessarily be possible to express water by mechanical means but this is not possible with the cellulose-jelly. He notes that extremely aqueous cellulose walls may even be brittle.

To account for both rigidity and swelling of the system, he postulates two forces, one attracting starch to starch, and the other attracting starch to water. He supposes the starch–starch force to be inversely proportional to some power of distance, and the starch–water force to be inversely proportional to some higher power of distance.

Thus, $F_{ss} = \frac{A}{D^p}$ and $F_{sw} = \frac{B}{D^{p+q}}$

where p and q are positive, and A and B are parameters.

At some distance determined by the parameters, which of course are constants for any one system, the forces will be equal. At any lesser distance than the distance of equality, the starch attracts water preponderantly; at any greater distance, starch attracts, preponderantly, starch. This gives us the repetitive pattern of blocks of starch separated by layers of water and allows for forced drying and re-imbibition with water.

An extremely elaborate development of this fundamental dynamic idea enables him to show that, given the initial postulates, the width of the layer of water would be greater if the particles of starch were smaller. Thus, if his mathematics and dynamics are correct, the postulates deliver a hypothesis connecting particle size with quantity of water. So, accepting that all particles are chemically starch, the notion that the compound molecules in the dense layers are larger than those in the loose layers acquires a certain status. That status remains, however, rather tenuously hypothetical. That he really came to believe in compound particles in this way is improbable, and he gives at least two much more direct arguments.

"Now it is possible to conceive of the relation of the particles of solid starch to the enveloping water in two ways: either that each atom of starch (consisting of 12 atoms of carbon, 10 of hydrogen and 10 of oxygen) is surrounded by an envelope of water, or that several atoms of starch are united to form a molecule, lying in an atmosphere of water . . . the fact that the water-content of solid starch varies between widely separated limits cannot be reconciled with the supposition that each atom of starch is surrounded by an envelope of water; for then . . . they would have to have a variety of properties . . . in the solid state; they would have to attract water in various quantities. In a word, there would have to be as many modifications of insoluble starch as there are different amounts of contained water. We are therefore led to adopt the second supposition as the more probable"[4]—that is, the supposition that different numbers of starch-atoms can combine to form starch-molecules of different sizes. When, in the passage just quoted, Nägeli speaks of starch containing very different quantities of water, he is thinking of the swelling of starch-grains on treatment with heat or with reagents. He must therefore argue that treatment of starch with the reagents concerned does not alter the starch chemically, and he writes:

"It is not possible to explain this phenomenon merely by the supposition that starch possesses for acids, for alkalis and for water at higher temperatures a greater affinity than for water at lower temperatures. Were this the cause of swelling, it would follow that, on the neutralization of the acids or alkalis and on cooling, the fluid taken up in excess must be released[4]."

He also notes that in all these cases, the swelling is a threshold phenomenon; heating must reach a certain temperature, and acids and alkalis a certain concentration before there is any swelling at all. The degree of polymerization he postulates can be judged by his computing the possible number of starch-atoms in a molecule to be "up to 9,000".

We may now consider how he attempts to prove that the compound (or polymer) molecules must be elongated in general form.

He argues that the rigidity of the whole structure (starch-grain or cell-wall) requires that the solid particles exercise different attractions in different directions, and he considers that it is "simpler and more natural" to suppose that this is due to their being anisodiametric, rather than to postulate different forces exercised in different directions by isodiametric particles[4].

This argument, which was probably correct in 1858, prevents him, I believe, from completing a most ingenious and characteristic argument based on the phenomena of cracking of starch-grains in shrinking or in swelling. From the space given to these phenomena in the more empirical part of his monograph, and from their being explicitly referred to in the theoretical part, it seems that they impressed his mind strongly.

Starch-grains are commonly composed of concentric spherical or ovoid shells, but when they are dried they do not, he says, show fissures between the shells, but very often do show radial cracking. This he interprets as proving that the particles "must have a radial as well as a concentric ordering". I cannot find that he anywhere makes clear that a mere geometrical ordering of particles in rank and file scarcely explains the observed phenomena. Unless I am greatly mistaken, what is required is a difference of attraction between particles; the attraction being greater along the radius of the starch-grain than in the tangential direction. If this could be conceded, we at once arrive at rod-shaped compound molecules with their length along the radius of the grain. But this would, of course, not be consistent with the postulate that isodiametric particles, such as he supposed the starch-atoms to be, must not be required to exercise different attractions in different directions.

Yet, in this discussion of cracking, which occurs early in the monograph, he does seem to have made up his mind on the presence of rod-shaped compound molecules; for, noticing that cracks occur also in the radial direction when the grain swells under reagents, he adopts as an explanation that in this case water-particles are inserted preferentially along the radius, which increases the radial dimensions relatively, just as in drying the radial dimensions are relatively increased by the removal of water lying principally tangentially between particles. Neither of these explanations seems to be very lucid unless we do assume that different forces are operative between the starch particles in the two directions, radial and tangential respectively.

Compared with the mass of work under review, my account, long as it is, is inadequately brief. But if it is of any value at all, what conclusions does it seem to hint at ? Some conclusion I feel called on to offer; for a case-history without a diagnosis is necessarily disappointing.

It seems to me probable that the elaborate theorizing in which Nägeli indulged was to a minor extent responsible for his conclusions: that its chief function was to fortify his mind; to give him courage to make his way through an immensity of tedious detail (he weakened his sight seriously by the extreme assiduity of his microscopic studies), and courage to enunciate with conviction conclusions partly, if not wholly, arrived at by a process of independent judgment exercised immediately on his observations. It is not without significance that both Hofmeister and Pfeffer praise him for his *Scharfsinn*; which seems to be the same quality as that referred to by Aristotle under the name ἀγχίνοια, and called by Kant, *Mutterwitz*. This is something the lack of which, as Kant remarks, no schooling can make good. We cannot analyse it, but one of its characteristics appears to be the ability to judge the relative importance of a piece of evidence; this is illustrated, to give only one example, by Nägeli's willingness to alter his model, which seemed to fit a number of the facts, when he found it inadequate to account for the tensile strength of plant fibres. It does appear to be a faculty which many, but not all, great scientists have had in an unusual degree. Mendel must have had it supremely or he would have been as unable as were his contemporaries to digest the mass of facts offered by Gärtner. Claude Bernard, on the other hand, is perhaps an example of a scientist who lacked this power. His results seem largely the product of an excellent method, and when called on to guess he commonly guessed wrong. Perhaps Faraday and Clerk Maxwell present a similarly contrasting pair.

The art of investigation, it may be, has, like other arts, its rules, by following which more can be accomplished than by the rules of other arts; but it has also, like them, an element which cannot be taught because it cannot be analysed.

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THE RADIO TELESCOPE INTERFEROMETER AT THE ROYAL RADAR ESTABLISHMENT

By Dr. J. S. HEY, M.B.E.

A NEW installation for radio astronomical and upper atmosphere research at the Royal Radar Establishment, Ministry of Aviation, has recently been constructed on the site of a former airfield at Defford, near Malvern. The equipment consists essentially of two steerable and mobile radio telescopes with parabolic reflectors 25 m. (82 ft.) in diameter, which can be used either separately or in conjunction as an interferometer (Fig. 1.) The radio telescopes can be operated over a wide range of wave-lengths down to a few centimetres, and their spacing can be varied up to about 1,000 m. in any orientation for interferometric observations of high angular resolution. It is planned to use the aerials both as radiometers for the reception of radio emissions and also with transmitters for radar astronomy. The aim was to devise a radio telescope system which would combine sensitivity, accuracy and versatility.

Before describing the equipment in more detail some of the guiding principles governing the choice of system will be outlined. A parabolic reflector is particularly suitable for a radio telescope which is to be steerable in any direction and capable of changing wave-length easily. The sensitivity of a radio telescope when receiving radio emissions is proportional to its collecting area A; the least angle that can be resolved is approximately λ/D where D is the diameter of the aperture; when used with a transmitter in radio reflexion experiments the echoed signals are proportional to A^2/λ^2. The shortest wave-length to be used determines the accuracy required in the surface contour of the reflector, and surface deviations should not exceed $\lambda/16$ in working conditions (about 6 mm. at $\lambda = 10$ cm.). While metre wave-lengths are appropriate for much radio astronomical work there is an important field of research at shorter wave-lengths; consequently, it was planned that the system should be able to operate at wave-lengths as short as 10 cm. Unfortunately, constructional problems and cost increase rapidly with size of reflector when high accuracy has to be maintained. A reflector diameter of 25 m. was therefore accepted, and it was decided to use two radio telescopes as an interferometer in order to increase angular resolution.

When two separated aerials are connected together as an interferometer the aerial pattern consists of interference lobes with maxima separated at intervals of approximately λ/L where L is the spacing between the two aerials. The half-power width of each lobe is approximately $\lambda/2L$, compared with λ/D for the half-power beam-width of a single aerial of diameter D. The angular resolution is thus greatly increased and is determined by the spacing; consequently, very accurate measurements of angular positions are possible on isolated sources. An interferometer with mobile aerials can be used for the determination of source diameters and the analysis of complex distributions of sources of radio emissions. This is achieved by the method of aperture synthesis in which observations obtained with the aerials at various spacings in different directions are combined. The resolution corresponds to that which would be achieved by exploring the sources with the very narrow beam produced by an aperture of dimensions comparable with the largest spacing.

The pointing errors of the radio telescopes must clearly be small compared with the beam-width. A 25-m. reflector gives a beam-width of about 20 minutes of arc at $\lambda = 10$ cm., so the pointing errors should not exceed 2–3 minutes of arc. The accuracy of the interferometer depends on the length and stability of the base-line. A variable spacing up to about 1,000 m. was chosen as appropriate for many purposes in the wave-length range of 10 cm.–1 m. To ensure an angular accuracy of a few seconds of arc it was specified that when operating at a given spacing the distance between the phase centres of two aerials should not vary more than 1 cm. and relative displacements at right angles to the line joining them be not more than a few mm.

The main contract for the equipment was placed with Werkspoor, which possessed unique qualifications in having previously constructed in Holland an accurate radio telescope of the required size as well as having special experience in the design and manufacture of traction equipment, bridges and large-scale structures. Several sub-contracts were arranged with British firms, and in particular the radio telescopes were erected at Defford by Carter-Horseley (Engineers), Ltd.

Several factors determined the choice of a site: it had to be nearly flat over distances of about 1 km. and with no screening of the field of view above a few degrees elevation, with freedom from sources of radio interference, and within a reasonable distance of the main Establishment at Malvern. The former Defford airfield fitted these requirements satisfactorily as well as providing runways suitable for the laying of tracks to support the mobile aerials, which are steel constructions each weighing about 240 tons. Two intersecting runways were required and the disposition of the tracks is shown in Figs. 1 and 2. The length of the tracks from the intersection is about 750 m. One aerial can run along the track in direction *AB* and the other along *CD*. It will be seen that any relative position can be achieved, the maximum spacing being 700–1,200 m. depending on direction. Along each runway are two parallel sets of standard double railway tracks 90 ft. apart. Each radio telescope is supported on a triangular carriage which can be electrically driven along the tracks. When they have been brought to the required positions for an experiment, the radio telescopes are levelled by means of hydraulic jacks. On each aerial, running, braking, levelling and locking are all controlled from one panel.

Although the radio telescope reflector is closely parabolic in contour, it is in fact polyhedral and consists of 1,464 triangular facets. These are in sets of 4, each set being separately adjusted to the correct position after mounting in the reflector framework. In this way a high accuracy of contour, within the specified limits for 10 cm. wave-length, can be attained. The reflector is constructed of wire mesh to minimize differential heating and consequent distortion, and also to reduce heat reflected to the focus during solar observations. The central region, of 20-m. mean diameter, is of fine mesh composed of wires 1·25 mm. in diameter with 7·25 mm spacing. The remaining outer part, to 25 m. diameter, is of slightly thicker wires with about twice the spacing. This concession in the

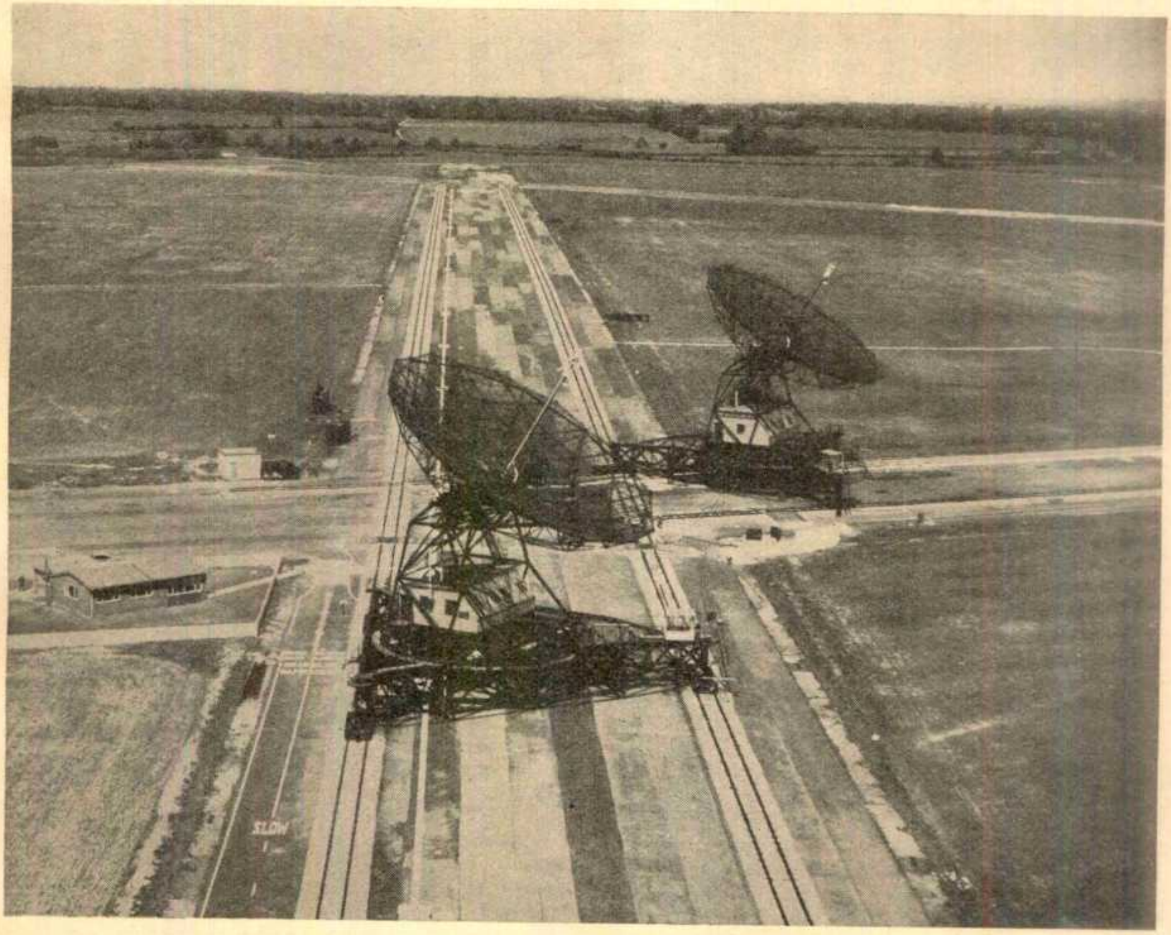

Fig. 1. The radiotelescope interferometer (Crown copyright)

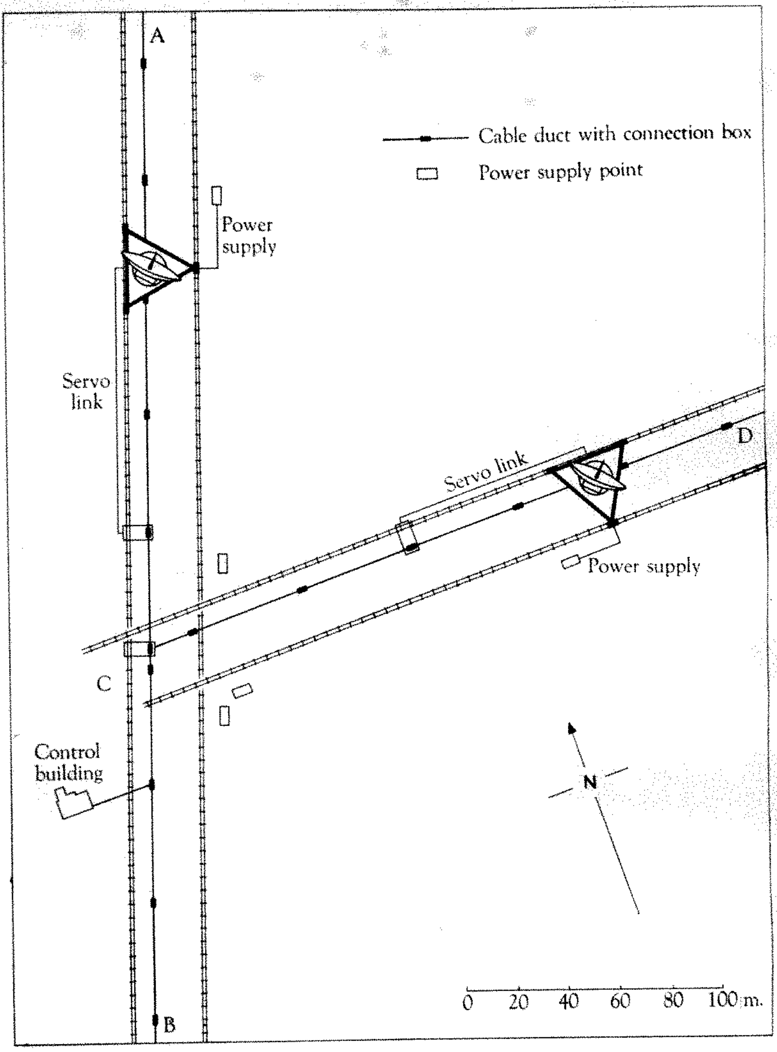

Fig. 2. Diagram of central part of the installation. The total length of the track in direction AB is 1,500 m. and that in direction CD is 750 m.

outer mesh eases mechanical construction without serious detriment to performance, since it entails only a small loss of reflexion efficiency at 10-cm. wave-length. In any event, when highly sensitive radiometers are operated at short wavelengths, it is sometimes advisable not to attempt to utilize the whole reflector since 'spill-over' tends to bring in unwanted radiation from the ground. The focal length of the reflector is approximately 12 m., and the position and polarization of the aerial feed at the focus are adjustable during operation by a mechanism behind the reflector.

The reflectors have alt-azimuth mounts, and are provided with servo-motor drives. The central pivot for azimuth rotation takes about half the load, the other half being shared by four wheels running on a horizontal circular rail of 16 m. diameter mounted on the mobile carriage. The system is designed to give a pointing accuracy of better than 3 min. of arc at azimuth and elevation-rates up to 18° and 12° per min. respectively in normal working conditions and wind speeds up to 20 m.p.h.

Each aerial is provided with a mechanical analogue converter or 'pilot' which automatically transforms celestial co-ordinates of declination and hour angle into azimuth and elevation. With this device a celestial object such as a radio star can be followed continuously. When the two radio telescopes are used together as an interferometer they must track simultaneously in the same direction, and they must be connected together to combine the radio signals they receive. The first requirement is met by providing a control room at the intersection of the runways where the directional data of the radio telescopes are displayed and from which both equipments can be controlled. In meeting the second requirement the interconnecting cables must satisfy certain stringent conditions: there must be little change of electrical length due to temperature fluctuations or moisture, and reflexions at connecting points and attenuation in the cables must be small. Coaxial cables are used of which the outer conductor is a solid aluminium tube filled with dry pressurized air, and the inner conductor is held by a helical membrane spacer. For thermal insulation and protection, the cables are surrounded by glass wool and bituminous felt and placed in concrete cable ducts. Connecting boxes are placed at intervals of about 50 m. along the cable run as indicated in Fig. 2. The connectors are specially designed to minimize discontinuities and consequent reflexions.

The cabins mounted behind the aerials are each large enough to accommodate two high-power radar transmitters as well as receiving and other apparatus. If desired, one aerial may be used for transmission and the other for reception to eliminate some of the difficulties which can arise in common transmitter-receiver systems with highly sensitive receiving devices. At present, a transmitter with 2½ MW. peak-power and 5 µs. pulse-length working at a frequency near 1,300 Mc./s. has been installed in one of the cabins, and in the other a transmitter with 100 kW. peak-power, 200 µs. pulse-length, with operating frequencies at 300 Mc./s and 500 Mc./s. At 3,000 Mc./s. it is planned to utilize the 2 MW., 5 µs. transmitter already available in the 45-ft. radio telescope at Malvern.

It will be appreciated from this description that the new installation is a major addition to British facilities for the radio exploration of space. It is a suitable instrument for observations of radio emissions originating from many different sources such as radio stars, the Sun, Moon and planets, Earth satellites and space probes. Within the solar system radar methods can also be applied. In the upper atmosphere, investigations can be made of meteors, auroræ, and ionospheric phenomena. The radio telescope system combines versatility and high angular accuracy for a wide variety of research in radio astronomy and the radio phenomena of the upper atmosphere.

NEWS and VIEWS

The Henry Dale Professorship of the Royal Society

THE Council of the Royal Society has accepted a generous gift of £100,000 from the Trustees of the late Sir Henry Wellcome to establish and endow a Royal Society professorship in medical research to be known as the Henry Dale professorship. The Wellcome Trustees have made this gift to commemorate the unique services of Sir Henry Dale to the Trust as its chairman for twenty-two years as well as his outstanding contributions to science and medicine in a wider context. In accepting the gift, the Society expresses its gratitude and great satisfaction in being associated with the Trustees in this act of honouring its distinguished Fellow, Sir Henry Dale, who has also rendered conspicuous service to the Society both as secretary (1925–35) and as president (1940–45). The Council of the Royal Society greatly values this outstanding benefaction, which will significantly increase the Society's capacity to promote medical research. The appointments to the new professorship will be especially, but not necessarily exclusively, in relation to research in physiology and pharmacology, which are the particular interests of Sir Henry Dale. This announcement of the establishment of the new professorship was made on Sir Henry Dale's eighty-sixth birthday, June 9.

The National Institute for Medical Research :

Sir Christopher Andrewes, F.R.S.

SIR CHRISTOPHER ANDREWES retired on June 6, after thirty-four years service as a member of the scientific staff of the Medical Research Council; he was head of the Division of Bacteriology and Virus Research at the National Institute for Medical Research, and had been deputy director of the Institute since 1953. Sir Christopher's scientific career coincides closely with the modern development of medical virology, and he is recognized internationally as an authority in this field. His name will always be associated with the outstanding work that led to the discovery of the influenza virus; in this he played a leading part, and the viruses of influenza and other respiratory diseases have remained his principal scientific interest. This is illustrated by the courageous decision that was taken by the Medical Research Council, on the personal initiative of Sir Christopher, to set up a research group under his direction in the Harvard Hospital at Salisbury for the long-term study of the elusive problem of the common cold. The combined laboratory and human volunteer organization that he established was universally recognized as being admirably designed for this type of work, and it is most gratifying that Sir Christopher's persistence in the face of repeated disappointments should have been rewarded by the discovery at Salisbury, a year before his retirement, of methods of isolating and cultivating common cold viruses. Sir Christopher has also contributed to the increasingly important subject of the nomenclature and classification of viruses. He has lectured on his subject in many countries and he has trained many pupils; the present flourishing state of medical virology owes much to his work and influence.

Dr. A. Isaacs

SIR CHRISTOPHER ANDREWES is succeeded as head of the Division of Bacteriology and Virus Research at the National Institute for Medical Research by Dr. Alick Isaacs. Dr. Isaacs qualified in medicine at the University of Glasgow in 1944. After house appointments and the tenure of a research scholarship in the Department of Bacteriology in Glasgow he spent a year in the Department of Medicine at Sheffield, and then worked for two years with Sir Macfarlane Burnet in Melbourne; this experience has had great influence on his subsequent career. Dr. Isaacs joined the scientific staff of the Medical Research Council in 1950 in order to work at the National Institute for Medical Research in the Division of which he is now taking charge, and during the past ten years he has established himself as a leading research worker in virology. His interests have lain chiefly in influenza and related viruses, and he has been responsible for the World Influenza Centre which was set up at Mill Hill, in co-operation with the World Health Organization, for the study of the epidemiological aspects of the disease, particularly in relation to the appearance of new strains of virus and their influence on the spread of epidemics from country to country. During the past few years Dr. Isaacs' work has attracted much attention through his discovery of interferon, a substance produced in cells as a result of viral infection, which has an inhibiting effect on viral growth. This is a discovery with widespread implications, and it has aroused interest and stimulated new research in laboratories throughout the world.

Mineral Technology at the Imperial College of Science and Technology: Prof. M. G. Fleming

DR. M. G. FLEMING, who has been appointed to the new chair in mineral technology in the Department of Mining at the Imperial College of Science and Technology, is a graduate of Queen's University, Kingston, Ontario. On leaving college he spent four years in the mining industry, finally holding the appointment of chief metallurgist at the Paymaster Gold Mine, Ontario. Following the outbreak of the Second World War, Fleming joined the Royal Canadian Air Force, and after various appointments in Training Command flew as flight lieutenant navigator with 435 Squadron from Down Ampney, Gloucestershire. On his return to civilian life in 1946, Fleming was appointed to the staff of the Royal School of Mines, which is one of the constituent colleges of the Imperial College, becoming senior lecturer in 1951 and reader in 1958. These years since the War have seen the establishment of the new degree course in mineral technology following the opening of the Mineral Dressing Laboratories in 1953. Fleming's main research interest has been connected with investigations into flotation phenomena, particularly the characteristics of secondary lead minerals, and he has also given much attention to the application of basic research to plant operation. He is well known for his work in both these fields and has been consulted frequently by industry and overseas Governments. As a consequence, he has travelled widely and has first-hand knowledge of mining conditions in many parts of the world, which, coupled with his research experience, will prove to be of particular value to the Royal School of Mines while building up the traditions of the new Department—a Department the work of which is devoted to one of those fascinating areas to be found lying between pure science on one hand and conventional engineering on the other, and which only now are beginning to be investigated systematically.

The National Institute of Agricultural Botany : Dr. P. S. Wellington, D.S.C.

DR. P. S. WELLINGTON has been appointed assistant director of the National Institute of Agricultural Botany as from July 1, 1961. The vacancy was caused by the death of Mr. G. W. G. Briggs (*Nature*, **190**, 861; 1961). Since 1952, Dr. Wellington has been the chief officer of the Official Seed Testing Station for England and Wales, which is one of the branches of the Institute. In this position he has been responsible for statutory tests under the Seeds Act and has initiated a programme of investigations into the problems arising from new methods of handling and conditioning the seed of farm and garden crops. Dr. Wellington is a graduate of the University of London, where he obtained first-class honours in botany at the Royal College of Science (Imperial College). During the War he served as an observer in the Fleet Air Arm and was awarded the D.S.C. in 1941.

International Meteorological Organization Prize for 1961 : Prof. K. R. Ramanathan

PROF. K. R. RAMANATHAN, of the Physical Research Laboratory, Ahmedabad, has been awarded the International Meteorological Organization Prize for outstanding work in meteorology and international collaboration. Prof. Ramanathan studied at Victoria College, Palghat, and Presidency College, Madras, and after obtaining a doctor's degree in science, he became a demonstrator in physics at Trivandrum College and later a research scholar at the University of Madras. In 1922 he joined Rangoon University College as a lecturer in physics. In 1925 he became a member of the Indian Meteorological Department. After having been director of the Kodaikanal and Bombay Observatories, he was appointed deputy director-general of observatories. In 1948 he was appointed director of the Physical Research Laboratory in Ahmedabad. Prof. Ramanathan has worked on problems of molecular scattering of light in fluids, atmospheric and solar radiation, study of the Indian monsoon, general circulation of the atmosphere, atmospheric ozone, physics of the ionosphere and aeronomy. For several years he was the president of the International Union of Geodesy and Geophysics, and was also president of the International Association of Meteorology of that Union. He has been, and still is, the president of the International Ozone Commission. The International Meteorological Organization Prize was established in 1955 by the World Meteorological Organization in honour of the former non-Governmental organization which had initiated international collaboration in meteorology in 1878, but which ceased in 1951 when the World Meteorological Organization took over. The Prize consists of a gold medal and 1,200 U.S. dollars.

Taxation of Scientific Bodies

ON the second reading of the Rating and Valuation Bill in the House of Lords on May 31 the position of the scientific societies was strongly represented by the Earl of Halsbury, the Earl of Cranbrook and Lord Douglas of Barloch, though Earl Jellicoe had not mentioned this question in moving the second reading. Lord Hailsham, in replying on the debate, said that there is a prospect of fairly general agreement between the Government and the learned societies about the way in which the problem should be tackled (see *Nature*, May 20, p. 657). He referred again to the deputation he had received on behalf of the societies, but repeated that he did not think it would be right to deal with the question within the scope of the present Bill beyond the 50 per cent mandatory relief. It would not be right to ask local authorities to undertake what was fundamentally a national burden. Lord Hailsham said that he recognized to the full what Lord Halsbury and Lord Cranbrook had said about the value of the work of these societies, that many of them are essential to the working of science, that they produce publications and maintain libraries of national importance, but his own opinion was that the difficulty could not be dealt with by rate relief at all, but that it must be dealt with by grants in some form. He thought we should consider using the Royal Society and the British Academy as vehicles for this kind of negotiation, and if they were prepared to act the Government would be glad to hear from them as and when the situation developed.

United Kingdom–Euratom Technical Collaboration

IN a written answer in the House of Commons on June 6, the Parliamentary Secretary for Science, Mr. D. Freeth, said that co-operation under the United Kingdom–Euratom Technical Collaboration Agreement had covered a wide range of subjects. These included basic reactor physics, reactor planning and programming, reactor economics and the costing of nuclear power, fast reactors and gas-cooled reactors, thermonuclear fusion, nuclear measurements, mass spectrometry, workshop organization, health and safety, corrosion, the construction of 'hot' laboratories, the industrial applications of radioisotopes, juridical problems arising from nuclear ship propulsion, radiation protection and third-party liability. Co-operation had proceeded by exchange of reports, visits of experts, conferences and symposia, and attachment of staff to specific projects and establishments. The United Kingdom also collaborated with Euratom and the United States Atomic Energy Commission in the translation pool "Transatom", which concentrated on translations from Russian and Japanese.

Funds for Cotton, Silk and Man-Made Fibres Research

THE Cotton Industry Development Council (Amendment No. 4) Order, 1961, which was approved by the House of Commons on May 8, enables the Cotton Board to collect a statutory levy from firms processing man-made fibres, to support the British Cotton, Silk and Man-Made Fibres Research Association, which is being formed by the merging of the British Cotton Industry Research Association and the British Rayon Research Association, as recommended last year in the report of the working-party appointed by the Councils of the two Associations (see *Nature*, 190, 589; 1961). Article 6 of the Amended Order enables the Cotton Board to collect from the man-made fibres section of the industry 11 per cent of the total being collected from the whole industry, which in 1959–60 and 1960–61 was £385,000. For 1961–62 the total proposed to be collected from the industry for all the Cotton Board's activities was £400,000, of which £45,000 would come from the man-made fibres section, and, with £200,000 from the cotton section, would go to the Research Association. The Order also enables the Cotton Board to assist scientific research on the man-made fibres side. In reply to a question, Mr. N. Macpherson, Parliamentary Secretary to the Board of Trade, who moved the approval of the Order, said that one object of the merger was to reduce expenditure; the income of the Shirley Institute was £462,000 in 1959–60 and was expected to be £531,000 in the first year of the next quinquennium: that of the Rayon Research Association had been £376,000.

The Devon Naturalists' Trust

THE Devon Naturalists' Trust was formally inaugurated on March 27. Its objects are to conserve the wild-life of the county against encroachment, to protect places of interest to naturalists, and to encourage the study of natural history. Lord Roborough is the first president. A public meeting to invite support was held in Exeter on May 16, and the speakers were Lord Roborough, Lord Hurcomb, Mr. H. G. Hurrell and Major J. Coleman-Cooke. Mr. Hurrell announced his gift to the Trust of Lady's Wood, its first reserve. This is an area of some 9 acres near South Brent which contains much of interest, including dormice and wood anemones. Apart from the reserve given by Mr. Hurrell, there is a strong possibility that the Trust will be offered another. It is also in contact with a farmer who has a large colony of *Lobelia urens* on his land, and it is hoped to do something to preserve this very rare plant. The Trust and the Devon Wild Fowlers Association are acting together to see if the management of the bird sanctuary on the River Exe can be improved. The prospects of introducing blackgame and *Zostera marina* in suitable places is being studied and may be put in hand if the initial inquiries indicate a good chance of success. The Trust is also making inquiries to discover if the commercial primrose picking reported in the Press last spring is likely to be damaging.

Conservation of Askham Bog

THE Nature Conservancy and the Yorkshire Naturalists' Trust have agreed to conserve Askham Bog. This area, near York, is a 100-acre remnant of a swamp which must at one time have covered much of the Vale of York. Now almost wholly covered by a thicket of birch, oak and alder, the Bog illustrates the later stages in the succession from open fen to woodland. The ground vegetation includes many bog and fen species uncommon in Yorkshire, among them the rare royal fern. Associated with this flora is a rich variety of insects, particularly beetles. The land is partly owned and partly leased by the Yorkshire Naturalists' Trust, which manages it as a nature reserve. At present it is managed successfully with due regard to the scientific interest and, accordingly, the Nature Conservancy does not feel it necessary to acquire a direct interest in the Bog or to declare it as a National Nature Reserve. By the agreement, Askham Bog is designated a Site of Special Scientific Interest (*A*). The Bog will continue to be managed by the Yorkshire Naturalists' Trust, advice from the Nature Conservancy being available when necessary. Permits to visit the site or undertake research there must be obtained from the chairman of the Management Committee, Mr. C. J. Smith, 7 Malton Way, Clifton, York.

Aerial Photographs in Geological Mapping

IN the construction of geological maps, increasing use has been made in recent years of photo-geology, and to-day even the most junior research student tends to regard aerial photographs as a pre-requisite to his field work. Notwithstanding an extensive periodical literature, however, comprehensive and up-to-date text-books on this subject have been generally unavailable, and geologists throughout the world will therefore be indebted to Dr. Richard G. Ray, of the United States Geological Survey, who has produced an excellent monograph, *Aerial Photographs in Geologic Interpretation and Mapping* (United States Department of the Interior: Geological Survey. Professional Paper No. 373. Pp. vi+230. Washington, D.C.: Government Printing Office, 1960. 2 dollars). This well-produced prestige publication, sold at a nominal price, lucidly describes the photo-geological techniques employed not only in routine geological work but also in the search for petroleum and ore deposits, in engineering geology, and in hydrological investigations; and it furnishes a readable account of the instrumentation utilized in these studies. The text is illustrated by 116 figures, principally stereoscopic-pair photographs chosen from the collections of Government departments in the United States.

The Australian Museum, Sydney

THE *Australian Museum Magazine* (13, No. 9; March 15, 1951. 3*s*.) includes an account of new aboriginal exhibits which have recently been placed on display in the Museum. One of the most successful of the exhibits illustrates how the aborigines make and use stone implements. Each technical process is demonstrated by using actual implements in cast hands and the impression is given of a native standing behind the exhibit and poking his arms through holes in the wall. The section on "Dress and Ornament" appeals to all visitors, and models of the various types of wind-breaks and huts, together with a painting of a camp scene, are equally arresting. The exhibits have been especially designed to be attractive and interesting to the casual visitor, instructive to those wishing to learn about the aborigine, and of service in the Schools Department. Other articles in this interesting *Magazine* deal with the gem-stones of Australia, fossil insects, freak fishes and the use of radioactivity in entomology and insect-pest control.

Site for Ulster Folk Museum

THE Trustees of the Ulster Folk Museum have announced the purchase of the Cultra Manor Estate in County Down for the purposes of a site for a permanent folk museum. It includes a manor house and 136 acres of delightfully situated land overlooking Belfast Lough and the Antrim coast. The estate is about six miles from the centre of Belfast and is in an area unlikely to suffer from suburban development. Initial work on the land will start in the summer, and as much material for the Museum has been gathered together since the Folk Museum was established by Act of Parliament some two years ago, it is hoped that developments will be well advanced in the foreseeable future.

University News :

Belfast

The following appointments have been announced: Dr. A. Dalgarno, to the chair of quantum mechanics in the Department of Applied Mathematics; Dr. E. A. Cheeseman, to the chair of medical statistics in the Department of Social and Preventive Medicine.

Bristol

Dr. R. G. Chambers has been elected reader in physics, also Dr. D. Bohm (until he takes up his appointment to the chair of theoretical physics at Birkbeck College). A. R. Paterson has been appointed lecturer in applied mathematics.

Hull

The following appointments to senior lectureships have been announced: Dr. G. C. Bond (chemistry); Dr. A. Cunliffe (physics); Dr. H. W. Parsons (mathematics).

London

The Wolfson Foundation has made a grant of £200,000 to University College, London. The money will be used to develop the Galton Laboratory and the Department of Eugenics, Genetics and Biometry in a building north of Euston Road which has been leased to the College by the University Grants Committee. The chair of biometry, vacant since Prof. J. B. S. Haldane left the University, will be re-established in the new premises.

Nottingham

Dr. James Crossland, lecturer in the Department of Physiology, University of St. Andrews, has been appointed reader in pharmacology in the Department of Pharmacy as from next session.

Sheffield

Dr. A. M. Murray has been appointed lecturer in physics.

The Night Sky in July

New moon occurs on July 12d. 19h. 12m. U.T. and full moon on July 27d. 19h. 51m. The following conjunctions with the Moon take place: July 8d. 20h., Venus 2° N.; July 9d. 11h., Aldebaran 0·4° S.; July 16d. 03h., Regulus 0·3° N.; July 17d. 02h., Mars 0·3° S.; July 27d. 07h., Saturn 3° S.; July 27d. 17h., Jupiter 3° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Aldebaran on July 16d. 06h., Venus being 3° N., and Mercury with Pollux on July 31d. 19h., Mercury being 6° S. Mercury is a morning star, and is visible low in the east before sunrise during the later part of the month. Venus is a morning star, rising at 1h. 20m., 1h. 10m. and 1h. 05m. on July 1, 15 and 31, respectively. Its stellar magnitude on July 15 is − 3·7; the visible portion of the apparent disk increases from 0·555 to 0·683 during the month, and its distance from the Earth increases from 73 to 96 million miles. Mars is too close to the Sun for easy observation. Jupiter and Saturn are visible almost all night, rising at about sunset and setting at about sunrise. Jupiter has stellar magnitude − 2·3 and Saturn + 0·3 on July 15, and their distances from the Earth on that date are 380 and 840 million miles, respectively, when they are in Capricornus and Sagittarius. There is one occultation of a star brighter than magnitude 6 visible at Greenwich: July 9d. 11h. 13·0 m., α Tau. disappearance; July 9d. 12h. 24·4m., α Tau. reappearance. The Earth is at aphelion on July 5, its distance from the Sun being 94·6 million miles.

Announcements

Prof. C. W. Wardlaw, professor of botany and director of the Laboratories and Experimental Grounds in the University of Manchester, has been elected an honorary foreign associate of the Royal Academy of Belgium (Section of Natural Sciences).

Dr. G. D. H. Bell, director of the Plant Breeding Institute, Cambridge, has been elected a vice-president of the National Institute of Agricultural Botany and a vice-president member of the Council.

Dr. I. M. Lifschitz of the Academy of Sciences of the Ukrainian S.S.R., Kharkov, has been awarded the Simon Memorial Prize by the U.K. Committee of the Low Temperature Group of the Institute of Physics and the Physical Society, for his work on the structure of the Fermi surface in metals and his work on liquid helium. The Prize, a memorial to Sir Francis Simon, a pioneer for the Low Temperature School of the Clarendon Laboratory, Cambridge, consists of £250.

From July 3, the address of the Director of the Service and all branches of the headquarters administrative office of the Public Health Laboratory Service will be: 24 Park Crescent, London, W.1; Tel. No. Museum 2223.

The opening of the new Creep Laboratories of the Electrical Research Association, Cleeve Road, Leatherhead, Surrey, will take place on July 7. Further information can be obtained from Leslie Jervis, press officer, Electrical Research Association, Cleeve Road, Leatherhead, Surrey.

A conference on "Behavior Genetics" will be held at the Center for Advanced Study in the Behavioral Sciences during August 14–September 3. Further information can be obtained from Preston S. Cutler, Center for Advanced Study in the Behavioral Sciences, 202 Junipero Serra Boulevard, Stanford, California.

An international conference on "Heat Transfer", presenting new developments in theory and practice, will be held at the University of Colorado during August 28–September 1. Further information can be obtained from Alan Brown, director, Bureau of Continuation Education, University of Colorado, Boulder, Colorado.

A meeting of the Photobiology Group will be held at the University of Sheffield during July 7–8. The programme will include papers on metabolism in photosynthetic bacteria and some aspects of photochemistry. Further information can be obtained from the secretary of the Group, Dr. D. Vince, Horticultural Research Laboratories, Shinfield Grange, Shinfield, Berks.

The United Nations conference on "New Sources of Energy" will be held in Italy during August 21–31. The programme has been divided into a number of sessions, including (*a*) geothermal energy; (*b*) wind power; (*c*) solar energy. Further information can be obtained from the United Nations Information Centre, 14–15 Stratford Place, London, W.1, or the Executive Secretary, United Nations Conference on New Sources of Energy, United Nations, New York.

RECENT SCIENTIFIC INQUIRIES IN PARLIAMENT

AT question time in the House of Commons on May 16, the Parliamentary Secretary for Science, Mr. D. Freeth, was faced with no fewer than 39 questions relating to scientific matters, ranging from the performance of the reactors at Calder Hall and Chapel Cross, progress with production of nuclear reactors for research and teaching purposes, to arterial disease and fire hazards of domestic equipment.

In the course of his replies, Mr. Freeth said that rather more than three-quarters of Great Britain has been covered by the Geological Survey in the primary 6-in. survey. At the present rate of progress the primary 6-in. solid and drift survey would be completed in about 55 years; it is hoped to reduce this to 35–40 years. Nearly as much secondary survey as primary survey is being carried out, and work is now being done on the more difficult mountainous regions. The Survey has also prepared the tectonic map of the United Kingdom as part of a co-operative scheme for a tectonic map of Europe.

On research into coal utilization, Mr. Freeth said that besides the British Coal Utilization Research Association, the British Coke Research Association and the Coal Tar Research Association are primarily concerned with the utilization of coal. All three research associations are doing very valuable work and there is a great deal of co-operation between them and between the National Coal Board, the Central Electricity Generating Authority, the Gas Council and the appliance and equipment makers. The total income last year of the Coal Utilization Research Association was £450,000, of the Coke Research Association more than £150,000 and of the Coal Tar Research Association less than £113,000. There was the fullest possible co-ordination on the matter between the Minister for Science and the Minister of Power.

Mr. Freeth was also asked what research was being conducted into the better use of the mineral resources of Britain; he referred to the basic information supplied by the Geological Survey and Museum in its maps and memoirs. The Warren Spring Laboratory and the National Chemical Laboratory undertake research on processing ores and extracting metals, and various research associations, industrial firms and universities undertake research into the better use of minerals.

Asked as to the action taken to apply scientific development to the machine tool industry, Mr. Freeth said that besides the scientific development work of the Machine Tool Industry Research Association and the Production Engineering Research Association, research of direct value to such scientific development was carried out at the National Engineering Laboratory and the National Physical Laboratory. In addition, 12 grants totalling more than £170,000 have been made for research and development work in this field in the past two years to six universities and two colleges of technology in England and Wales. The Machine Tool Advisory Council is reviewing progress made in implementing the recommendations of the Mitchell Sub-Committee. Mr. Freeth also referred to a ten-fold increase in the accuracy of large modern gears over the past twenty years, to which research and development work at the National Physical Laboratory and the National Engineering Laboratory had contributed. Gears up to 16 ft. in diameter can now be made with an error of one-quarter of a thousandth of an inch from the true form and spacing of the teeth and the automatic error-correcting system developed recently by the National Engineering Laboratory have been applied to a gear-cutting machine.

While the Department of Scientific and Industrial Research has not sponsored any research into the use and operation of multi-purpose pipe-lines for the transport of both liquids and solids, it is itself carrying out research into the subject, and the Department is always willing to discuss with industry the part it could play in assisting research and developing techniques in every field.

In response to a specific request which had been made for a breakdown of the figure of £42·9 million for Government expenditure on civil research and development in 1958–59 given in the report of the Advisory Council on Scientific Policy, Mr. Freeth gave the following figures for the source of the funds concerned: defence departments, £2·9 million; civil departments, £34·7 million; private industry, £4·5 million; other organizations, £0·8 million.

MAWSON INSTITUTE OF ANTARCTIC RESEARCH

ON April 15 the Prime Minister of Australia (the Rt. Hon. R. G. Menzies) inaugurated, within the University of Adelaide, the Mawson Institute of Antarctic Research. In his inaugural address Mr. Menzies referred to the great achievements of Sir Douglas Mawson in science and exploration, and affirmed that Mawson had accomplished so much because he possessed, in the highest degree, the qualities of imagination, courage and endurance.

The University of Adelaide has established the Institute, believing that it would provide the best means to commemorate one of its most distinguished members. Mawson joined the University staff in 1905 as lecturer in mineralogy and petrology. In 1921, he was appointed professor of geology and mineralogy, and he retired in 1952. He remained a member of the University Council until his death in 1958.

The main purpose of the Institute will be to foster polar studies and research. In order to do this it will maintain and develop reference collections of geological and biological materials from the Antarctic.

PHYSICS DEPARTMENTAL LIBRARY

It will maintain and develop a library of papers and maps as well as books on Antarctica. It will provide accommodation for workers engaged in antarctic research. It will promote public interest in polar matters by means of lectures and a museum devoted to antarctic materials and equipment.

The University plans to accommodate the Institute in a major annex to the geology school. This would contain a library in which the map collection would be included, a museum which would serve as a meeting-room, generous storage space and a number of small research rooms.

The basis of the reference collections will naturally be the materials collected by Mawson and members of the expeditions which he led, although valuable collections from other parts of Antarctica have been obtained by exchange with the British Museum and other institutions. At present these are largely housed in the Geology Department of the University. Many periodicals and other books have been segregated in a 'polar' room in the University library, and their number will soon be greatly increased as Lady Mawson has announced that she will present to the Institute the extraordinarily fine library collected by Sir Douglas.

To mark the inauguration of the Institute a small 'Antarctic Room' has been partitioned from the geological museum. This is mainly occupied by rocks arranged on a regional basis and well documented with maps and geological sections. It also serves to illustrate the achievements of the expeditions of which Mawson was a member or which he led.

The interest created by the inauguration of the Institute has been very wide. Representatives of the Royal Society and the Academy of Science of the U.S.S.R. were present and the Australian Academy of Science was represented by its president. Other institutions which were represented included the Royal Geographical Society, the American Geographical Society, the Argentina Institute of Antarctic Affairs and the Scott Polar Research Institute. Messages of goodwill and congratulations were read. In addition, the Governments of the United Kingdom and New Zealand sent representatives and, from the South Australian Government, two Ministers of the Crown attended. Many men with personal experience of antarctic exploration and research were present. The occasion revealed wide approval of the University's move and paid high tribute to a very great Australian. A. R. ALDERMAN

TRAINING IN OCCUPATIONAL HYGIENE

THE twelfth Conference of the British Occupational Hygiene Society was held in London on April 11. The morning session, with Dr. J. C. Gilson in the chair, dealt with the need for training, and the afternoon, with Dr. L. G. Norman as chairman, covered present and future facilities in universities.

Dr. Gilson opened the proceedings by contrasting the considerable effort now being put into radiation monitoring with the meagre resources available for checking dust and fumes, which he estimated to be a risk to some 150 times as many people as were exposed to nuclear hazards. The disparity of effort on the job was reflected in training resources, and no university course in occupational hygiene existed.

The interest of management in working conditions was emphasized by Mr. H. F. Spencer, managing director of Richard Thomas and Baldwins, Ltd., who criticized the term 'occupational hygienist'. Dr. J. M. Rogan, of the National Coal Board, who directs one of the largest epidemiological investigations at present being made in the world, considered that the existing employment of industrial medical officers in large concerns, with specialist laboratory help when required, might be more satisfactory than the appointment of broadly trained occupational hygienists.

Dr. K. P. Whitehead of Imperial Chemical Industries, Ltd., and Dr. P. G. Swann of Esso gave accounts of the ranges of activity of the personnel whose health, as industrial medical officers, they had to safeguard. The former emphasized the diversity of the chemicals handled. In his firm an industrial hygiene research laboratory studied toxicity and advised on works policy and plant design. He could see little need for the employment of industrial hygienists at factory-level in large, well-organized industries although they might be of considerable benefit to smaller firms.

Dr. Capel's firm employed an industrial hygienist, a graduate in chemistry, who had been trained in the United States. His reports were countersigned by one of the medical staff. It was advantageous for the candidate for such a post to have a degree related to the activities of the firm, or possibly a degree in general science, in which case a six months' course in the principles and practice of industrial hygiene would be sufficient. The parent American company maintained a medical research division which served affiliates all over the world and provided toxicological data on all materials handled.

Mr. H. Collinson, chairman of the Trades Union Congress Social Insurance and Industrial Welfare Committee, concentrated on the problem of the small employer with a back-street factory where conditions were often appalling, although work in the highest tradition of craftsmanship might emanate from it. To some extent this was a matter of educating the employer who was unaware and unco-operative towards suggestions for improving working conditions, but it was unlikely that voluntary action was going to be sufficient. The responsibility for making occupational hygiene available to the whole range of industry rested on the Government. The T.U.C. attached great importance to the pioneer work being done by group health services, such as that at Slough. The Nuffield Foundation had recently placed a quarter of a million pounds at the disposal of the Ministry of Labour for the encouragement of such schemes.

It was important that training should include practical experience in factories, which could only be achieved by the persuasive powers of those planning courses.

Ten years experience in providing an occupational hygiene service for the 200 member firms of the Slough Industrial Health Service was described by Dr. D. Hickish. The firms ranged in size from 2 to 2,000 employees and the backing of the group medical ice was available. Recently, consultant work utside the group has been undertaken. The function of the occupational hygienist was : (*a*) to recognize environmental stress affecting health ; (*b*) to evaluate the stress ; (*c*) to prescribe control measures.

The occupational hygienist needed to have studied elementary physiology, industrial medicine and toxicology. Training in sampling and analysis, including dust, radiation and noise, detailed knowledge of heating, ventilating, air-conditioning and lighting, and a familiarity with statistical methods were desirable. Courses at a technical level, in addition to those for graduates, would be advantageous.

The deputy chief inspector of factories, Miss K. Crundwell, described a recent attempt to catalogue the facilities which at present exist for testing factory air. It was going to be difficult to convince industry of the need for testing to such an extent that they were willing to pay for it. Some employers were anxious to improve their working conditions but others might not take kindly to the suggestion that they should pay, as they might see it, for the evidence on which to hang themselves.

The last speaker in the morning session was Dr. S. G. Fortuin, director of the Philips Medical Services, Eindhoven, Holland. He explained that in every plant they tried to have a full-time medical man, whose services might also be shared by smaller firms in the same town. The checking of environmental conditions was the task of the medical department and there was no place for a more or less independent industrial hygiene department. The physician alone should be responsible for the evaluation of health hazards.

The industrial hygiene laboratory was part of the Medical Service ; chemists and engineers were employed in this work and no university programme existed in the Netherlands for training them in the specific theoretical and practical problems of industrial hygiene ; postgraduate courses should be organized for this purpose.

In the discussion which followed the first session it was generally agreed that a need for testing facilities existed, and this involved the training of occupational hygienists to carry out environmental measurements. The cost and method of paying for such services was debated and it seemed unlikely that small firms would be willing, or even able, to carry this individually, although trade associations might offer a way round the difficulty. Another problem was the cost of remedial measures, involving re-design of plant or exhaust equipment, which might be crippling to a small undertaking.

The afternoon session was opened by Dr. L. G. Norman. In a survey of courses related to occupational hygiene which are at present available, Dr. C. H. Wood said that very little was offered in Britain. British employers of occupational hygienists, when questioned, were unanimous in stating that the only training provided was in-service, augmented, occasionally, by short courses given, as a rule, by senior members of the staff.

It was difficult to find out what British teaching institutions gave in the way of special courses, as distinct from the comprehensive type in the United States, since no general list was published; nevertheless quite a number of subjects could be investigated in this way, lectures on radiation hazards being liberally provided.

Mr. S. A. Roach gave an account of a nine-month course in industrial hygiene engineering which was being planned at the London School of Hygiene and Tropical Medicine and which was to open in October 1961.

Prof. R. E. Lane, who is responsible for the teaching of occupational hygiene in the University of Manchester, stated that the current course for industrial medical officers lasted two years but required attendance for only a day and a half a week. The department also appointed research fellows who were postgraduate science candidates for M.Sc. or Ph.D. degrees.

Prof. R. C. Browne, who occupies the Nuffield chair of industrial health at the University of Durham, felt that the chemistry graduate was usually the best equipped for the work, followed, in order, by physicist, engineer and doctor, though special risks, such as radiation, clearly affected this. Such graduates were in short supply at present.

During the discussion on the afternoon papers the question of payment for training was raised. Several speakers thought that industry, while willing to send employees on short courses lasting a week or two, might not be prepared to lose their services for a year. The suggestion that Government bursaries might be paid drew the reply that all the students taking a somewhat analogous course in public health engineering had been from overseas for several years past, supported by the World Health Organization or their own Governments ; no Treasury support was available for Britons. Other speakers considered it likely that bursaries would become available from industry.

The conference proceedings will be reported in full in the *Annals of Occupational Hygiene*.

C. N. DAVIES

CLOSED CIRCUIT TELEVISION IN THE MEDICAL SCHOOL

A SYMPOSIUM on the applications of television in medical research and teaching was organized at the London Hospital Medical College during May 10–11 by the Medical Committee of the Scientific Film Association in collaboration with the Association for the Study of Medical Education. Since the 2-hr. demonstration of a land-line colour broadcast by a team from Guy's Hospital in 1957 (*Nature*, **181**, 91, 1958) no collective attempt had been made to evaluate and discuss the potential usefulness of closed-circuit television in medicine. This meeting is therefore a welcome sign that the universities are ready to concede that television need not necessarily be equated with mass entertainment. In fact, the conference was well timed, as many of the seventy senior representatives from British medical schools who participated are concerned with the planning of new buildings and the possible incorporation of closed-circuit television.

At least three schools are about to install such facilities and several others may shortly follow their example.

Apart from isolated *ad hoc* demonstrations by manufacturers of electronic equipment, and the somewhat elaborate presentations organized at congresses by a pharmaceutical firm, there is little practical experience in Britain. To ensure, therefore, that facts rather than opinions could be discussed, the organizers invited the co-operation of acknowledged experts in television engineering: on the use of television in a medical school in the United States, on research applications and on its uses in a military medical centre—both from the United States—and a surgeon in charge of television facilities at a German university.

While television has serious limitations, such as restricted resolving-power and relatively high cost, the conference has shown that television offers considerable advantages in a number of fields—with the obvious reservation that the usefulness of any device must depend on the ability and knowledge of the user, whether research worker or teacher. The main properties of television are: its ability to convert invisible radiation into visible images, its high sensitivity to light, electronically adjustable contrast, and above all, its ability to present instantaneously moving images, even of very small subjects, to an unlimited audience, while the camera may well be in a location too restricted or too dangerous for direct human observation.

Radiology makes full use of these properties. Small 'Vidicon' tube cameras can display enlarged pictures from the X-ray image converter. Similarly, large 'Orthicon' tube cameras equipped with systems of wide-aperture mirror lenses can reproduce the radiographic image from a life-size fluorescent screen with sufficient brilliance so that the radiologist need no longer work in the dark, and consultants and students can study simultaneously the same information on receiver screens in any number of different rooms. These methods make it possible to reduce the radiation received by the patient, so that the film records from the television screen can be used almost as a matter of routine for the study of processes within the human body. Image storage on special tubes, on ferro magnetic disks and on magnetic tape ('Video' tape) are now well beyond the laboratory stage, and the development in the United States of a really large television tube sensitive to X-rays will probably replace the use of the conventional fluorescent screen in the none too distant future.

Ultra-violet radiation, sufficient for direct photomicrography, will destroy biological tissues in a very short space of time, and similarily cine-micrographic studies of the retina would require an impracticable intensity of light. It is in these examples that the enlarging, intensifying and radiation-converting properties of television can be of value to research work. Camera tubes sensitive to ultra-violet radiation will produce images of the contents and behaviour of cells with greatly reduced radiation, and highly sensitive 'Orthicon' tubes can be used to record colour images of retinal blood vessels with light intensities which are well tolerated by the human eye. Tubes sensitive to infra-red radiation may be used instead of infra-red photography for the direct examination of the subcutaneous venous circulation.

A straightforward electronic method of superimposition makes it possible to display and record in accurate register on one receiver-screen two images from separate television cameras. Eye movem can thus be studied by superimposing the reflexio of a spot-light on an eye on to the image of the object which is being scanned by the subject.

Filming from the television receiver can also be used for selective studies of movements or behaviour. The television camera, with its automatic aperture compensation for changes in the intensity of light, can be operated from a distance by remote control. While the camera is in a relatively inaccessible or even dangerous location, the observer can aim and focus accurately by watching a television screen and record the action with a film camera in front of another receiver.

Applications in university teaching are obvious and numerous if it is remembered that television can ensure that every member of the audience can see equally as well as the demonstrator; the teaching of dental procedures is an outstanding example. The lecturer can use this enlarging device either in the classroom itself or in another room while he directs the demonstration with the help of a two-way communication system. The teacher can determine the area and degree of magnification of the subject to be displayed on a television projection screen or on receivers by adjusting the zoom lens on the camera from a small remote control panel. Chemical reactions, anatomical dissections or experiments under the microscope can be televised from within the lecture theatre itself, but other demonstrations, such as a psychiatric interview or a surgical operation, must take place elsewhere and are then relayed to the students and lecturer through closed circuit television.

Endoscopy, the exploration of body cavities, is perhaps a good example where television is equally applicable in both research and teaching. Here the television camera enables several research workers or a virtually unlimited number of students to study simultaneously what, until recently, could be seen by the endoscopist alone. Both in Britain and in the United States flexible bundles containing several thousands of very thin glass-fibres are being developed. Each fibre will, by internal reflexion, transmit a part of the image from a small lens to the television camera tube. The diminutive diameter and flexibility of these bundles make it possible to explore normal as well as pathological processes of organs inside the body which are not readily accessible to conventional optical endoscopes.

Like any other device, television should only be used where it is superior to other methods. It does not take the place of lantern slides or films, but there will clearly be occasions when an enlarged and detailed display of something which is happening now is likely to be more effective. Few films suit more than one teacher. They are too rigid, and it is impossible to modify what they present to suit the reaction of a particular audience.

Relatively inexpensive equipment, which is simple to operate and maintain, is now available, so that active experimentation should shortly replace the somewhat theoretical arguments on the assumed merits and demerits of television. Baylor University, where basic instruction on techniques in physiology are shown to students on television receivers in their individual laboratories, has demonstrated that television may contribute to better and more effective teaching, without in any way attempting to take the place of the teacher or individual practical experience by the student.

C. E. Engel

THE FOURTH WORLD FERTILIZER CONGRESS

IT is generally believed that nitrogen is a key element in crop production. This is evident from the figures in Table 1, showing the yield in kilograms in different countries expected for an application of 1 kgm. of plant nutrient:

Country	Crops in rotation			Permanent pastures		
	N	P	K	N	P	K
Norway	9	3	5	11	6	4
Sweden	14	11	7	14	11	7
Denmark	18	4	2	12	5	3
U.K.	16	5	5	—	—	—
Ireland	20	8	8	—	—	—
Holland	19	6	3	10	6	4
France	19	5	2·1	—	—	—
Germany	19	8	4	9	10	—
Switzerland	18	8	4	9	10	5
Greece	15	5	3	—	—	—
Italy	11	3	—	12	4	3
Average	16	8	4	11	7	4

In the *Oxford Economic Atlas of the World* (Oxford University Press, 1959) the following increased yields per kilogram of nitrogen are recorded:

	Wheat	Rice	Potatoes	Grass (or hay)
Kgm. per hectare	17	17	84	17

The Indian Council of Agricultural Research has reported that the average production of rice in India is ten times the amount of nitrogen applied. The United Nations Korean Reconstruction Agency has stated that 1 kgm. of nitrogen as ammonium sulphate produces 12–14 kgm. of brown rice and 14–28 kgm. of rough barley in South Korea. Moreover, 1 kgm. of phosphate as superphosphate can yield 4–18 kgm. of brown rice. In the United States large amounts of fertilizers have been applied in special cases and the results show a big response to nitrogen, but the responses to phosphate and potash are smaller. Doses in amounts larger than 200 lb. of nitrogen or phosphate or potassium per acre show a depressing effect in ordinary soils, but in soils of prairie lands naturally rich in humus, beneficial results are obtained with heavy doses of fertilizers.

Because of the importance of fertilizers in crop production, the world fertilizer industry is well organized by the International Confederation of Engineers and Technicians of Agriculture (C.I.T.A.) at the International Centre of Chemical Fertilizers (C.I.E.C.), Zurich. During May 9–12, the fourth World Fertilizer Congress, organized by the International Centre, met in Opatija on the Adriatic coast of Yugoslavia, under the presidency of Prof. S. Nikolic of Belgrade. Some 290 scientists, industrialists and technicians from twenty-eight countries met to consider six general reports and sixty-five special communications. At this Congress, besides French, English and German, Russian and Yugoslav were also recognized as official languages.

Prof. Nikolic gave an account of the soils of Yugoslavia and the application of fertilizers, micronutrients and organic matter. Prof. M. R. Braconnier, of Paris, reported on fertilizer production and consumption, and stated that in 1958–59, 8·75 million tons of nitrogen, 9·03 million tons of phosphate and 7·88 million tons of potassium were produced by the world fertilizer industries, and the average $N : P_2O_5 : K_2O$ ratio of fertilizers applied has shifted from 1 : 3·85 : 0·77 in 1900 to 1 : 1·03 : 0·9 in 1958–59. In Holland the ratio is 1 : 0·5 : 0·7. Prof. Y. Coic, of Versailles, discussed the adaptation of fertilizers by cultivated plants. Prof. L. Schmidt, of Darmstadt, discussed the experimental results obtained by compound fertilizers and trace-elements. The influence of irrigation on mineral fertilization was dealt with by Prof. L. Cavazza, of Italy. The application of radioisotopes was introduced by Prof. A. C. Schuffelen, of Holland; papers on work with phosphorus-32 carried out in Italy, Germany, Hungary, Poland and Yugoslavia were given.

The functions of light, temperature, acidity, coatings on fertilizers and humus in the absorption of nutrients by plants were discussed in several papers.

From a survey of crop production and fertilizer application in different countries, it appears that in countries not using large amounts of fertilizers the nitrogen response to cereals is very marked; the law of diminishing return is in operation in Holland, Belgium and Norway, where large amounts of nitrogen are applied. But in Japan, China and Taiwan, where composts and other plant and animal wastes are utilized along with fertilizers, better crop yields per unit nitrogen are still obtained. In the United States, yields of wheat and corn are increased by producing organic matter through rotations.

Approximately 1,000 million tons of cereals, 700 million tons of pulses, potatoes, vegetables, sugars, etc., are being produced annually in the world, and the world grassland production is about 1,600 million tons. Hence assuming the yield is 15 times the nitrogen application, the production of the world's food, fodder and fibre requires approximately 250 million tons of available nitrogen. It has been already stated that the industrial supply of nitrogen is only 8·75 million tons; legumes contribute 5 million tons and precipitation 7–10 million tons of nitrogen. Hence the world soil humus is the chief supplier of crop nitrogen. It is well known that the recovery of nitrogen by crops is smaller than that of phosphate and potash, chiefly due to loss of nitrogen as nitrogen gas in the processes of nitrate formation from manures and fertilizers, and this loss of nitrogen is retarded by organic matter and minerals. Experiments have shown that all nitrogenous fertilizers and manures when applied to land intensify the oxidation of soil humus due to the formation of nitrates in the soil. Organic matter such as straw, plant residues, grasses, etc., when incorporated in soils, not only improve the physical properties of the soil but also fix atmospheric nitrogen and protect the soil nitrogen and increase the humus capital and this is aided by different calcium phosphates. The world reserve of phosphate rocks is of the order of 21,000 million tons and its present utilization is 30 million tons annually. Moreover, the expanding world steel industry is supplying large amounts of basic slags rich in phosphate, lime, magnesia, manganese, copper, vanadium and other useful nutrients. Hence increased utilization of these cheap phosphates incorporated with organic matter could go a long way towards meeting the world-wide lack of nitrogenous fertilizers.

N. R. DHAR

THE HAFFKINE INSTITUTE

THE annual report of the Haffkine Institute for 1959, which refers to the continued difficulty of recruiting qualified staff, includes lists of grants and research fellowships and scholarships as well as of papers published during the year[*]. Research activities in the Department of Antitoxins and Sera included the antigenic analysis of cholera vaccine and the use of submerged or shaken cultures for growing diphtheria toxin and pertussis vaccine. In the Department of Chemotherapy, the synthesis of compounds of possible anti-tubercular activity continued, including heterocyclic derivatives of thiourea and thiocarbanilide. Compounds with the *p*-nitrophenyl and/or dichloroacetyl groups attached to the pyrimidine, thiazole and pyrazole ring were also synthesized and tested *in vitro* against *Salmonella typhosa*, while other pyrazole and pyrazolene derivatives have been synthesized for examination as anti-convulsants. Two 1 : 3-disubstituted sulphonyl ureas have shown promise as antidiabetics.

The Department of Clinical Pathology and Diagnostic Reagents has finally identified *E. coli* from urine by sero type (preliminary) of 'O' antigen determination and is pursuing its investigations on the antigenic structure of the typhoid–paratyphoid group under partial anaerobiosis for possible application in preparing improved agglutinable suspensions. Besides epidemiological studies of enteric infection, it has continued its antigenic work on cholera vaccine, its investigations on otomycoses and, in collaboration with the Entomology Department and the Director's Laboratory, on the alimentary canal of the different rat-fleas with reference to the growth-promoting factors of some protozoa and bacteria. The Department of Entomology completed its studies on *Drosophila muris*, and started work on the morphological structure of the salivary glands of different non-poisonous snakes in comparison with the parotid glands of the poisonous snakes. Besides work on cholera autotoxin, the Department of Pharmacology continued its work on indigenous drugs, and has developed a new colorimetric method for determining 5-chloro-7-iodo- and 5 : 7-diiodo-8-hydroxyquinoline by coupling with *p*-nitroaniline-2-sulphonic acid, and modified methods for determining *m*-aminophenyl in *p*-aminosalicylic acid have been examined as well as methods for determining 'Novalgin', 'Avil' and thymol.

In the Department of Virus Diseases the immunogenicity of viral vaccines was investigated with the view of standardizing testing procedures and work on the adaptation of locally isolated rabies street virus strains to embryonated eggs. The Biochemistry Section reports on preliminary methods of preserving tissues from cadavers, the screening of vegetable extracts for blood-group activity and the preparation of anti-*A* and anti-*B* serum, while the Department of Nutrition continued its nutritional evaluation of foodstuffs commonly consumed in Bombay State, and its investigations into the nutritive value of some leafy vegetables and their supplementary effect, as well as its work on fermented foods and on the effect of commercial sterilization on the nutritive value of milk.

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[*] Government of Bombay. Annual Report of the Haffkine Institute for the Year 1959. Pp. ii+74. (Bombay: Director, Government Printing and Stationery, Maharashtra State; London: High Commissioner for India, 1960.) Rs. 0·60; 1*s*.

HOSPITAL IN-PATIENTS IN ENGLAND AND WALES

THE hospital in-patient inquiry for the years 1956–57 was organized jointly by the Ministry of Health and the General Register Office to provide statistics which will assist in the administration of the hospital services and contribute towards the knowledge of illness (morbidity) in the community[*].

Information for the inquiry is supplied by hospitals, where the form is completed for one patient in every ten discharged, giving a confidential summary of the case record. Hospitals of all types except psychiatric take part in the inquiry which, since 1958, has covered virtually all hospitals in England and Wales. In 1956 and 1957, the years to which the present report relates, the inquiry covered 68·5 and 82·1 per cent respectively of all hospital discharges and deaths in England and Wales.

Apart from the main reference tables the report contains, for 1957, a series of tables devoted to duration of stay. This is related directly to bed-usage, and the figures should be of value for planning purposes.

Other features of the report are: a discussion of the statistical and other problems which arise in the interpretation of hospital morbidity data; some analyses according to the type of hospital in which patients were treated, and, for 'acute' hospitals, their size; a table showing age distribution for the 800 categories of the *Detailed List of the International Statistical Classification of Diseases*, and tables for six of the fully represented regions showing the local administrative area in which patients resided and the hospital group in which they were treated. These help to determine the 'catchment areas' of hospital groups.

Altogether, there were 3,485,000 discharges and deaths in 1957 in hospitals during the inquiry. The average duration of each spell of hospital care was slightly shorter than in 1955 or 1956, being 22 days for males compared with 24 days in each of the two preceding years and 20 days for females compared with 22; in half the cases the duration of stay was less than 10½ days.

During 1957 maternity cases stayed an average of 10 days after delivery compared with 11 days in 1955. The proportion of mothers discharged within 7 days of delivery increased from 7 per cent in 1955 to 13 per cent in 1957. 51 per cent of mothers stayed very in 1957 and 36 per cent 11 ged 5–9 years, had the shortest average spital (11 days) and patients, aged 75 and longest (45 days for men and 63 for women). h this is mainly due to more chronic illness in r people, small children and older people stayed nger than older children and young adults for treatment of the same condition.

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[*] Ministry of Health; and General Register Office. *Report on Hospital In-Patient Enquiry for the two years 1956–1957*. Pp. vii+335. (London: H.M.S.O., 1961.) 18*s*. net.

Of the patients aged 65 and more, women usually stayed longer than men; for circulatory diseases the average stay for men was 53 days and for women 72, and for injuries other than head injuries 29 days for men and 41 days for women.

In 'acute' non-teaching hospitals the average length of stay was shortest in medium-sized hospitals. The same was true at all ages and for both sexes, and for many specific diagnoses.

From data on the duration of stay, the number of hospital beds required by different groups of patients can be calculated. The number of beds required per 10,000 population increases greatly with the patient's age, ranging from 20·8 at ages 15–24 to 188·88 at ages 75 and more for men, and from 18·7 at ages 5–14 to 187·3 at ages 75 and more for women.

Of the 40·8 beds per 10,000 population used for male cases, 13 per cent were taken up by cases of respiratory tuberculosis; 7 per cent were occupied in treatment of malignant neoplasms, 13 per cent for cardiovascular diseases, 10 per cent for respiratory diseases and 11 per cent for digestive diseases. For females, 48·9 beds per 10,000 population were in use; 7 per cent of them for respiratory tuberculosis, 6 per cent for malignant neoplasms, 8 per cent for digestive diseases and 15 per cent for maternity cases.

RESPONSE TO HOT ENVIRONMENTS

A REPORT* prepared by the Medical Research Council owes its origin to the concern felt by the Board of Admiralty regarding the thermal conditions to which men were exposed during the Second World War when serving in His Majesty's ships in tropical waters. This led, in 1944, to the appointment of a committee to inquire into and advise on the problems in this field. It was recognized from the outset that investigations would eventually have to be made in a tropical climate, and, in 1948, the Royal Naval Tropical Research Unit was established in Singapore under the joint auspices of the Admiralty and the Medical Research Council.

Before the establishment of the unit at Singapore, however, investigations were initiated in London at the National Hospital for Nervous Diseases. In the experiments carried out in London, the physiological effect on men wearing different types of clothing of exposure to high air temperatures was examined at different humidities and air speeds, both when the men were resting and when they were working. On the results of these experiments, for which the subjects had been artificially 'maximally acclimatized' by previous exposure to hot conditions in the laboratory, it was possible to base tentative recommendations concerning the conditions of warmth that were acceptable in ships at sea in tropical waters. The results also seemed to indicate that the 'effective temperature scale', which had been accepted for use as a measure of the severity of environment in ships, was misleading in certain respects. This led Dr. B. McArdle to devise a new index, the 'predicted four-hour sweat-rate' (P4SR), for the assessment of hot environments.

With the establishment of the unit at Singapore similar investigations were carried out. These confirmed largely the previous findings in London and, though they did not entirely support the criticisms of the 'effective temperature scale', they did demonstrate that the 'predicted four-hour sweat-rate' was an accurate and convenient measure of the severity of indoor hot-environments. The work at Singapore was, however, extended to compare the relative tolerance to heat of men who had been artificially 'maximally acclimatized' in the laboratory, with those who had been 'naturally acclimatized' by residence in a tropical climate. Investigations were also made of the stress imposed by radiant heat of long wavelength and by different rates of energy expenditure. Work carried out to determine the upper limits of warmth to be permitted in ships was extended to determine the upper limits that could be physically tolerated and the position of the 'comfort' zone. In addition, a number of physiological problems associated with these main lines of research was examined.

Now that the results of all the investigations in London and in Singapore have been brought together, it is clear that considerable additions to knowledge have been achieved. Much information has been obtained on the relative effects of various levels of air temperature, humidity, air speed, and radiant heat, and about the limits of heat which people can tolerate.

Answers have been provided to some of the practical problems of the Navy; the critical examination of a widely used scale of environmental heat-stress and the production and testing of a new index have improved and added to the techniques available for evaluating the severity of different climatic environments. As well as achieving these practical ends, this work has extended knowledge of the physiological mechanisms involved in the process of adaptation to hot environments and has indicated the direction for future research.

The report has been compiled by Dr. R. K. Macpherson with the assistance of an editorial board composed of Dr. T. Bedford, Dr. O. G. Edholm, Surgeon Captain F. N. Ellis, and Dr. N. H. Mackworth. It brings together much patient and arduous work by a number of service-men and civilians, and it is a fine example of how long-term investigations should be planned, organized and controlled. Many of its findings should be of interest to industrial organizations where hot processes are involved; managements in the new Indian steelworks should find much of value.

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* Privy Council. Medical Research Council Report Series, No. 298. *Physiological Responses to Hot Environments.* Compiled by R. K. Macpherson. (An account of work done in Singapore, 1948–1953, at the Royal Naval Tropical Research Unit, with an appendix on preliminary work done at the National Hospital for Nervous Diseases, London.) Pp. xv + 323. (London: H.M. Stationery Office, 1960.) 35*s.* net.

SEA SURFACE TEMPERATURE IN COASTAL WATERS OF T BRITISH ISLES

INVESTIGATIONS of the sea temperatures in British coastal waters have hitherto been neglected despite their value to workers interested in the ecology of the seashore and to meteorologists in connexion with fog forecasting. Mr. Lumb's short paper on the variations of these temperatures is therefore to be welcomed*.

The paper is really concerned with the whole of the shelf seas around the British Isles and not just with the waters within a few miles of the coasts. The typical winter and summer isotherm patterns, as derived from charts published by the International Council for the Exploration of the Sea, are first discussed. The winter pattern is explained in terms of cooling and vertical mixing in relation to the depth of the water column, but no mention is made of the part played by currents. In the North Sea, for example, the winter isothermal pattern is dominated largely by two tongues of warm water which are the result of currents bringing water into the sea from the Altantic through the Straits of Dover and to the north of Scotland. To explain the summer pattern the work of Dietrich is used: this shows that stratification of the water column due to the absorption of solar radiation cannot take place in areas where the tidal streams are sufficiently powerful to provide a strong vertical mixing agency. In this connexion it is stated that the English Channel is nearly isothermal throughout the year. This is not so, as a thermocline develops in the western English Channel in summer.

The possibility of drawing quasi-synoptic temperature charts for five-day periods on the basis of sea-temperature observations made by light-vessels and a few selected ships is then examined, the typica seasonal isotherm patterns being taken as a guide to the course of the isotherms in places where the observations are scanty. Such charts are considered to be a possible aid to local weather forecasting. Five examples of the charts that can be produced by using this technique are given, and at first sight they appear to give reasonable pictures of the prevailing conditions. If, however, one goes beyond this paper and compares them with the corresponding monthly charts published by the International Council for the Exploration of the Sea, a few discrepancies are found even when allowance is made for the fact that the Councils' charts are based on a thirty-day period. They are probably due to different observational methods being employed. Indeed, the relative merits of the various ways of taking sea surface temperatures are a vexed question, but Mr. Lumb does not discuss the accuracy of the observations on which his charts are based. Further, there is some loss of detail, particularly as regards temperature gradients, in his charts compared with those of the Council, as the latter are based to a large extent on observations made by research vessels and merchant ships fitted with recording thermographs or sampling at close intervals. This loss might be important in so far as local forecasting is concerned. However, if charts are needed for forecasting purposes they must be produced rapidly, and the delay which usually occurs while oceanographic data are processed cannot be tolerated. Mr. Lumb is therefore wise to use only data which are readily available; but it would appear that if further investigations of this nature are to be carried out, then the marine meteorologists and oceanographers concerned should endeavour to standardize their methods and pool their data.

A. J. LEE

* Air Ministry: Meteorological Office. Scientific Paper No. 6: Seasonal Variation of the Sea Surface Temperature in Coastal Waters of the British Isles. By F. E. Lumb. Pp. ii + 21 (13 plates.) (London: H.M. Stationery Office, 1961.) 3s. net.

CORRECTION FOR EXTINCTION ERRORS IN CRYSTAL STRUCTURE ANALYSIS

By S. CHANDRASEKHAR and D. C. PHILLIPS*

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IT was shown recently[1,2] that it is possible to correct for primary and secondary extinction errors in the X-ray analysis of crystals by the use of plane polarized X-rays. If the incident beam is polarized with the electric vector perpendicular to the plane of incidence the integrated reflexion from a crystal suffering from primary or secondary extinction, or a combination of the two, is:

$$\rho'_{\perp} = \alpha|F|^2 - \beta|F|^4 \qquad (1)$$

to a good approximation when the proportional reduction of intensity by extinction ($\beta|F|^2/\alpha$) is not greater than about 25 per cent. If, on the other hand, the incident beam is polarized parallel to the plane of incidence, the polarization factor is $|\cos 2\theta|$, where θ is the Bragg angle, and since all other factors remain unchanged:

$$\rho'_{\parallel} = \alpha|F|^2\cos^2 2\theta - \beta|F|^4\cos^4 2\theta \qquad (2)$$

The true integrated reflexion for normal polarization:

$$\rho_{\perp} = \alpha|F|^2 = \frac{\rho'_{\parallel} - \rho'_{\perp}\cos^4 2\theta}{\cos^2 2\theta - \cos^4 2\theta} \qquad (3)$$

thus may be determined from measurements of $\rho'_{\perp}$ and $\rho'_{\parallel}$.

The theory has been verified experimentally in the determination of extinction in small crystals of rock-salt[2,3]. The main advantage of the technique is that

* External staff of the Medical Research Council.

it corrects for both primary and secondary extinction simultaneously in a crystal of any shape. Its principal limitation is that the equations become ill-conditioned for values of θ approaching 0°, 45° and 90°; the exact limits of applicability depend on the accuracy of the intensity measurements.

This article describes a simple apparatus which can be used conveniently in crystal structure analysis. The apparatus is shown in Fig. 1. Use is made of the fact that the angle 2θ for the 311 reflexion of copper K_a rays by diamond is close to 90° (actually 91° 34′ mean for $\alpha_1\alpha_2$). The reflected X-rays are therefore almost completely polarized with the electric vector perpendicular to the plane of incidence. The X-ray tube and diamond crystal are mounted on a disk which can be rotated about a horizontal axis collinear with the collimator of the diffractometer, in such a way that the reflected beam passes along the axis for every setting of the disk. The diamond is in the form of a plate (approximately 8 × 7 × 2·5 mm.) with (111) faces and the asymmetry of the 311 reflexion produces a broad beam convenient for use with small crystals completely bathed in X-rays. This beam is sufficiently strong for measurements of reflexions from a crystal mounted on the diffractometer to be made in the usual way by means of a proportional counter and crystal oscillation mechanism[4].

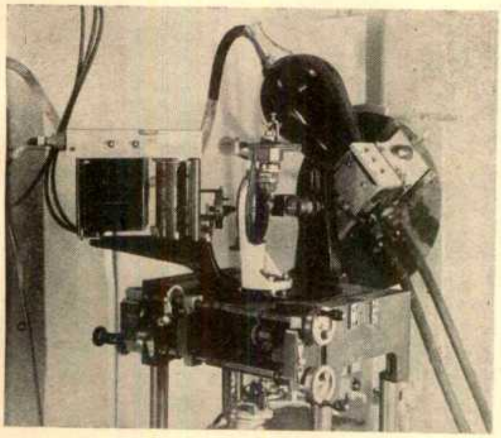

Fig. 1. Single-crystal diffractometer with apparatus for rotating plane of polarization of incident X-ray beam

The apparatus has been tested by performing Barkla's polarization experiment, using the 311 reflexion from small diamond crystals mounted on the diffractometer. The variation of integrated intensity with polarization angle was found to agree closely with the theoretical value and the ratio of the intensities of the two perpendicularly polarized components of the incident beam was estimated to be 0·0064.

The elimination of extinction errors by means of this technique has been tested in a study of reflexions from hexamethylene tetramine[5]. These crystals were chosen because some reflexions are known to suffer heavily from extinction[6], and because it seemed likely that liquid air shock treatment[7] would change the state of perfection of the crystals and enable a comparison to be made of corrected intensities from the same crystal with different amounts of extinction.

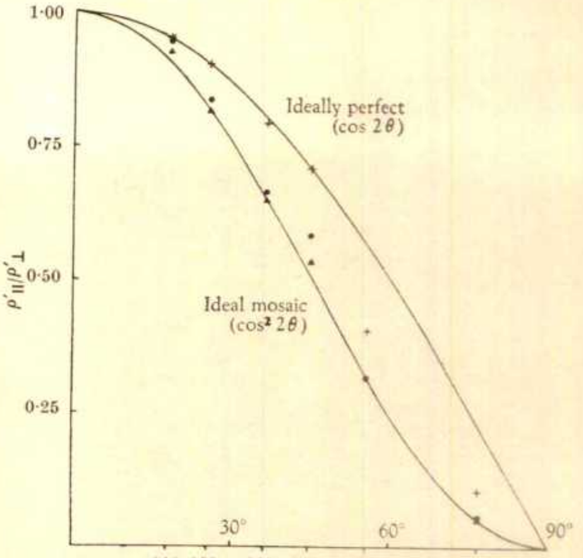

Fig. 2. $\rho'_{\|}/\rho'_{\perp}$ for reflexions from hexamethylene tetramine: +, freshly grown crystal; ●, after one liquid air treatment; ▲, after two liquid air treatments

Fig. 2 illustrates the application of the method to one crystal with diameter about 0·05 cm. The ratio $\rho'_{\|}/\rho'_{\perp}$ for a range of reflexions is plotted against 2θ. The upper curve is the theoretical curve for a perfect crystal, the lower that for an ideal mosaic. The measured values are those for a fresh crystal (crosses), and for the same crystal after one liquid air treatment (circles) and after a second treatment (triangles). Clearly strong reflexions from the fresh crystal have values appropriate to a perfect crystal, but the double liquid air treatment has reduced all the reflexions to values near those for an ideal mosaic. It must be remarked, however, that the strongest two reflexions 110 and 222 were found to be extinguished even after repeated liquid air treatment. Seven other crystals of different sizes were examined and in none of them was it possible to eliminate the extinction completely

Table 1. MEASUREMENTS ON HEXAMETHYLENE TETRAMINE, $C_6H_{12}N_4$; SPACE GROUP $I\bar{4}3m$; a = 7·02 Å.

	Freshly grown crystal						
hkl	$\rho'_{\perp}$ (obs.)	$\rho'_{\|}$ (obs.)	$\rho'_{\|}/\rho'_{\perp}$	Extinction (exp.)	$\rho_{\perp}$	Theoretical $\rho'_{\|}/\rho'_{\perp}$ Ideally perfect cos2θ	Ideal mosaic cos²2θ
110 θ = 8° 56′	5,294	5,047	0·953	Highly perfect	—	0·9517	0·9057
200 θ = 12° 42′	2,056	1,860	0·905	Highly perfect	—	0·9033	0·8159
220 θ = 18° 6′	993	788	0·794	Highly perfect	—	0·8070	0·6512
222 θ = 22° 22′	3,374	2,397	0·710	Highly perfect	—	0·7104	0·5047
330 θ = 27° 46′	603	245	0·406	28 per cent	841	0·5660	0·3203
440 θ = 38° 24′	1,529	162	0·106	> 30 per cent	—	0·2286	0·0523
	After liquid air treatment:			Per cent			
110	22,148	20,947	0·946	> 30	—		
200	3,654	3,057	0·837	12	4,160		
220	1,898	1,264	0·666	6	2,020		
222	10,290	6,010	0·584	24	13,556		
330	805	255	0·317	0	805		
440	2,664	149	0·056	7	2,862		
	After further liquid air treatment:			Per cent			
110	28,400	26,355	0·928	20	35,800		
200	4,000	3,260	0·815	0	4,000		
220	1,953	1,270	0·650	0	1,953		
222	12,220	6,535	0·535	11	13,692		
220	817	—	—	—	817		
440	2,792	147	0·053	0	2,792		

Further liquid air treatment did not increase the intensities of any reflexions.

for these two reflexions by liquid air treatment. Nor was there any relationship between crystal size and the amount of residual extinction, indicating that primary extinction was probably taking place in these crystals[3]. These results again bring out the well-known fact that a crystal may behave as an ideal mosaic for a weak reflexion and yet show a high degree of perfection for a very strong one.

The measurements for this crystal are shown in Table 1, together with the integrated intensities corrected for extinction by the use of equation (3). These corrected values agree well for the different states of perfection of the crystal when the extinction is less than 25 per cent.

The complete set of measurements on this crystal took 8 hr. (with no breaks). There was evidence of a slight decrease in the intensities of reflexions from hexamethylene tetramine on prolonged exposure to air—approximately 6 per cent in 24 hr. No allowance has been made for this effect in the results presented here. Furthermore, the measurements have not been corrected for normal crystal absorption and they are, of course, on an arbitrary scale. The data have been corrected, however, for the small depolarization of the incident beam and the results for the seven other crystals, appropriately scaled, are in very good agreement.

We thank Dr. H. J. Milledge for the loan of the diamond plate, Dr. U. W. Arndt for help in setting up the counting system, Prof. R. King for his support, and finally, Sir Lawrence Bragg, whose interest and advice have been of the greatest value.

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GIANT GRAIN GROWTH IN ALPHA FERRIC OXIDE

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AN investigation of the electrical properties of α ferric oxide revealed an anomalous resistivity-temperature curve for semi-spectroscopically pure iron oxide supplied by Johnson, Matthey and Co., Ltd. (Fig. 1, *b*). Roasted ferric oxide gel gives a linear plot over the same temperature-range (Fig. 1, *a*). The deviation shown by the curve *b* material is consistent with inhomogeneity of composition within the grains.

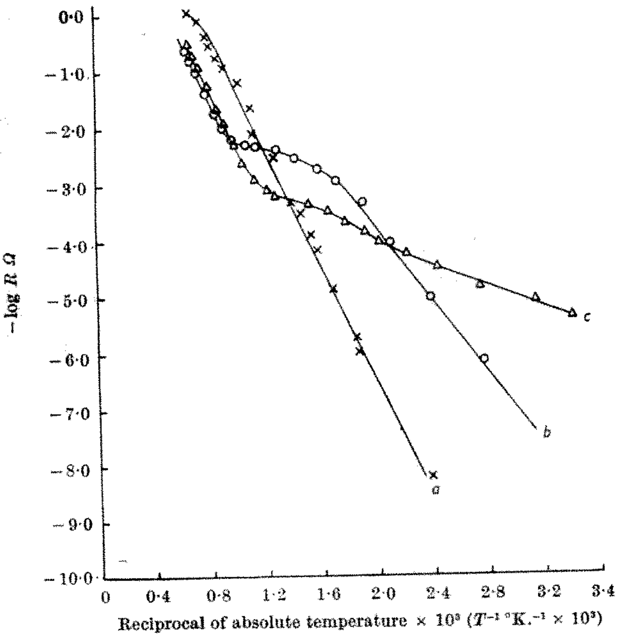

Fig. 1. The variation of resistivity of α ferric oxide with temperature: rectangular pellets, four probe. *a*, Ferric oxide from ferric nitrate by precipitation and roasting at 1,180° C. for 16 hr. in air; *b*, semi-spectroscopically pure ferric oxide 'as received'; roasted at 1,200° C. for 19 hr. in air; *c*, semi-spectroscopically pure ferric oxide treated as aqueous slurry with ultrasonic waves, 20 kc./s., 500 W. for 10 min.; roasted at 1,200° C. for 14 hr. in air

Attempts to remove the inhomogeneity included treatment of the material with ultrasonic waves prior to firing. A tuned stainless steel rod attached to an ultrasonic transducer (500 W., 20 kc./s.) was dipped into a slurry of 20 gm. semi-spectroscopically pure iron oxide in 200 ml. distilled water in a 'Pyrex' beaker. The slurry was agitated by slowly moving the beaker during the 10-min. treatment ; the temperature rose to 40° C. The oxide was separated by decantation and dried in an air oven. Pellets were pressed without binder at 25 tons/in.^{2} and fired in air at temperatures from 1,100° C. to 1,400° C.

The resistivity-temperature curve is modified by the treatment (Fig. 1, *c*). A polished section of this pellet showed giant grain growth throughout. The grains are distinguishable by anisotropy in polarized light and by etching with hydrochloric acid. Contrary to the usual pattern of crystallite growth, the inner grains are larger than those at the surface ; the grain sizes in a pellet of unirradiated material are much smaller. Giant grain growth in ultrasonically treated material has been observed several times (Fig. 2*a* and 2*b*). The effect is not destroyed by preheating the ultrasonically treated material at 700° C. for 15 hr. before pelleting. After firing at 1,300° C. the unirradiated material shows hexagonal grains with straight sides, ranging from 10 to 40μ in diameter. The grains in the ultrasonically treated material show very irregular grain boundaries and contain many pores ; the range in size is from 20μ for occasional grains near the periphery to 500μ for grains at the centre. The large grains show cracking both along grain boundaries and across grains, suggesting comparable strength of intra- and inter-crystalline material. The cracks probably occur during cooling, due to the anisotropy of the oxide. The temperature at which giant grain growth occurs in semi-spectroscopically pure iron oxide has been observed to vary from 1,200° C. to 1,375° C. The general appearance of the grains suggests rapid and irregular movement of the grain boundaries unhindered by pores.

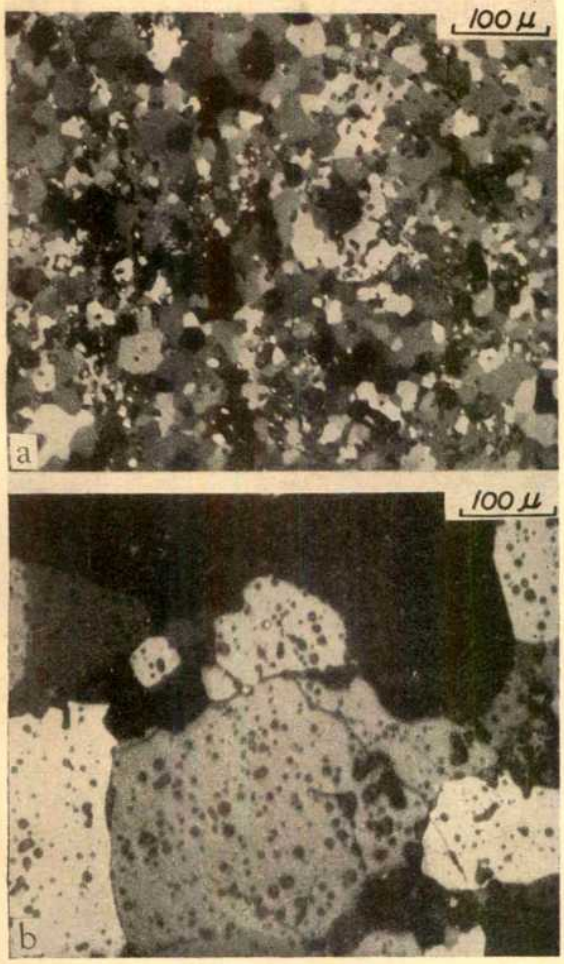

Fig. 2. Polished sections of pellets of semi-spectroscopically pure iron oxide fired in air at 1,300° C. for 15 hr.; incident polarized light: *a*, untreated; *b*, treated with ultrasonic waves before pressing and firing

Giant grain growth has not been observed in 'as-received' semi-spectroscopically pure iron oxide at temperatures up to 1,400° C., nor in the oxide slurried and dried without ultrasonic treatment.

Four possible explanations can be suggested. The first is that some impurity has been introduced during the ultrasonic treatment. The addition of 0·2 per cent titania to alumina causes giant grain growth[1], probably due to the formation of a eutectic melting below the firing temperature and assisting secondary crystallization. Spectroscopic analysis of semi-spectroscopically pure iron oxide gave: calcium, sodium $\leqslant$ 0·1 per cent; silicon $<$ 0·05 per cent; chromium, manganese, aluminium, titanium, lead, each $<$ 0·02 per cent; nickel, copper, tin, each $<$ 0·01 per cent; molybdenum $<$ 0·005 per cent. No difference could be detected between the untreated and the treated material.

Two other explanations, that grain-growth is due to improved crystallite orientation, or to cleaning of the grain surfaces, are not easily evaluated. Cavitation will clean and probably de-gas the surface of a solid, will de-gas a liquid and produce very localized high temperatures. Chemical effects in aerated distilled water include the formation of traces of hydrogen peroxide. The iron oxide surface may be chemically modified, increasing the number of carriers (Fig. 1, *c*). Increased conductivity may also be due to the elimination of grain boundaries.

Finally, excessive growth might be attributed to a change in particle-size distribution. The fines in the slurry settle more slowly after ultrasonic treatment. Although ultrasonic waves in the 20 kc./s. range will de-laminate graphite and mica[2], it seems unlikely that comminution occurs at these wave-lengths. The fines probably result from the dispersion of agglomerates. Giant grain growth occurs in beryllia if the starting material has a wide particle-size distribution[3], though other factors are important[4]. Giant grain growth in iron oxide may be due to separation and dispersion of the fines throughout the material: it is observed in a temperature-range where grain growth becomes visible and where dissociation is incipient. Fine particles will be thermodynamically less stable at these temperatures and their resistance to re-orientation by an advancing grain boundary may be correspondingly reduced.

This work forms part of the research programme of Warren Spring Laboratory and is published by permission of the director.

We are greatly indebted to Mr. Owen-Roberts of Mullards, Ltd., for providing facilities for ultrasonic irradiation and for his helpful discussion ; and to Mr. L. D. Muller of Warren Spring Laboratory for preparing the sections.

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DERIVATIVES OF ISATIN BETA-THIOSEMICARBAZONE WITH ANTI-VIRAL CHEMOTHERAPEUTIC ACTIVITY AGAINST ECTROMELIA INFECTION

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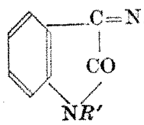
THE anti-viral chemotherapeutic activity of isatin β-thiosemicarbazone (I) in mice infected intracerebrally with neurovaccinia virus was investigated by Bauer[1], who found that mice infected with 100,000 *LD*50 of neurovaccinia virus could be protected against death by the repeated subcutaneous administration of the compound in doses of 2 mgm. Bock[2] also studied the effect of isatin β-thiosemicarbazone in mice infected intracerebrally with vaccinia, and made the additional observation that the compound had no antiviral effect in mice infected with ectromelia. In view of the close antigenic relationship between the two viruses, this finding was very surprising, and cast the first doubt on the generally held view that an anti-viral compound would have to act by selectively affecting some host-cell system which was required for the multiplication of the virus. The observed fact that isatin β-thiosemicarbazone is active against vaccinia but not against ectromelia suggests that it is acting against the virus rather than against the host cell, which is the same in both infections. The force of this argument is not essentially reduced by the subsequent observation of Sheffield, Bauer and Stephenson[3] that isatin β-thiosemicarbazone is active against ectromelia virus in tissue culture, since the activity was very low and at the very limit of detectability. Bauer and Sadler[4] tested 19 derivatives of isatin β-thiosemicarbazone and related compounds for activity against ectromelia in mice, but none showed any evidence of activity. It is therefore of considerable interest that further work on the modification of the molecule of isatin β-thiosemicarbazone has now revealed a series of derivatives with high activity against ectromelia infection, and with little or no activity against vaccinia.



(I)

The compounds so far found to possess anti-ectromelia activity are all 4′ : 4′-dialkylthiosemicarbazones of isatin, N-methylisatin or N-ethylisatin. Dimethyl-, diethyl- and dibutyl-thiocarbamylthioglycollic acids were obtained by Jensen's method[5]. These, when heated in aqueous alkaline solution under reflux with excess hydrazine hydrate, produced the corresponding 4 : 4-dialkylthiosemicarbazides. The dibutyl compound, which is an oil, was converted to the hydrochloride by passing dry hydrogen chloride through its benzene solution. The 4′ : 4′-dialkylthiosemicarbazones (Table 1) were prepared by heating under reflux equimolar quantities of the isatin and reagent in ethanolic solution. In the case of the dibutyl derivatives, 50 per cent aqueous ethanol was used with the addition of one equivalent of sodium acetate. The melting points and analytical results for the 4′ : 4′-dialkylthiosemicarbazones are shown in Table 1.

Table 1. ANALYTICAL RESULTS FOR 4′: 4′-DIALKYLISATIN β-THIOSEMICARBAZONES

R	*R′*	Melting point (°C)	Formula	Required percentages C	H	S	Found percentages C	H	S
CH_3	H	232	$C_{11}H_{12}N_4OS$	53·2	4·8	12·9	53·2	4·8	12·6
CH_3	CH_3	196	$C_{12}H_{14}N_4OS$	54·9	5·3	12·2	54·7	5·0	12·4
CH_3	C_2H_5	193	$C_{13}H_{16}N_4OS$	56·5	5·8	11·6	56·3	6·1	11·9
C_2H_5	H	211	$C_{13}H_{16}N_4OS$	56·5	5·8	11·6	56·5	6·0	11·7
C_2H_5	CH_3	129	$C_{14}H_{18}N_4OS$	57·9	6·2	11·1	57·8	6·0	10·9
C_2H_5	C_2H_5	112	$C_{15}H_{20}N_4OS$	59·2	6·6	10·5	59·4	6·8	10·7
C_4H_9	H	151	$C_{17}H_{21}N_4OS$	61·4	7·2	9·7	61·5	7·3	10·0
C_4H_9	CH_3	110	$C_{18}H_{23}N_4OS$	62·4	7·5	9·3	62·3	7·7	—
C_4H_9	C_2H_5	87	$C_{19}H_{26}N_4OS$	63·3	7·8	8·9	63·1	7·9	8·8

The tests of anti-viral activity were carried out by two methods. In the first method, serial decimal dilutions of ectromelia virus (Sandom strain of pseudolymphocytic choriomeningitis virus) in the form of a suspension of infected mouse brain were inoculated intracerebrally or intraperitoneally in parallel in two series of mice of uniform weight, two groups of 6 mice being infected with each dilution; one series was treated with the compound under investigation and the other served as a control. The compound selected for test was inoculated subcutaneously twice daily for 5 days, the first dose being given some hours after infection. The mice were observed over a period of 14 days and the deaths were recorded. The results of five typical tests are given in Table 2. Isatin β-4′ : 4′-dimethylthiosemicarbazone (I; $R = CH_3$, $R' = H$) confers a significant degree of protection in mice infected with 100 *LD*50 of virus intracerebrally, and N-methylisatin β-4′ : 4′-dimethylthiosemicarbazone (I; $R = CH_3$, $R' = CH_3$) confers some protection against 1,000 *LD*50. With intraperitoneal infection the results were less regular, but the first compound nevertheless gave some protection against 1,000 – 10,000 *LD*50, and the second against 1,000 *LD*50. In the tests described so far the compounds were given at dose-levels of 25 and 50 mgm./kgm., but an antiviral effect was still

Table 2. EFFECT OF ISATIN AND N-METHYLISATIN 4′: 4′-DIMETHYL THIOSEMICARBAZONES IN MICE INFECTED WITH ECTROMELIA VIRUS

Compound	Dose of compound (mgm./kgm.)	Route of infection	Dose of virus (*LD*50)	Treated	Control
Isatin β-4′: 4′-dimethylthiosemicarbazone	25	Intracerebrally	1,000	6/6	6/6
			100	3/6	6/6
			10	0/5	6/6
			1	0/6	3/6
	25	Intraperitoneally	10,000	2/5	6/6
			1,000	5/6	6/6
			100	5/6	6/6
			10	0/6	5/5
N-methylisatin β-4′ : 4′-dimethylthiosemicarbazone	50	Intracerebrally	1,000	3/5	6/6
			100	1/5	6/6
			10	0/5	6/6
			1	0/5	3/6
	50	Intraperitoneally	1,000	0/6	12/12
	25		1,000	3/6	12/12
	12·5		1,000	4/6	12/12

Numerator, number of mice dying; denominator, number in group.

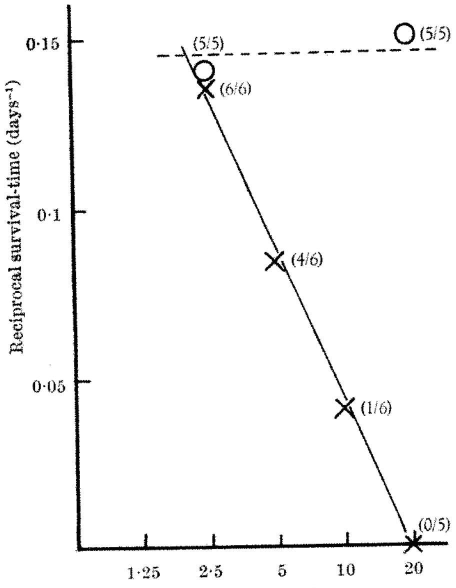


Fig. 1. Dose-response curve of antiviral chemotherapeutic effect of isatin β-4′ : 4′-diethylthiosemicarbazone in mice infected intracerebrally with 100 *LD*50 of ectromelia virus
○, control; ×, treated. Numerator, number of mice dying; denominator, number of mice in group

observed with the N-methyl derivative at a dose-level of 12·5 mgm./kgm. as shown in the last test of Table 2.

In tests carried out by the second method, six groups of mice of uniform weight were inoculated intracerebrally with about 100 *LD*50 of ectromelia virus; the first and last groups were left untreated as controls and the remaining groups were treated with the test compound in doses increasing by multiples of 2 given subcutaneously twice daily for 5 days. The animals were examined for 14 days; the times of death were recorded to the nearest half-day and converted to reciprocals and the mean reciprocal survival-time for each group was plotted against the dose on a $\log_2$ scale. A result obtained with isatin β-4′ : 4′-diethylthiosemicarbazone (I; $R = C_2H_5$, $R' = H$) is shown in Fig. 1. The dotted line represents the mean response (reciprocal survival-time) obtained in the two control groups, and the continuous line has been fitted to the combined responses of the treated mice by the method of least squares. The responses do not differ significantly from linearity, and the anti-viral action of the compound can thus be represented by a linear dose-response curve, with complete protection against 100 *LD*50 of virus at a dose-level of 20 mgm./kgm., as represented by a reciprocal survival-time of zero.

In further tests carried out by one or both methods, marked activity against intracerebral or intraperitoneal infection with ectromelia virus was also found with isatin β-4′ : 4′-dibutylthiosemicarbazone (I; $R = C_4H_9$, $R' = H$), N-methylisatin β-4′ : 4′-diethylthiosemicarbazone (I; $R = C_2H_5$, $R' = CH_3$), N-methylisatin β-4′ : 4′-dibutylthiosemicarbazone (I; $R = C_4H_9$, $R' = CH_3$), and the three corresponding N-ethyl derivatives (I; $R = CH_3$, C_2H_5 or C_4H_9, $R' = C_2H_5$). Anti-viral activity of the same extent was also found in similar experiments with the Hampstead and Moscow strains of ectromelia virus. The compounds so far described had little or no activity against vaccinia infection in tests carried out by the same method.

The discovery of high activity against ectromelia in compounds of this type thus significantly extends the spectrum of anti-viral action of the isatin β-thiosemicarbazone derivatives, and gives reason for supposing that further modifications may lead to the acquisition of activity against other viruses.

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NEURAMINIDASE IN THE CHORIOALLANTOIS OF THE CHICK EMBRYO

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IN 1941, Hirst[1] and McClelland and Hare[1] described hæmagglutination by influenza virus. The enzymic nature of this reaction was first suggested by Hirst[2], who showed that following agglutination of red cells, the virus eluted spontaneously, leaving the cells inagglutinable by the same strain of virus; the liberated virus could agglutinate fresh red cells. Following the discovery that other viruses (including mumps and Newcastle disease) had similar activity, Burnet, McCrea and Stone[3] found that cells from which one strain of myxovirus had eluted were still agglutinable by certain other strains; the arrangement of virus strains in the order of their progressive action on cellular receptors was termed the 'receptor gradient'. In 1946, Burnet, McCrea and Stone[3] discovered in filtrates of *Vibrio cholerae*, a soluble factor, the receptor-destroying enzyme[4], which rendered red cells inagglutinable by myxovirus: the progressive action of this enzyme destroyed the agglutinability of the cells for different virus strains in a characteristic sequence—the receptor-destroying enzyme receptor gradient—and this differed in some respects from that of myxoviruses. Both viral and bacterial enzymes are now called neuraminidase[5] or sialidase[6], as their action has been identified as the hydrolytic cleavage of the glycosidic bond joining the keto group of sialic acid to D-galactose or D-galactosamine[5].

Myxoviruses are commonly grown in cells present in the chorioallantoic membrane (hereafter referred to as membrane) of chick embryos 10–12 days old. A previous attempt using biological methods to show the presence of neuraminidase activity in normal membrane was unsuccessful[7]. In this article we report the presence of neuraminidase activity in such extracts. The enzyme has been partially purified and concentrated, and its activity is described in biological and chemical terms. The possible relationship between this enzyme and the myxovirus enzyme is discussed.

Detection of the enzyme. The enzyme was detected by incubation of a sterile brei of membranes from 12-day-old embryos with excess sialyl lactose, a substrate of neuraminidase. The mixture was incubated (20 hr., 37° C., *p*H 5·7), samples taken at intervals and the amount of split product determined by the Warren test[8]. At a concentration of 1 mgm./ml. of sialyl lactose, the rate of release of split product, estimated as N-acetylneuraminic acid, was about 1·5 μgm./ml./hr.; the rate of action decreased slowly over the 20 hr. period. This activity was shown to be present in extracts heated to 56° for 30 min., but absent in extracts heated at 80° C. for 15 min.

Age of the membranes. The neuraminidase activity was found to vary with the age of the embryos from which the membrane was obtained. In one experiment, membranes from 8, 10, 12, 15, 18 and 19 day embryos (3 membranes of each group) were collected and ground with powdered 'Alundum' into *tris*-maleate buffer (0·1 *M*, *p*H 5·7, 2 ml./membrane). After clarification, the enzymic activity of each extract was determined (Table 1). In another experiment, twelve membranes from 19-day-old embryos were collected and extracted separately. All showed high levels of enzymic activity although the values showed considerable spread as would be expected. (Rate of release of N-acetylneuraminic acid (μgm./ml./hr.) = 18 ± 3·8 *S.D.*; average protein concentration = 4·0 mgm./ml.). Membranes of 19-day embryos from two different poultry farms showed similar high levels.

Table 1. Liberation of Split Product estimated as N-acetylneuraminic Acid (N-ANA) from Sialyl Lactose (1 mgm./ml.) by Extracts of Avian Chorioallantoic Membrane

The reaction mixtures were incubated at 37° C. for 4½ hr. Protein was estimated by the method of Lowry *et al.* (ref. 24)

Age of embryo (days)	Protein concentration of extract (mgm./ml.)	Release of N-ANA (μgm./ml./hr.)
8	3·1	< 1·0
10	2·7	< 1·0
12	4·0	1·2
15	3·5	2·8
18	5·1	16·1
19	4·5	19·0

Addition of a membrane extract from 10-day-old embryos to a membrane extract from 19-day-old embryos did not significantly depress the enzymic activity of the latter. Membrane extracts from both 11- and 18-day-old embryos were found to contain approximately similar amounts of N-acetylneuraminic acid–aldolase, the enzyme which splits N-acetylneuraminic acid to N-acetylmannosamine and pyruvate[9]. The amount present was not sufficient to interfere seriously with the estimation of neuraminidase at pH values of 5·7 or lower.

Bacterial sterility of membranes and extracts. To eliminate the possibility that bacterial contamination of membrane or extract was responsible for the enzymic activity, four membranes from 19-day embryos were ground with 'Alundum' under aseptic conditions, clarified and enzyme assays carried out on each extract (37° C., 2 hr.; no antibiotics added). Each extract showed a high enzymic activity. Samples of the extracts before clarification and after the assay procedure were tested for aerobic and anaerobic bacteria, for fungi and for pleuropneumonia-like organisms. No growth of any organisms was detected over a period of 2 weeks incubation. In most work penicillin (100 units/ml.) and streptomycin (50 units/ml.) were included in the reaction media.

Concentration and purification of the enzyme. Membranes from 250 embryos (19 days old) were ground with 'Alundum' and the mixture extracted with *tris*-maleate buffer (pH 5·7, 0·1 M). The mixture was centrifuged and to the supernatant (about 1 litre) was added calcium chloride to a final concentration of 0·1 per cent (w/v). The solution was heated (56° C., 30 min.), centrifuged (70,000g, 10 min.) and ammonium sulphate added to the supernatant to a final concentration of 60 per cent (w/v). After standing (0° C., 16 hr.), the suspension was centrifuged (70,000g, 10 min.), and the precipitate dissolved in and dialysed against potassium phosphate buffer (0·1 M, pH 6·8). The solution was clarified by addition of protamine sulphate (0·3 gm.) and chromatographed on a column of hydroxyl apatite[10] (gradient 0·1 M – 2·0 M phosphate buffer, pH 6·8). The enzymically active fractions were pooled and concentrated to about 5 ml. The enzyme was purified about 50-fold in this way and was suitable for the tests described below.

Properties of the membrane neuraminidase. Action on sialyl lactose. Purified enzyme (0·5 ml.) was incubated with 10 mgm. of sialyl lactose (37° C., 16 hr.). The identity of the split products was established as follows. In the Warren test, the absorption spectrum (500–600 mμ) of the chromophore derived from the enzyme substrate mixture was identical with that given by crystalline N-acetylneuraminic acid. Following paper chromatography in butanol/pyridine/water (6 : 4 : 3), three spots corresponding in R_F to sialyl lactose, N-acetylneuraminic acid and lactose were observed. In these experiments, more than 50 per cent of the sialyl lactose was split. The pH of optimum activity was about 4·5. The rate of action of the membrane enzyme was unaffected by 0·01 M ethylenediamine tetraacetate. K_m at pH 4·4 was about 1×10^{-3} M.

Action on ovine submaxillary gland mucin. Ovine submaxillary gland mucin was dissolved in the purified enzyme to a final concentration of 0·15 per cent (w/v) and the solution incubated (37° C., 16 hr.). Samples taken before and after incubation showed that (1) more than 60 per cent of the bound N-acetylneuraminic acid was liberated from the ovine submaxillary gland mucin. (2) The capacity of the treated ovine submaxillary gland mucin to inhibit hæmagglutination by heated *WSE* virus[12] was abolished (inhibitory titre reduced from $1{\cdot}56 \times 10^5$ to < 50).

Action on fowl red cells. The agglutinability of the fowl red cells for the different strains of myxovirus was removed in the order shown in Table 2. This receptor gradient was not the same as that given by neuraminidase from *V. cholerae* determined under the same conditions. It is also different in several respects from the receptor gradient established by enzymic treatment of red cells with myxoviruses[3]. It is difficult, however, to obtain a satisfactory gradient following viral action when the technique used in the present work is adopted, so the virus results are not fully comparable to those obtained with soluble neuraminidases.

Table 2. Receptor Gradient of Fowl Red Cells treated either with Neuraminidase from the Chorioallantois of the Chick Embryo or with Neuraminidase from *V. cholerae*

Source of neuraminidase	Receptor gradient					
Membrane	*MEL*	*NDV*	$\frac{Mu}{WSE}$	*LEE*	*MIL*	
V. cholerae	*MEL*	*WSE*	*NDV*	*LEE*	*Mu*	*MIL*

Serial two-fold dilutions of the purified enzyme and the crude enzyme from *V. cholerae* were allowed to act at pH 6·2 on 0·05 ml. amounts of washed packed red cells. The cells were then washed thrice with saline and their reaction to different strains of myxovirus tested (ref. 13).

Discussion

The results show that the chorioallantois of the chick embryo contains an enzyme which, in many respects, behaves like bacterial and viral neuraminidases. Thus the membrane enzyme splits both sialyl lactose where the carbon atom 2 of N-acetylneuraminic acid is linked (α) to carbon atom 3 of galactose and also ovine submaxillary gland mucin where the carbon atom 2 of N-acetylneuraminic acid is linked (α) to carbon atom 6 of N-acetylgalactosamine[14]; it also abolishes the capacity of ovine submaxillary gland mucin to inhibit hæmagglutination by heated influenza virus (although it has not been formally excluded that this may be due to a contaminating protease[15]) and destroys receptors of red cells for viral hæmagglutination. In contrast to the neuraminidase from *V. cholerae*, the activity of both the membrane and viral neuraminidases are unaffected by ethylenediamine tetraacetate[16].

We have been very much aware of the possible objection that the neuraminidase discovered in the membrane is of bacterial origin. It should be pointed out that the enzyme content of unconcentrated extracts of membranes from 19-day-old embryos is equivalent to about 400 biological units/ml. of bacterial (*V. cholerae*) neuraminidase[17]. Heavy growth of an organism such as *V. cholerae* would be required to yield this amount of neuraminidase. Careful bacteriological examination failed to detect any micro-organisms in the membranes or in the incubated extracts. We have not tested for latent viruses, but, for several reasons, this seems a very unlikely possibility.

The chief interest of this report is the finding for the first time of neuraminidase in vertebrate tissues. The possibility that it is present in all or most warm-blooded vertebrate cells cannot be excluded. One aspect which may be functionally significant is the demonstration that, as the fertile egg progresses to the stage where hatching of the embryo will occur, the neuraminidase content of the membrane increases ten- to twenty-fold. There is a suggestion here that the enzyme may play a part in preparing the membrane for its rather sudden loss of function as soon as the chick becomes air-breathing.

The possibility of a relationship between the membrane neuraminidase and the viral enzyme requires some consideration. At present it is reasonable to assume that the membrane enzyme is present in those cells which serve as host cells for the virus. The first question that arises is whether viral enzyme represents a component non-specifically adsorbed to the virus particle. This is most unlikely; the evidence is overwhelmingly in favour of the view that the enzyme is an integral component of the virus itself. Both serological[18] and chemical[19] analyses of purified myxoviruses support the view[20] that certain fractions of the myxovirus particle are very similar to components present in the normal host cell. Is the virus enzyme also in this category? Does virus invasion stimulate a change in cell metabolism which results in greatly increased neuraminidase production—a change that may in some way be analogous to the increase that takes place during the last days of incubation? In developing this suggestion we have to bear in mind: (*a*) that the enzymic activity of different myxoviruses shows a wide range of quantitative and qualitative differences; (*b*) that according to most authors the viral enzyme is related to the protein surface antigen *V*. To accommodate these findings, our hypothesis could take the form that the specific enzyme structure is determined by genetic information provided by the host but that this is incorporated into the virus under the influence of the virus genome, either during the intracellular synthetic process or during emergence from the host cell surface. If the specificity of the viral enzyme were of host origin this would help to explain why the myxovirus particle contains such a small proportion of ribonucleic acid[21]—an amount per virus particle of the same order as that present in much smaller viruses such as poliomyelitis[22], and presumably containing an equally limited amount of genetic information.

The present work does not, of course, exclude the possibility that the viral enzyme is as fully determined by the viral genome as any other component of the virus. It does, however, raise an important alternative. To choose between them will become easier if neuraminidases from the membrane and from representative myxoviruses are obtained in such a form as will allow a valid comparison of their physical, enzymological and serological properties.

While this work was in progress, Warren and Spearing[23] reported the presence of neuraminidase in commercial preparations of bovine and human serum glycoprotein. The enzyme was not detected in unprocessed plasma or serum. Their enzyme is stimulated by calcium ions and inhibited by ethylenediamine tetraacetate, so is different from the factor described in this report.

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MECHANISM OF THE TRANSFORMATION OF THE POLYSACCHARIDE CAPSULE IN PNEUMOCOCCUS

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AFTER an interval of more than a year in the transformation of the heterologous polysaccharide capsule I found that the *R* form, which was obtained in our laboratory in 1957, and which prior to this had been very easily transformable into the encapsulated form of the type III pneumococcus with the aid of the transforming principle isolated from the same by use of the transforming medium in the presence of salts with univalent cations[1–3], is now not easily transformable when using similar conditions as before. Either it does not transform at all and remains in the *R* form, or changes more or less massively into the encapsulated form of type II, that is, into the original parental strain. Only very rarely, and no longer under control or to a very small extent, if at all, is it transformed into the encapsulated form of pneumococcus of type III with the aid of the transforming principle isolated from this strain.

Ravin[4], using the strain *R36A* obtained originally in the laboratory by Avery *et al.* and successfully transformed in other laboratories[5,6] into various capsular states, found that the factor for antibiotic resistance was still transferable but not the capsular factor. I am of the opinion that the strain *R36A* may possess a gross deletion at one or more capsule-influencing loci.

Now the question arose whether in the course of preservation my *R* strain did not change to such a degree that it is unable to accept the transforming principle and to incorporate the relevant segment of deoxyribonucleic acid (DNA) into its genome or whether it had not been changed in another sense; on the other hand, whether my transforming principle had not been damaged (for example, by deoxyribonuclease) even though the isolation was always carried out in citrate medium and using all necessary precautions for the maintenance of its biological activity[7]. If this were the case, the damaged transforming principle would not function as a carrier of genetic information, that is, of information for the synthesis of the polysaccharide capsule of type III, but having been damaged would serve rather by its breakdown products to stimulate the synthesis of a non-specific DNA and thus also of a non-specific polysaccharide capsule in the recipient cell, in this case of type II.

I have in mind a similar effect to that described by Firshein and Braun[8], where the DNA, partly digested by enzyme and its products of cleavage, stimulates selectively the synthesis of the DNA in the *S* cells, in a mixture where there is a preponderance of *R* cells over *S* cells.

I examined first my *R* form, which had been originally fully avirulent, and found that, by testing in a solid medium or in glucose blood broth, the *R* form is not changed, but that in a selective medium, for example, in broth with anti-*R* serum, it is possible to intercept sporadically the maternal *S* form of type II both after 72 hr. of growth and *in vivo* when infecting mice.

The transforming principle was also checked. I investigated whether mild lesions of the polymeric molecule of the principle, due to small doses of deoxyribonuclease, would not result rather in a non-specific stimulation of the synthesis of the polysaccharide capsule of type II than in an induction of the synthesis of type III. I used a transforming principle (crude extracts of the DNA with 11 deproteinizations[7]) with a content of 2 μgm. DNA in 0·025 M veronal buffer *p*H 7·5 per 1 ml. of the medium and of deoxyribonuclease in a magnesium sulphate solution of a final concentration of 0·003 M. The transformation test was carried out either in the presence of sodium citrate of a final concentration of 0·01 M[9] or without it. The mixture of transforming principle and deoxyribonuclease with the citrate as well as without it had been activated for 15 min. at 30° C. and inactivated for 15 min. at 60° C. The control with the same content and with citrate in the medium had been neither activated nor inactivated. It was surprising to find that in the presence of deoxyribonuclease with a content of approximately 0·01 μgm./1 ml. of the medium and with a lower content (10^{-4}–10^{-5} μgm.) in the presence of citrate and in test-tubes, where the activation and inactivation had been carried out in the stated time-intervals, transformation of the *R* form into the *S* form of type III occurs regularly.

It is evident from Table 1 that the citrate added alone to the transforming medium inhibits the growth of the pneumococci and the test-tubes remain sterile. Among all variations of arrangements of components newly introduced into the transforming medium, transformation occurs solely in the presence of deoxyribonuclease in the concentrations already given in the presence of citrate and only when the mixture of the transforming principle and the deoxyribonuclease had been activated and inactivated, or only inactivated. If the deoxyribonuclease is left in the medium without inactivation in the presence of citrate, it prevents the transforming reaction.

Table 1. EFFECT OF THE COMPONENTS ADDED TO THE TRANSFORMING MEDIUM

(Composition of the medium (ref. 2): beef heart infusion, 1 per cent neopeptone (Difco), 0·5 per cent sodium chloride, potassium chloride, 1·5 × 10^{-4} M, 10 per cent anti-*R* serum), *p*H 7·6–7 8

TP	*R* rarely *S* III
TP activ. and inactiv.	*R*
TP + $MgSO_4$	*R*
TP + citrate	0
TP + $MgSO_4$ + citrate	*R*
TP + $MgSO_4$ + citrate + DNase	*R*
TP + $MgSO_4$ + citrate + DNase activ.	*R*
TP + $MgSO_4$ + citrate + DNase inactiv.	*S* III
TP + $MgSO_4$ + citrate + DNase activ. and inactiv.	*S* III
TP + $MgSO_4$ 3 × 10^{-5} M + DNase + citrate activ. and inactiv.	*R*
DNA + $MgSO_4$ + DNase + citrate 10^{-4} M activ. and inactiv.	*R*
DNA + $MgSO_4$ 3 × 10^{-5} + DNase + citrate 10^{-4} M activ. and inactiv.	*R*

$MgSO_4$ in the final concentration 0·003 M; sodium citrate in the final concentration 0·01 M; transforming principle (TP) (2 μgm. DNA/ml.) in veronal buffer 0·025 M, *p*H 7·5, if DNase is present, otherwise in physiological solution; deoxyribonuclease (Light, and Hungarian lyophil.) in a concentration from 10^{-2} to 10^{-5} μgm./ml. of the medium. The transformation was carried out in 2–3 control series with 1,000–3,000 cells of the recipient *R* cultures and the results read off after 16–96 hr. of growth in a liquid medium by sub-culturing twice daily on to glucose blood agar twice from each test-tube. The macroscopic identification was confirmed by agglutination with a homologous immune serum.

R, non-encapsulated form of the pneumococcus; *S* III, encapsulated form of the type III pneumococcus; 0, sterile growth.

The question has been posed whether a similar mechanism, that is, the mutual interaction of the DNA and the deoxyribonuclease with the participation of the blocking system, acts also normally in the processes of transformation. The problem of the penetration of the DNA as a high polymeric molecule into the cell still remains unknown. In experiments with newly isolated 9 new *R* strains, of which 12 single-colony cultures were chosen for the experiment, it was found that both the stable and the unstable strains are liable to transformation into the heterologous type, and that out of the twelve single-colony *R* cultures examined only 4 were transformed without deoxyribonuclease; the remainder only when it was present. As the experiments were carried out with two transforming principles obtained from the isolation of the same culture of the pneumococcus of type III, differing from each other only in age by 3 hr. (6 and 9 hr. culture) it was ascertained that it was possible to transform without deoxyribonuclease the newly gained strains only when the principle isolated from the younger culture was used.

It is evident from the experiment that the process of transformation of the heterologous polysaccharide capsule is by no means simple. There exist mutual interactions between the physiological state of the recipient cell and the biological activity of the transforming principle, certainly depending on the ucture of the DNA, which depends again on the ysiological state of the cells from which the transorming principle had been isolated. It is possible that the deoxyribonuclease which those who work with pneumococci have always strived to eliminate by using only a small number of recipient cells, to avoid the depolymerization of the DNA by the deoxyribonuclease which is liberated by those cells, plays an active part in the transformation, even though the deoxyribonuclease in minimal concentration, acting only within a certain time-span, is blocked moreover by citrate (or another blocking system). Details of this work will be published in *Folia Microbiologica*.

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PROTEINS OF HEN'S EGG YOLK

IN a recent communication on the proteins of the plasma and egg yolk of the hen, Schjeide and Urist[1] remark that much confusion exists in "the nomenclature, chemical constitution and physical orientation" of these proteins. As the portion of their analysis dealing with current knowledge of the yolk proteins is limited in scope and somewhat speculative, another view of the biochemistry of yolk proteins is offered here. While this article is based largely on experience in this Laboratory, I do not regard these findings as absolute and irrefutable facts; but the criticisms of this work implicit in the article by Schjeide and Urist do not suggest to me any radical revision of our views.

Fevold's[2] review of ten years ago provides an adequate reference to earlier investigations. At that time, four proteins had been isolated from egg yolk, although it was recognized that none of these had been shown to be homogeneous by modern criteria. Recently, Schjeide and Urist[3] detected, by ultracentrifugation, both a floating and a sedimenting component in the fluid portion of egg yolk and two more sedimenting components in a solution of the solid granules. The nomenclature used infers that these components differ from the known fractions of egg yolk, although most of their distinguishing properties appear to be the same. Similar flotation patterns of the low-density fraction (about 90 per cent lipid) have been published[4], and this fraction has been shown[5,6] to yield lipovitellenin (about 40 per cent lipid) on ether extraction. They[3] called the sedimenting component in the fluid fraction 'ovalbumin' and defined it as "one of the components of livetin", a statement that does little to clarify nomenclature, but quite correctly suggests that there is more than one livetin. In the granule fraction their phosphoprotein X_1 is said, indirectly, to be an impure form of Mecham and Olcott's[7] phosvitin (containing 9·7 per cent phosphorus) and that their lipoglycoprotein X_2 "seems to be synonymous with" the α-lipovitellin of Sugano[8], or lipovitellin. Evidently Schjeide and Urist have detected four ultracentrifugal components corresponding to the four proteins previously isolated and described by other workers. This is in general agreement with earlier reports[4,9] which, depending on the conditions employed, indicate the resolution of three to five ultracentrifugal components in solutions of egg yolk.

The ultracentrifuge, however, cannot resolve all the yolk proteins shown to be present in substantial proportions by electrophoretic analysis. Sugano's[8] patterns suggest at least five components in ether-extracted egg yolk, and Bernardi and Cook[10] identified six in the sedimenting material from which the floating fraction, containing lipovitellenin (β-lipovitellin of Sugano), had been removed. These components have all been isolated and characterized[7,10–15] as to molecular weight, N-terminal amino-acids, as shown in Table 1; and some by amino-acid composition or other properties. Clearly, seven proteins are present in egg yolk, and there are indications that some of these are not homogeneous. Consequently, one or more of Schjeide and Urist's four ultracentrifugal components must have contained more than one protein.

The procedures developed in this Laboratory for the isolation of these proteins (Table 1) are typical of widely used biochemical techniques. The usual precautions were taken at all stages with respect to temperature and other conditions. Naturally, some of the steps must be repeated to obtain homogeneous preparations, and some may be omitted; for example, the chromatographic procedure can be used directly to obtain certain proteins. Schjeide and Urist[1,3] term these 'chemical' procedures and state that it is 'obvious' that at least phosvitin so obtained is a derived protein that loses most of its calcium, often part of its phosphorus, and may even be derived from breaking the 'phosphoprotein backbone'. In support of these statements they point to the lower sedimentation coefficient of our isolated preparations. Phosvitin is a polyelectrolyte that occurs in egg yolk as an insoluble salt, which is converted partly to the sodium salt with a loss of calcium when dissolved in sodium chloride solutions, the commonly used solvent also employed by Schjeide and Urist for their sedimentation experiments. They have not demonstrated the loss of phosphorus but rather the reverse, since they state[1] that their X_1 phosphoprotein and phosvitin, prepared according to Joubert and Cook[11], had the same phosphoserine content. Later they say that both phosvitin and lipovitellin are 'known' to be altered to a greater extent than other proteins isolated from physiological fluids, regardless of the method employed for their isolation. This may be so; but, as no evidence is presented to support this generalization, the reader must decide *quaecumque vera*.

With a knowledge of the N-terminal amino-acids of the several proteins[15,16], the protein phosphorus content of α- and β-lipovitellin (Burley, R. W., and Cook, W. H., unpublished work) (Table 1), and further physical determinations[10,14], it has been possible to estimate the composition of the ultracentrifugal components in solutions of the high-density fraction of egg yolk. Clearly, six proteins are present in this

Table 1. PREPARATIVE PROCEDURES AND PROPERTIES OF EGG YOLK PROTEINS

Preparative procedures Egg yolk dissolved in 0·45 *M* magnesium sulphate; centrifuged at high speed	Designation	Characteristic properties				Refs.
		$M \times 10^{-4}$	Principal N-terminal amino-acids	Lipid content (per cent)	Protein phosphorus content (per cent)	
Low-density floating fraction; no known method of fractionation	This fraction yields lipovitellenin (about 40 per cent lipid) on ether extraction	480	Arg, Lys	90	< 0·5	5, 6, 15
High-density sedimenting fraction						
Precipitated at 0·15 *M* mag. sulphate	Phosvitin	3·6	Ala		9·6	7, 11, 16
Precipitated at 0·05 *M* mag. sulphate (α- and β-forms separated by column chromatography)	α-Lipovitellin	40	Arg, Lys	20	0·50	9, 10*
	β-Lipovitellin	40	Arg, Lys		0·27	14, 15*
Soluble in 0·05 *M* mag. sulphate Soluble in 37 per cent sat. ammonium sulphate (α- and β-forms separated electrophoretically)	α-Livetin	8·0	Arg, Lys	0·1	*c.* 0·10	12, 16
	β-Livetin	4·2	Lys + 2 others			
Precipitated by 37 per cent sat. ammonium sulphate	γ-Livetin	15	Ala	0·5	0·03	13, 16

* Also unpublished work by Burley, R. W., and Cook, W. H.

fraction, although they sediment as only two major components (S_1 and S_2) in 5 per cent sodium chloride. The faster sedimenting S_1 component is a complex of α- and β-lipovitellin with phosvitin, plus an aggregated form of γ-livetin, a protein which has been isolated and described[13], but which is not resolved from this complex by ultracentrifugation in 5 per cent sodium chloride. After precipitation by dilution, the S_2 component is absent and other analyses show that α-, β- and γ-livetin and part of the phosvitin are removed. Evidently γ-livetin does not interact strongly with the lipovitellins, as originally suggested[9]. The original S_1 component obviously contains phosvitin, since its protein phosphorus content approaches 2 per cent, and is only reduced to about 1 per cent after repeated precipitation from 5 per cent sodium chloride by dilution, a result in agreement with the protein phosphorus content reported by earlier workers who used this method[17]. Since this is twice the phosphorus content of the mixed lipovitellins prepared from magnesium sulphate solutions[9], or by chromatography (Table 1), this complex, which sediments as one component, must contain about 5 per cent phosvitin of 10 per cent phosphorus content. In fact, the protein phosphorus contents reported[14] for isolated α- and β-lipovitellin are now known to be high owing to the difficulty of removing phosvitin from lipovitellin by hydroxyapatite chromatography, and more accurate values are reported (Burley, R. W., and Cook, W. H., unpublished work) in Table 1. Schjeide and Urist[1] state "that no significant amount" of phosvitin is complexed with lipovitellin. A complex containing 5 per cent phosvitin is not likely to be detected by sedimentation, but this amount is highly significant analytically. These authors do not report the results on which their conclusion is based, but it seems doubtful that the partition-cell procedure would yield their X_2 (lipovitellin) free of X_1 (phosvitin).

In alkaline solvents a third component of about 7 *S* (not 4–5 *S*) appears in the high-density fraction and increases in amount as the *p*H is increased. At *p*H 7·6 (pattern reproduced as Fig. 2 by Schjeide and Urist), this component is predominantly a non-aggregated form of γ-livetin[14]. At higher *p*H values a second component of about 7 *S* also appears in lipovitellin solutions now known to be free of phosvitin and γ-livetin by protein phosphorus and end-group analyses. We have also used the method of Schmidt, Bessman, Hickey, and Thannhauser[18] employed by Schjeide and Urist for isolating the yolk granules, and found that the three livetins are absent from solutions of the granule proteins. Without the livetins, phosvitin and the lipovitellins are clearly evident as two components in neutral solvents, but in alkaline solvents a third component of about 7 *S* is also present.

Clearly, the additional ultracentrifugal component observed in alkaline lipovitellin solutions is not γ-livetin. Before the present criteria were available, we erroneously called this 7 *S* component γ-livetin, since it had a similar sedimentation-rate, was reduced in amount by repeated precipitation[9], and had a type of dependence on *p*H suggesting that lipovitellin contained two distinct proteins. Moolenaar[19] also observed this second component in lipovitellin at *p*H 7·8 or higher and correctly called it a sub-unit of lipovitellin. The identity of this 7 *S* component was clarified by the work of Bernardi and Cook[10,14,20], who found that lipovitellin had two electrophoretic components, α- and β-, separated them by chromatography, and established that both forms dissociate reversibly into two sub-units in alkaline solvents. Both lipovitellins behave as *p*H-dependent reversible association-dissociation systems, but β-lipovitellin dissociates at a lower *p*H than α-lipovitellin. These findings explain our earlier observations indicating that lipovitellin contained two proteins.

I cannot reconcile these results with those reported by Schjeide and Urist[1]. They claim discovery of an alkaline breakdown product of lipovitellin of 4–5 *S* and suggest this may be the second component called γ-livetin by Joubert and Cook[9]. Evidently they have observed the second component reported earlier[9,19], and correctly identified by Moolenaar[19]; but they do not describe its properties. They do report the isolation of a trailing component which was found to be the natural form of phosvitin containing 10 per cent phosphorus. Clearly, this was not the 7 *S* component observed in alkaline solutions of our phosvitin-free lipovitellin. In fact, it appears that these workers isolated their trailing component from a solution of the granule proteins in 10 per cent sodium chloride, a solvent in which the sub-unit of lipovitellin does not exist. Alternatively, if they did use an alkaline solvent, they should have resolved three components in the granule proteins. Their results appear, therefore, to have no bearing on the identification of the 7 *S* component under discussion.

Since lipovitellin, the classical lipoprotein of egg yolk, has now been fractionated, it is desirable to clarify nomenclature. Although the three fractions that have been characterized are, correctly speaking, lipoglycophosphoproteins, in this Laboratory we have continued to use modifications of the older, simpler names, α- and β-lipovitellin, and lipovitellenin; corresponding names for the three lipid-free protein moieties are provided by omitting the prefix 'lipo'. Our α- and β-lipovitellin are not identical with those so designated by Sugano[8], who used ether-extracted egg yolk and identified his α- and β-components as purified forms of lipovitellin and lipovitellenin, respectively. In our work lipovitellenin was removed by centrifugation and the terms α- and β- used to describe the two electrophoretic components of lipovitellin proper. Our α- and β-forms are not resolved by ultracentrifugation in sodium chloride solutions, but the parent molecules and their dissociated sub-units can be resolved in alkaline solvents. Lipovitellin may therefore occur in at least four forms.

Since the second component in lipovitellin solutions is a dissociated sub-unit and not γ-livetin, the molecular weights observed in sodium chloride solutions are now quite consistent[4,9,14]. Those reported by Joubert and Cook[9,11], however, are subject to correction for a computational error affecting the diffusion coefficient, which increases all those reported by about 15 per cent. The correct values have been published[14]. The phosvitin–lipovitellin complex precipitated from, and examined in, sodium chloride solutions had $M = 4\cdot0 \times 10^5$ by light scattering[4], and $4\cdot1 \times 10^5$ by sedimentation-diffusion[9]. At *p*H 9·0 the presence of 10 per cent or more of the dissociated sub-unit reduced the molecular weight of lipovitellin to $3\cdot7 \times 10^5$ (corrected value), but in sodium chloride solutions both α- and β-lipovitellin had $M = 4\cdot0 \times 10^5$ (Table 1)[14]. Since the lipovitellin–phosvitin complex contains only about 5 per cent phosvitin, its molecular weight would not be expected to differ, beyond experimental error, from that of undissociated lipovitellin.

The molecular weights of X_1 and X_2 proteins are reported by Schjeide and Urist[3] to be $7\cdot7 \times 10^4$ and $2\cdot0 \times 10^5$, respectively, which differ considerably from our values of $3\cdot6 \times 10^4$ and $4\cdot0 \times 10^5$ for phosvitin and lipovitellin. They suggest that our phosvitin was degraded, but do not advance a specific reason for the discrepancy in the estimated size of lipovitellin. An evident explanation is that their X_2 contained X_1, since the latter could not have been completely removed by separation in a partition cell. They found that X_1, X_2 and serum albumin all showed comparable boundary-spreading in the ultracentrifuge, but do not report diffusion coefficients. The molecular weights were evidently calculated from the sedimentation coefficients and the "reasonable assumption" that the X_1 and X_2 proteins have an axial ratio comparable with serum albumin. This assumption, however, is neither reasonable nor adequate for the estimation of molecular weights. It is not reasonable for phosvitin, since the results of Joubert and Cook[11] show that this protein and its soluble complexes with calcium all had frictional ratios far larger than that of serum albumin, and no evidence is presented to show that X_1 differs in this respect. The assumption is inadequate, since this calculation also assumes that both X_1 and X_2 have the same partial specific volume as serum albumin, which is neither in accord with expectation (composition) nor experimental observation[9,11]. These considerations also have a bearing on their conclusions[3] regarding the relative sizes of their X_1 and X_2 proteins in egg yolk and hen plasma.

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Dr. Cook's article constitutes an excellent summary of the large amount of work that he and his associates have carried out on the subject of egg yolk proteins, and brings into focus several crucial points where disagreements seem to exist among workers in the field. We shall take these up, but first would like to recapitulate the reasons why we submitted our original communication[1].

Basically, we were concerned with what seemed to be a general failure to recognize that the chemically derived phosvitins and lipovitellins of serum and egg yolk may be considerably different from these proteins as they occur in the native state. Secondly, we wished to explain our reasons for employing the serum and egg yolk X_1–X_2 nomenclature, and wished to caution against use of terms already ascribed to the chemically derived proteins when referring to the 'native proteins' as they might be resolved during gentler procedures of ultracentrifugation, etc. Thirdly, we wished to explain our view that serum X_1-phosphoprotein and serum X_2-lipoglycoprotein do not exist together as complexes in serum and that egg yolk X_1-phosphoprotein and egg yolk X_2-lipoglycoprotein are not present as complexes in 5 per cent sodium chloride at neutral *p*H as suggested by Cook and by Common and co-workers[2]. We wished, further, to make it clear that the reasons for the non-existence of such complexes constitute some of the most important facets of our understanding of the nature and role of those unique proteins adapted to the sustenance of the embryo bird, reptile and amphibian.

In the preceding article Dr. Cook has reopened certain of these issues. For the sake of brevity, we shall mention only a few points on which we still differ:

(*a*) We have not changed our view that both phosvitin and lipovitellin obtained chemically as described by Joubert and Cook in 1958 (ref. 3) differ in certain respects from the ultracentrifugally resolved X_1 and X_2 proteins and are probably (at least in the case of the phosphoprotein) less 'physiological'. (i) Phosvitin definitely lacks most of the calcium which was originally associated with this molecule, and because of its lower sedimentation constant appears to be either (or both) broken or of increased axial ratio. (The loss of calcium, in its simplest terms, would result in only a 5–7 per cent reduction in molecular weight.) (ii) Joubert and Cook[3] have shown that the correct molecular weight for phosvitin is about 36,000. Thus, it appears that phosvitin obtained chemically is at most only half the molecular weight of its counterpart obtained more simply by dissolution of the yolk granules with 10 per cent sodium chloride followed by differential ultracentrifugation. Our estimation of the molecular weight of egg yolk X_1-phosphoprotein (77,000) was based on the premise that the phosphoprotein was as globular in shape as albumin. If a greater axial ratio actually obtains, the molecular weight of X_1-phosphoprotein is even greater than indicated by our original estimate. (iii) With respect to lipovitellin, Joubert and Cook[3] have, themselves, pointed out that, as originally obtained, it was not a pure fraction.

(*b*) Another of the views that has been strictly maintained by Dr. Cook and co-workers is that their faster sedimenting S_1 component of whole egg yolk in 5 per cent sodium chloride (our X_2-lipoglycoprotein) is a complex of the 'alpha' and 'beta' lipovitellins with phosvitin. (i) It has been our experience that the association of phosphoprotein with lipoglycoprotein (as by dilution or change in *p*H in the presence of sodium chloride) results in macro-complexes possessing sedimentation constants considerably in excess of S 10. Even if a complex of only one molecule of phosvitin per molecule of 'alpha' or 'beta' lipovitellin were postulated—such a physical orientation should produce a detectable difference in the rate of sedimentation as compared with phosvitin-free lipovitellin. (ii) Dr. Cook states specifically that "the S_1 [our egg yolk X_2] component obviously contains phosvitin since its protein phosphorus content approaches 2 per cent and is only reduced to about 1 per cent after repeated precipitation from 5 per cent sodium chloride by dilution".

Two misconceptions are evident here: in the first place, the S_1 material (S 10) as present in the preparatory ultracentrifuge tube necessarily contains phosvitin since the S_1 deflexion simply defines the boundary of a molecular species which is sedimenting through a medium containing sodium chloride, phosvitin, etc. The material causing the S_1 deflexion cannot be isolated from the centrifuge tube alone. Only a mixture of this component together with the other materials in the medium through which it is passing can be obtained.

In order to ascertain which component in the centrifuge tube contains phosphorus or calcium (and hence phosvitin) one can proceed indirectly as follows: the mixture of proteins is centrifuged in a partition cell for the preparatory ultracentrifuge[4] and is then partitioned into several vertical sections. Differing amounts of the various proteins in the mixture are present in each section, their concentrations being functions of their rates of sedimentation. The content of each section is ascertained by analytical ultracentrifugation and analysis for protein phosphorus. One can now determine by inspection whether the phosphorus rises in proportion to the deflexion caused by a given protein component. Neither protein phosphorus nor calcium increases detectably when the S_1 (our X_2) component is present in greater concentration in any particular segment[5].

Secondly, our experimental results[5] indicate that the reason the S_1 (our X_2) component is precipitated with dilution is that large complexes form between X_1-phosphoprotein on one hand, and X_2-lipoglycoprotein on the other. Hence, repeated precipitation should not result in complete elimination of phosvitin phosphorus from lipovitellin prepared by the method of dilution.

(iii) The occurrence of a precipitate is apparently due to the many negative charges appearing on the X_1-phosphoprotein (following the removal of calcium by dilution) reacting with positive charges on the X_2-lipoprotein. So long as these two molecules are present in a neutral medium of high ionic strength such as 5 per cent sodium chloride, the complexes do not form, even when much of the calcium has been removed. We have successfully separated each of these molecules (X_1 by differential ultracentrifugation and X_2 by extraction from frog larvæ cells (tail-bud stage) in which X_1 is absent[6] and have found that they are each perfectly soluble in distilled water by themselves. However, mixing the two clear solutions will cause the formation of a copious precipitate.

(*c*) It cannot be reasonably disputed that the following sedimentation differences exist when egg yolk and serum components are centrifuged in the same 5–10 per cent sodium chloride medium: egg yolk X_1 sediments faster (S 4·7) than phosvitin (S 3·5); serum X_1 sediments faster (S 8–9) than egg yolk X_1 or phosvitin derived from serum (S 3·5); serum X_2 sediments faster (S 16·5) than egg yolk X_2 (S 10). However, the sedimentation constants of Dr. Cook's purified lipovitellin and our egg yolk X_2 agree exactly (S 10), indicating that they may be identical substances. Also, our latest calculations of the sedimentation constants for the alkaline-produced sub-units of X_2 agree exactly (S 7) with those of Dr. Cook's sub-units of α- and β-lipovitellins. All molecular weights of the above substances, given in our papers, have been approximations and this has been so stated.

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THE DISTRIBUTION OF ANTIGENIC GROUPINGS ON MOUSE GAMMA-GLOBULIN MOLECULES

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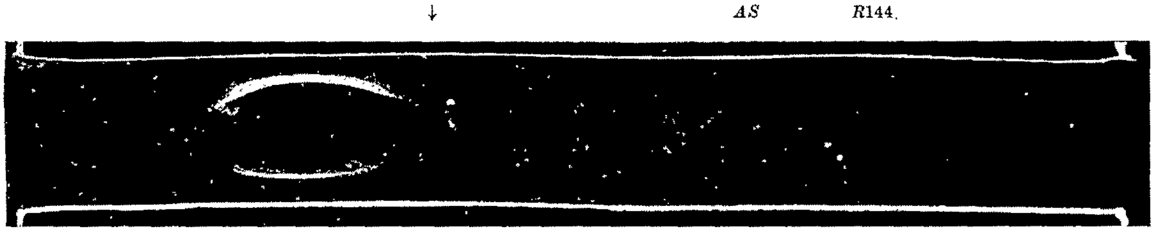
EDELMAN *et al.*[1] observed that certain antisera prepared in rabbits against human γ-globulin or human γ-myeloma proteins consistently gave two precipitation lines when tested by immunoelectrophoresis against normal human γ-globulin. When the antigenic properties of human γ-globulin fragments, obtained by digestion with papain and cysteine by the method of Porter[2], were examined by means of these antisera, the papain digests were found to contain fragments with two distinct antigenic groupings; these were referred to as 'fast' or *F* fragments and 'slow' or *S* fragments according to their relative rates of migration towards the anode during electrophoresis. Immunoelectrophoretic comparison of the original human γ-globulin with the papain digest indicated that the two precipitin lines given by human γ-globulin were due to antibodies against antigenic groupings shared exclusively with the *F* fragments and exclusively with the *S* fragments respectively. These findings are hard to explain if all molecules of undigested human γ-globulin have both *F*- and *S*-antigenic groupings on the same molecule, but would easily be accounted for if the *F*- and *S*-antigenic groupings were present on distinct γ-globulin molecules.

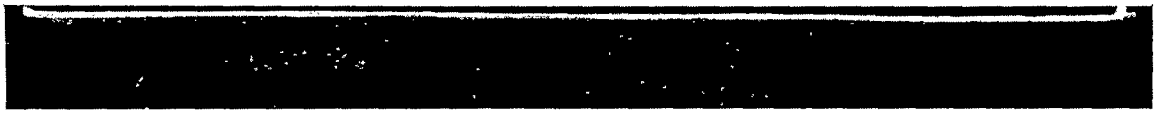
In the course of experiments on normal mouse γ-globulin and γ-myeloma globulins formed by transplantable plasma cell neoplasms, it was observed that rabbit antisera against normal mouse γ-globulin or the γ-myeloma protein formed by the plasma cell tumour line 5563 in C_3H mice[3] could also give double precipitation lines with normal mouse γ-globulin on immunoelectrophoresis (Fig. 1). Mouse γ-globulin and myeloma protein, on treatment with papain and cysteine, have been found to form fragments with distinct antigenic groupings (*S* and *F* groupings)[4]. It was therefore possible to design an experiment to test directly whether *F*-antigenic groupings and *S*-antigenic groupings are present on distinct molecules in the undigested γ-globulin.

Normal mouse γ-globulin, prepared by zone electrophoresis on 'Geon 425' (British Geon Co.) by the method described by Fahey and Horbett[5], and 5563 myeloma protein purified by fractionation on

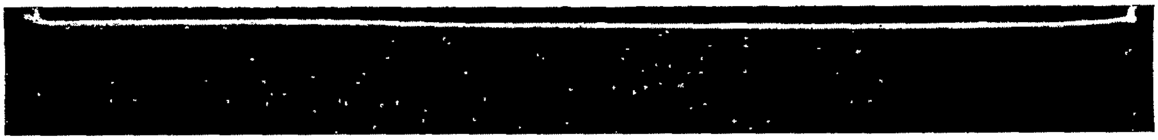


AS partly absorbed with *S* fragment

AS absorbed with excess of *S* fragment



AS partly absorbed with *F* fragment



AS absorbed with excess of *F* fragment

Fig. 1. Double precipitin lines with normal mouse γ-globulin and absorbed antisera on immunoelectrophoresis. The arrow indicates point of application of purified normal mouse γ-globulin at a concentration of 5 mgm./ml. *AS R*144 rabbit antiserum against purified 5563 myeloma protein. This antiserum was absorbed with *S* and *F* fragments isolated from 5563 myeloma protein after treatment with papain and cysteine

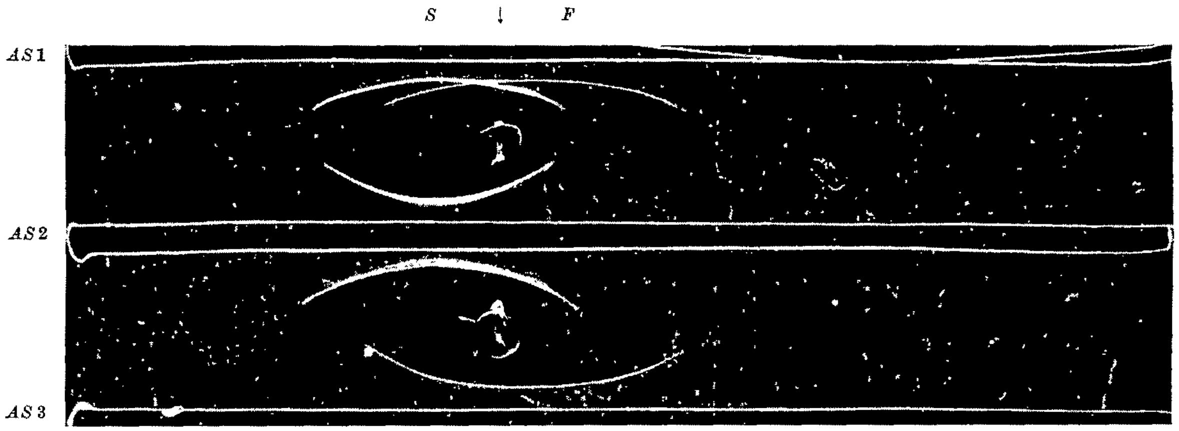


Fig. 2. Immunoelectrophoretic analysis of absorbed antisera. Arrow indicates point of application of papain and cysteine-treated 5563 myeloma protein. Fragments with distinct *S*- and *F*-antigenic groupings may be seen
AS 1, rabbit antiserum (*R* 91) against purified 5563 myeloma protein diluted by 1/2; *AS* 2, R 91 absorbed with a slight excess of fragment with *F*-antigenic grouping from papain and cysteine-treated 5563 myeloma protein.; *AS* 3, *R* 91 absorbed with fragment with *S*-antigenic grouping

DEAE cellulose[6] were labelled with iodine-131 at an average level of 0·5 atom/mole using the technique of McFarlane[7]. At least 99 per cent of the radioactivity was attached to protein having electrophoretic mobility characteristic of normal mouse γ-globulin and 5563 myeloma protein respectively. Samples of one rabbit antiserum prepared against normal mouse γ-globulin (*R*15) and of two antisera against 5563 myeloma protein (*R*144, *R*91) were absorbed with a slight excess of fragments (papain digest of 5563 myeloma protein) which had been purified so as to contain either *S*- or *F*-antigenic groupings only[4]. The absorbed antisera reacted exclusively with fragments possessing either *S*- or *F*-antigenic groupings, in contrast to the unabsorbed sera which reacted with both (Fig. 2).

Aliquots of the unabsorbed and of the absorbed antisera were mixed in a standard volume of saline with varying amounts of iodine-131 labelled normal mouse γ-globulin or iodine-131 labelled 5563 myeloma protein, giving ratios of antigen to antibody ranging from less to more than equivalence, and after 24 hr. at 2°, the proportion of iodine-131 labelled protein precipitated was estimated by centrifuging at 0°, and measuring the distribution of radioactivity between the supernatant and the antigen–antibody precipitate. The results (Table 1) showed that not only the unabsorbed antisera but also the absorbed antisera, specific for either *F*- and *S*-antigenic groupings, could precipitate 95 per cent or more of the normal mouse γ-globulin or 5563 myeloma protein labelled with iodine-131. Furthermore, by analysis of the precipitates at the equivalence points (Table 2), it was apparent that the total precipitable antibody was, within experimental error, equal to the sum of the antibody precipitable after each absorption, and there was no significant antigenic overlap between *S* and *F* fragments.

Table 1

Rabbit antiserum	Absorbed with 5563 papain digest fragment	Per cent iodine-131 labelled protein precipitated in antibody excess	
		Normal mouse γ-globulin (iodine-131 labelled)	5563 myeloma protein (iodine-131 labelled)
R 15	—	98·4	99·2
R 15	*S*	98·3	99·2
R 15	*F*	99*	95·8
R 91	—	98·9	99·5
R 91	*S*	99*	99·7
R 91	*F*	99*	99·1
R 144	—	97·3	99·5
R 144	*S*	92·6*	99·5
R 144	*F*	98·5*	99·5

* Only 89–96 per cent of the iodine-131 labelled normal mouse γ-globulin was precipitated under the conditions described, but a further amount was co-precipitable with the rabbit antibody, on adding excess of goat antibody against rabbit γ-globulin. Iodine-131 labelled normal mouse γ-globulin in normal rabbit serum was not precipitated in these circumstances.

Table 2. AMOUNT OF γ-GLOBULIN AND MYELOMA PROTEIN PRECIPITATED BY ANTISERA REACTING ONLY WITH *S*- OR *F*- ANTIGENIC GROUPINGS

Antiserum	Mgm. antibody per ml. antiserum precipitated at equivalence by:	
	normal mouse γ-globulin	5563 myeloma protein
R 91 (anti-5563 myeloma protein)	5·74	6·9
R 91 absorbed with *S* fragment	2·76 } 5·42	3·28 } 7·4
R 91 absorbed with *F* fragment	2·66 }	4·12 }

Our conclusion is that, in normal mouse γ-globulin as well as in the 5563 myeloma protein, the antigenic groupings of both the 'fast' and 'slow' papain digest fragments must be present on all the molecules. The double precipitin lines which we observed to occur between normal mouse γ-globulin and rabbit antisera cannot therefore be explained simply as due to a heterogeneous distribution of *F*- and *S*-antigenic groupings in the original normal mouse γ-globulin molecules. In fact we found (Fig. 1) that antisera partially or completely absorbed with *F* or *S* fragments still gave double lines with normal mouse γ-globulin on immunoelectrophoresis, although none of our antisera gave double lines with the 5563 myeloma protein. It is possible that an alternative explanation may lie in the presence in the antisera of antibodies with varying avidity for groupings the availability of which depends on the folding of the γ-globulin molecules, which is likely to be much more variable in normal mouse γ-globulin than in the more homogeneous myeloma protein.

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FAVISM AND THALASSÆMIA IN SARDINIA AND THEIR RELATIONSHIP TO MALARIA

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GLUCOSE-6-PHOSPHATE dehydrogenase deficiency of the red cell is a hereditary trait that predisposes to hæmolytic anæmia from *Vicia faba* beans, drugs (see review by Beutler[1]), and infections[2]. It has been suggested that this trait[3] and thalassæmia[4] have attained their world distribution by protecting their carriers against death from malaria. It would therefore be expected that populations exposed to severe and intense malarial infections in the historical past would have a high incidence of these traits—provided that the appropriate mutations had occurred or had been introduced into such populations. Inhabitants of the Sardinian plains, which until 1946 were among the most highly malarial areas in Europe[5], are known to have a high incidence of thalassæmia and favism. The island is even further suited for study because of the existence of isolated mountain villages where malaria is not prevalent.

Male school children of nineteen Sardinian villages that had in the past experienced varying incidences of malaria were studied. A dye decoloration test was used to detect glucose-6-phosphate dehydrogenase deficiency[6,7], and an osmotic fragility screening test and blood films served for the diagnosis of thalassæmia[8]. More detailed preliminary tests for thalassæmia, involving red cell enumeration, hæmoglobin, hæmatocrit, and hæmoglobin A_2 determination, and calculation of red-cell constants showed that the error of misclassification with the more rapid screening tests did not exceed 1 per cent[8]. The type of thalassæmia seen in one hundred randomly chosen families in different areas in Sardinia was associated with increased hæmoglobin A_2 levels[8,9].

Investigation of fifty-two families confirmed the fact that the enzyme deficiency is inherited as a sex-linked trait[10]. Linkage investigations in which colour-blindness was used as an X-chromosome marker showed no instance of recombination in 12 families among 4 large kindreds in which the two traits co-existed[10]. This finding formally rules out sex limitation or partial sex-linkage and indicates close linkage of the genes for colour-blindness and enzyme deficiency.

The data on the incidence of thalassæmia and enzyme deficiency are summarized in Table 1. Previous work on blood group distribution had indicated the similarity between populations in the central mountain villages (Desulo and Tonara) and east coast villages (Orosei and Galtelli)[4]. When this work was extended to west-coast villages (Terralba and Cabras), the low *Rh* (*cde*) and high blood group *M* gene frequencies characteristic of Sardinians were again found, suggesting that all population groups studied were ethnically homogeneous[11].

Fig. 1 demonstrates the positive correlation between thalassæmia and enzyme-deficiency gene frequencies, suggesting selection by a common ecological agent, namely, malaria by *Plasmodium falciparum*. It is apparent that the maximum gene frequency for thalassæmia (14 per cent) is significantly lower than that for enzyme deficiency (35 per cent). This finding is assumed to be related to the lethality of the thalassæmia gene in the homozygote state in contrast to milder selection against enzyme deficiency. It is also possible that the rate of accumulation of the thalassæmia gene is slower in populations with higher frequencies of enzyme deficiency, since this less harmful gene may already provide sufficient protection against malarial mortality.

Estimates of malarial morbidity were derived from the data of Fermi and Missiroli (see ref. 11 for literature), whose extensive investigations, carried out between 1929 and 1938, provide detailed information on the incidence of malaria in Sardinian villages. The relationship of the two genetic traits to the past incidence of malaria is shown in Fig. 2. There is good positive correlation between past malarial incidence and glucose-6-phosphate dehydrogenase deficiency

Table 1. ENZYME DEFICIENCY AND THALASSÆMIA IN SARDINIAN VILLAGES
(See also Bernini *et al.*, ref. 11)

Village	Altitude (m.)	Malarial frequency (per cent)*	No. examined	Enzyme deficiency in males = gene frequency (±*S.E.*)	Thalassæmia	
					Heterozygote frequency	Gene frequency† (±*S.E.*)
Luras	508	78	100	0·070±0·025	0·225	0·112±0·022
Usini	190	95	99	0·061±0·024	0·142	0·071±0·019
Lodé	345	90	163	0·294±0·035	0·221	0·110±0·017
Siniscola	42	55	198	0·113±0·023	0·258	0·129±0·024
Benetutti	406	50	100	0·090±0·028	0·120	0·060±0·017
Suni	333	85	98	0·143±0·035	0·250	0·125±0·023
Orosei	19	93	180	0·140±0·026	0·188‡	0·094±0·012‡
Galtelli	40	96	175	0·120±0·024	0·213‡	0·106±0·014‡
Fonni	1,000	19	100	0·030±0·014	—	—
Desulo	891	33	313	0·031±0·009	0·037‡	0·018±0·005‡
Tonara	935	10	148	0·040±0·016	0·047‡	0·023±0·010‡
Lanusei	595	25	100	0·040±0·019	—	—
Tortoli	15	90	50	0·160±0·052	—	—
Cabras	9	97	200	0·350±0·034	0·280	0·140±0·024
S. Giusta	10	97	42	0·309±0·075	—	—
Terralba	9	97	100	0·300±0·046	—	—
Isili	523	17	100	0·090±0·029	0·190	0·095±0·021
Gergei	374	66	92	0·185±0·040	0·131	0·065±0·018
Teulada	50	90	101	0·169±0·037	0·250	0·125±0·023

* For refs. see Bernini *et al.*, ref. 11. † The gene frequency was calculated as one-half the heterozygote frequency because of lethality of homozygous thalassæmia. ‡ From Ceppellini, 1956 (includes both sexes).

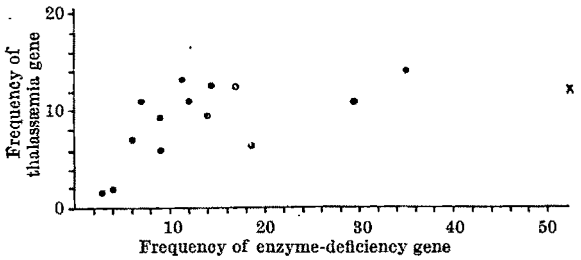

Fig. 1. Gene frequencies of thalassæmia and enzyme deficiency. Each dot represents one Sardinian village. The X refers to the data by Cohen *et al.* (ref. 15) on Kurdish Jews in Israel. (Note that the gene frequency of thalassæmia is approximately one-half the trait frequency, while the gene frequency of enzyme deficiency is identical with the trait frequency in males)

(or negative correlation with altitude; Table 1). To a lesser extent a similar correlation was observed for thalassæmia. Since the malarial frequency index refers to all malarial species, a still better correlation might have been obtained if the data could have been restricted to the lethal *P. falciparum* malaria. It is of interest that the sickling and the enzyme deficiency genes show a similar positive correlation in African populations[3]. There is extensive evidence now that sickle-cell heterozygotes are protected against death from *P. falciparum* malaria[12]. Data suggesting a similar protective effect on the part of the enzyme deficiency are now accumulating for African populations[3,7,13,14]. These results, as well as those presented here, therefore strongly implicate *P. falciparum* malaria as a selective agent for the maintenance of these red cell polymorphisms.

It was found that the presence of thalassæmia increased enzyme-levels, as measured quantitatively, in all genotypic classes, but especially in heterozygote females[8]. This increase probably was related to the presence of young blood cells, which are known to contain high enzyme-levels. Similar findings, based on the glutathione stability test, were obtained by Cohen *et al.*[15] in Kurdish Jews with the combined traits of thalassæmia and enzyme deficiency. Such an increase in enzyme-levels might be expected to decrease the frequency of clinical episodes of favism, and we investigated this possibility by surveying the medical records and carefully questioning 295 males and females more than fifty years of age in Lodè, where there was a frequency of enzyme deficiency of 29 per cent among males and a 22 per cent trait frequency of thalassæmia. No favism had occurred in individuals without enzyme deficiency. Of 50 subjects with both enzyme deficiency and thalassæmia, only 3 had had hæmolytic episodes, while among 121 with enzyme deficiency alone, 23 had had such episodes ($\chi^2 = 4\cdot6$, $P = < 0\cdot05 > 0\cdot02$ for 1 d.f.). It is therefore suggested that the presence of thalassæmia makes enzyme-deficient individuals less liable to acute hæmolysis from *V. fava* beans and probably also from infections and drugs. On the other hand, if malarial protection is related to low enzyme-levels[3], the presence of thalassæmia would partially deprive enzyme-deficient individuals of the malarial protection afforded by the enzyme deficiency while maintaining that given by thalassæmia. These considerations indicate the intricate interaction of two polymorphic red cell traits in a population. It is likely that full understanding of the population dynamics of these and many other human genes will require further detailed investigation of interactions of this type.

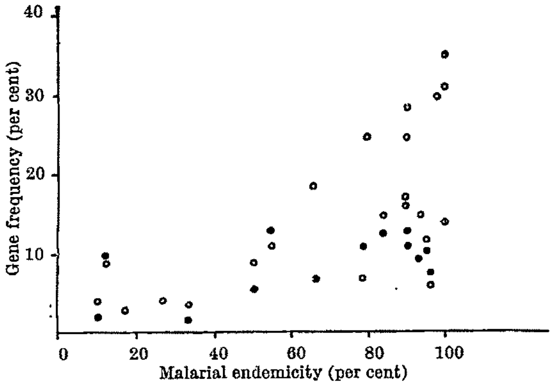

Fig. 2. Present gene frequency of enzyme deficiency (○) and thalassæmia (●) against malarial prevalence before eradication of malaria in Sardinia. Each dot represents a village. Not all villages were screened for both traits

These investigations were supported by grants of the Rockefeller Foundation, the Italian National Research Council, and Grant *H*-3091 of the U.S. National Institutes of Health.

Note added in proof. The very close linkage between the genes for enzyme deficiency and colour-blindness is supported by additional observations made during the past nine months or so by us in Sardinia. From these it has become clear also that the incidence of colour-blindness itself is positively correlated with the past incidence of malaria and therefore with the frequency of the enzyme deficiency. The situation is interpreted as an example of the selection of a neutral gene through the close linkage with a highly adaptive one. The hypothesis is further strengthened by the finding of an excess of families of the coupling type in the isolated village so far studied with almost complete ascertainment (Lodè), as would be expected if the fittest genotype versus malaria were represented by the enzyme-deficient male.

The recent results of Adam (*Nature*, 189, 686; 1961) confirm the close linkage between the two genes in Israel but show an excess of families of the repulsion type. It would be interesting to know in this connexion: (i) whether or not this was due to ascertainment; (ii) if all the families reported were derived from a single breeding unit and if this is of recent formation; (iii) if the propositi are the sons of people coming from different populations where the frequencies of the two genes were fixed at different equilibrium-levels.

Further discussion on these problems and the additional results from Sardinia will be reported in detail elsewhere.

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LETTERS TO THE EDITORS

PHYSICS

Coesite as an Inclusion in G.E.C. Synthetic Diamonds

In the course of X-ray diffraction work on synthetic diamonds[1], many specimens were found to contain polycrystalline inclusions. A sample of coesite[2] made at the National Physical Laboratory, and kindly given to us by Sir Gordon Sutherland, has enabled us to identify it as the most prominent of the inclusions encountered (see, for example, ref. 1, Plate X, Figs. 1, 2).

This result is of interest for two reasons. In the first place, coesite has recently been identified in the Meteor Crater in Arizona[3], where its presence is taken as evidence of the occurrence of an impact shock-wave of sufficient intensity to result in the production of crypto-crystalline diamond[4], a possibility which has actually been demonstrated by de Carli and Jamieson[5]. One specimen in which coesite was found was actually the first synthetic diamond we ever examined, being a group of parallel black cubes which was selected from a batch in the G.E.C. Laboratories in Schenectady because of its superficial resemblance to cliftonite, the cubic graphite found in meteorites[6]. However, no graphite was detected in this specimen.

The second reason is that geologists have long suspected that reported inclusions of quartz in natural diamond were in reality *intrusions*, as quartz could scarcely be expected under the conditions likely to prevail during the genesis of diamond[7]. The identification of coesite strongly supports this point of view, since it indicates that if crystalline SiO_2 were present at all, it would almost certainly have been the high-pressure form. Some X-ray photographs of natural diamonds indicate the possible presence of coesite. A search for further specimens is in progress. If substantiated, this would represent the first natural occurrence of coesite except in meteor craters.

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Thermal Diffusion of Oxygen and Nitrogen in Zirconium

The diffusion of oxygen in zirconium under the influence of an electric field has already been studied qualitatively[1]. Before beginning a further investigation of this phenomenon, it seemed worth while to find out whether a diffusion of the 'Ludwig Soret-type' occurred. If, under the influence of a temperature gradient, oxygen were indeed to diffuse preferentially in the direction of increasing or decreasing temperature, such an effect would be superimposed on the diffusion under the influence of an electric field if resistance heating were applied on the zirconium wire or sheet. The reason for this is that when the oxygen moves to the anode, the concentration of oxygen in that region rises, the electrical resistance increases locally and thus a temperature gradient develops along the wire or sheet. Darken and Oriani[2] have found a thermal diffusion of carbon and nitrogen in iron under the influence of a temperature gradient. They could show that both nitrogen and carbon diffuse to the region of the highest temperature. In order to evaluate such an effect in the case of oxygen in zirconium, we tried several experimental methods. The most reproducible results were obtained in the following circumstances.

As the hardness of zirconium and of tantalum is very sensitive to small amounts of oxygen (or nitrogen)[3], we used measurements of the hardness to determine the oxygen content. We carried out our experiments with ribbons of zirconium (free of hafnium, brand I.C.I. and Hereaus) first 140μ thick and 6 mm. broad, later 200μ thick, 6 mm. broad and in both cases 70 mm. long. An amount of oxygen was measured by volume and pressure and then absorbed in the zirconium by subjecting the strip to resistance heating (a.c.) at about 1,200° C. The solution was homogenized by subsequent heating at 1,350° C. during 4 hr. in a high vacuum. At various sites on the strip the homogeneity was checked with hardness measurements.

It proved very difficult to operate a furnace (to obtain a temperature gradient) in a vacuum of about 10^{-7} mm. mercury, without producing contaminating gases. Zirconium readily absorbs these gases. Therefore, resistance-heating (with a.c.) was applied in the experiments on thermal diffusion. The temperature gradient was realized by cutting away part of the ribbon so as to decrease its width towards one end, or so that the width diminished from both ends towards the centre (Fig. 1). In cases where the sheet was too brittle (more than 5 atm. per cent oxygen) to be cut, part of the material in the middle was etched away. After the gradient heating, several micro-hardness tests were made on each of a number of distances from one of the ends of the ribbon. Good reproducibility was obtained in the tests at constant distance from the end.

It was found that the oxygen moves in the direction of the lower temperature. Strips of zirconium with 1 and 2 at. per cent oxygen subjected to heating with a temperature gradient of 1,100–1,700° C. over a length of 50 mm. gave a decrease of the hardness (or oxygen content) on the site which had been at

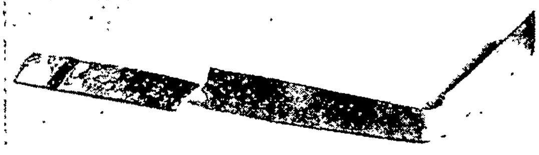

Fig. 1. Strip of zirconium with 6 at. per cent oxygen. after gradient-heating. The thinnest part is ductile and bent, the thick parts remain brittle and one end is broken

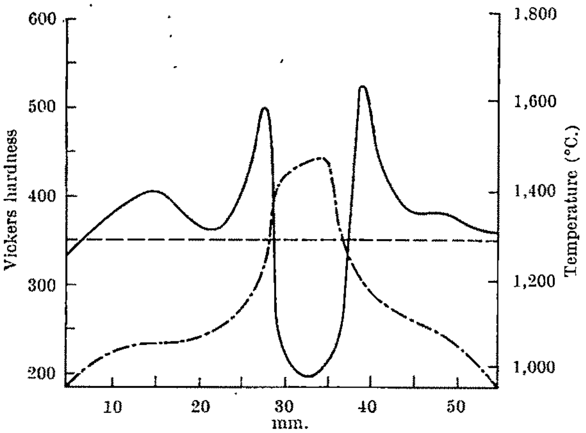

Fig. 2. Hardness and temperature along a strip of zirconium with (initial) 6 at. per cent oxygen. Annealing for 20 hr. at 1,000°–1,460° C. — — —, Hardness before annealing; ——, hardness after gradient annealing during 20 hr.; — · —, temperature along the strip during annealing

the highest temperature, and a rise in hardness (oxygen content) in the region which had been at a lower temperature. The same phenomenon occurred in strips with an oxygen content of 4 at. per cent after gradient-heating 1,350–1,700° C. In all these cases every part of the strips could be assumed to be in the region of the β-phase, according to the phase diagram of zirconium–oxygen as given in ref. 4. Strips with 4 at. per cent oxygen or more, heated for 20 hr. with a gradient 900–1,450° C., had (according to the same phase-diagram) their high-temperature parts in the β-region. The low-temperature part would be in the α-region, therefore the different solubilities of oxygen in the α- and in the β-phase (more oxygen will dissolve in the α-phase of zirconium) would have a part to play. This difference in solubility of the oxygen in α- and β-zirconium might enhance the movement of the oxygen away from the high-temperature part.

In some experiments the diffusion reduced the oxygen content of the high-temperature part almost to zero and, as a result, this part became as ductile as pure zirconium, whereas the other parts remained brittle. The phenomenon is demonstrated in Fig. 1, Fig. 2 giving the plot of hardness and temperature along the length of the strip. A strip of zirconium not loaded with oxygen, but subjected to the same gradient heating, showed scarcely any difference in hardness along its length.

There are indications that nitrogen in zirconium and oxygen in titanium behave in the same way as oxygen in zirconium.

We conclude, therefore, that when oxygen or nitrogen is dissolved in zirconium a 'Ludwig Soret-effect' occurs, which causes the oxygen or nitrogen to move to that part of the metal which has the lower temperature. We shall report more extensively on these experiments and also try to determine this diffusion effect quantitatively.

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Elastohydrodynamic Lubrication of Rollers

It is now well known that the lubrication at such nominal line contacts as are found in roller races and between gear teeth is hydrodynamic in character. It is also known that this lubrication possesses peculiar features not found in journal bearings and which arise from the high pressure—a pressure, for example, of tens of tons per square inch as compared with 100 lb. in.$^{-2}$. Because of the pressure the surfaces are deformed, in general elastically, and in addition within the pressure zone the oil acquires a much higher viscosity than it normally possesses; the viscosity might be greater by a thousand-fold. These circumstances have excited much inquiry as to the shape in cross-section of the hydrodynamic film. This communication reports an experimental measurement of that shape.

The measurements have been made with 3-in. diameter disks, one of which was of glass and which rolled with other disks of hardened steel. All were polished. The glass disk carried the evaporated chromium electrode shown in plan in Fig. 1*a* and electrical connexions were made as shown in Fig. 1*b*. As the leading edge of the electrode approaches and passes through the conjunction of the surfaces the capacitance formed by the electrode and the steel disk rises, and it may be shown that to a sufficient accuracy the potential appearing across the resistor is proportional to ε/h, where h is the gap separating the disks at the leading edge and ε is the dielectric constant of the intervening medium. Results have been obtained up to loads of 600 lb. per in. of face-width and peripheral speeds of 30 ft. per sec.

A typical result is shown in Fig. 2. This is an oscillograph showing the potential across the resistor as a function of time and will be treated as a plot of ε/h against distance. The film has a parallel section which, in the example shown, was found to be 1μ in thickness in agreement with previous measurements

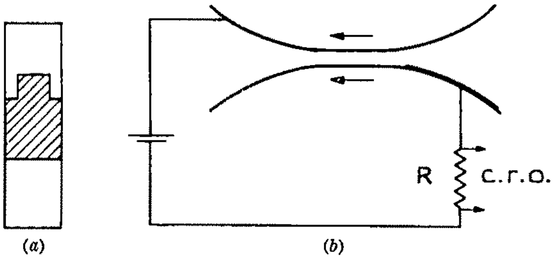

Fig. 1. *a*, Plan of chromium electrode; *b*, electrical connexions

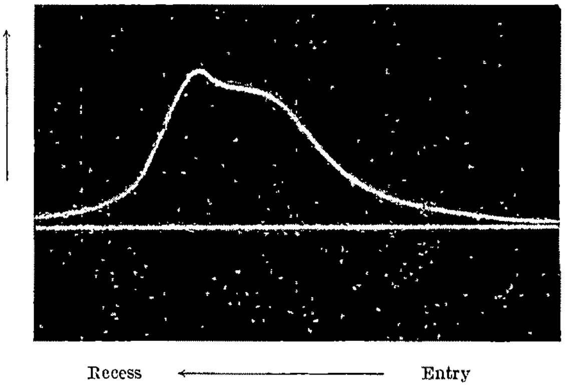

Fig. 2. Oscilloscope trace of potential across *R* (Fig. 1*b*)

by another method[1]. Another notable feature of the figure is that the parallel section is followed by a constriction in which the film thickness falls by approximately 10 per cent. These features confirm qualitatively theoretical ideas propounded in an approximate analysis by Grubin[2] and recently by Dowson and Higginson[3] and Archard, Gair and Hirst[4] in more exact analyses. The parallel section confirms that the pressure distribution is approximately Hertzian, while the constriction implies that somewhere towards the recess edge the pressure exceeds that of the Hertzian distribution. The practical implication of the excess pressure is that the material of the disks might be more severely stressed than is suggested by the Hertzian analysis on which design is at present based. The practical implication of the constriction is that it is the most probable site for breakdown of the oil film and the initiation of surface damage. A fuller account of the results will be published later.

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Phase-Velocity of Ultra-sound in the Primary Aliphatic Amines

Continuous-wave electronic measurements of the speed of sound in liquids commonly depend on the presence of appreciable standing-waves in paths of low absorption, the half-wave-length being determined by detection of successive resonances as the path-length[1] or frequency[2] is changed. Alternatively, for liquids of high absorption in which the energy content of the standing-wave is necessarily small, the wave-length may be found by direct comparison of the phases of the transmitted and received signals. In conditions of very high absorption it has been necessary to use pulse techniques to obtain adequate acoustic intensity at the receiving transducer consistent with tolerable energy dissipation in the medium.

I have devised an alternative method of measurement employing continuous-wave frequency-modulated ultra-sound. The lower permissible signal/noise ratio in a frequency-modulation system allows operation at much-reduced acoustic intensities. The path occupied by a complete period of the modulation in a liquid sample is determined. The liquid is contained in an interferometer having separate transmitting and receiving quartz transducers. The frequency of the ultra-sound is varied sinusoidally by approximately ±40 kc./sec. about a mean of 10·7 Mc./sec. The modulation of the received ultra-sound is detected by a ratio discriminator preceded by a limiting stage. The observed absence of distortion of the detected modulation indicates that any dispersive effect in the liquids tested is too small to have appreciable effect for the frequency deviation used. Phase-lag of the received modulation is measured by inversion and linear addition to a sample of the transmitted modulation of equal amplitude. In the absence of phase-shift of electronic origin, the resultant is zero when the time of transit is an integral number of periods of the modulation, a condition obtained by adjustment of the modulating frequency. The phase-velocity is then given by:

$$v = Lf/N$$

where L is the length of the column, f is the modulating frequency and N is an integer. A range of path-lengths is used as a check on diffraction effects, and N-values are varied up to 4 as a check on consistency of behaviour of the electronic equipment. The temperature of the liquid is stabilized at 19·3 ± 0·1° C.

The first application of the method has been to the homologous series of primary aliphatic amines. Measured phase-velocities (v) appear in Table 1, the uncertainties being about 3 m.sec.$^{-1}$.

Table 1

Liquid	v (m.sec.$^{-1}$)	R $\frac{\text{cm.}^3}{\text{mole}}\left(\frac{\text{m.}}{\text{sec.}}\right)^{1/3}$	ΔR $\frac{\text{cm.}^3}{\text{mole}}\left(\frac{\text{m.}}{\text{sec.}}\right)^{1/3}$
n-Propylamine	1,234	877	194
n-Butylamine	1,269	1,071	187
n-Amylamine	1,296	1,258	186
n-Hexylamine	1,317	1,444	189
n-Heptylamine	1,345	1,635	191
n-Octylamine	1,354	1,826	198
n-Nonylamine	1,372	2,024	

Values of the molecular velocity defined in ref. 3 as:

$$R = v^{1/3} M/\rho$$

appear in the second column, M being the molecular weight and ρ the density. The increments ΔR with addition of successive $-CH_2-$ groups are tabulated in the third column. It is evident these are approximately constant, the mean being 190.

The constancy of ΔR was demonstrated by Rao[4] for six homologous series, an overall average of 195 being obtained. Weissler[1] obtains 190 for series of alcohols including branched isomers and ring molecules. More recently, Leon[5] quotes Mikhailov and Nizhin (1947) as obtaining 189 for the *l*-olefines, and gives an average of 188 for some higher paraffins.

It is hoped to publish a detailed account of the research elsewhere.

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METALLURGY

Effect of a Thin Oxide Film on the Reaction of Iodine Vapour with Copper

In experimental work on the oxidation of metals the preparation of specimens is directed to the removal of contaminants such as grease, the production of a consistently smooth or rough surface and the realization of a stable metallurgical structure defined by the grain size and texture. The congenital

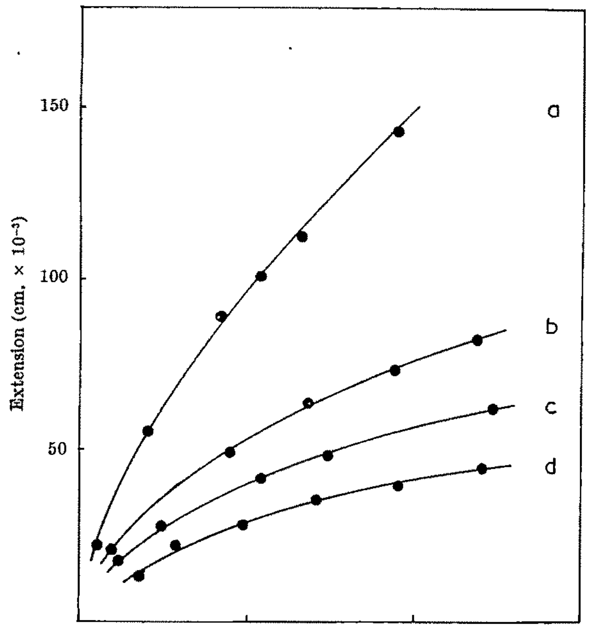

Fig. 1. Reaction of iodine vapour with copper at 285 ± 1° C., iodine reservoir at 16 ± 0·2° C. Times of room temperature exposure of specimens to the atmosphere before reaction: *a*, about 10 min.; *b*, 1 day; *c*, 3 days; *d*, 8 days

thin oxide film, which may increase in thickness on abrasion and polishing, or change in identity on electrolytic treatment, or disperse on heat-treatment *in vacuo*, is recognized as influencing the extent of the subsequent oxidation, but is largely ignored in discussions of the oxidation mechanism. In cases where the gaseous reactant is not oxygen, the thin oxide film, having no identity with the reaction system, assumes a different significance. Intuitively this is thought of in terms of protection and some results obtained in a preliminary study of the reaction between copper and iodine vapour demonstrate this.

Specimens (1 × 3 cm.) were cut from the centre of a 6-in. wide strip of 0·004-in. thick cold-rolled copper, de-greased with acetone and heated in an evacuated glass capsule at 500° C. for 1 hr. After cooling and opening the capsule the specimens were stored in a desiccator until required. The reaction was followed gravimetrically using a calibrated 'Pyrex' glass spring of sensitivity 17·37 cm.gm.$^{-1}$, and a cathetometer which could be read to 0·001 cm. The specimen was suspended from the spring by a thin silica rod which carried a fiduciary mark, and this assembly was further suspended in a vertical glass reaction tube, the lower closed end of which served as the iodine reservoir. The reaction zone was provided by a tube furnace, its temperature being controlled with a conventional circuit incorporating an auto transformer and 'Electroflo' controller. Temperature measurement was carried out potentiometrically, using a calibrated platinum – 13 per cent rhodium couple placed in a well extending into the reaction zone and sealed into the reaction tube.

With an acetone–solid carbon dioxide bath surrounding the iodine reservoir and a specimen in position, the apparatus was evacuated (10^{-3} mm. mercury) while the reaction zone was heated. When the steady controlled temperature was achieved, the reaction tube was isolated from the pump, the cold bath replaced by a water jacket, and readings of the cathetometer were made at suitable intervals.

The reactions were carried out at 284 ± 1° C. with the iodine reservoir at 14 ± 0·2° C. The results of these measurements are shown in Fig. 1, where the extension of the spring is plotted as a function of time, and each specimen is identified by the time of its exposure to the atmosphere between its removal from the heat treatment capsule and its suspension in the reaction tube.

It is seen that the increase in this time of exposure results in a retardation of the formation of cuprous iodide, which is due apparently to the presence of an oxide film of increasing thickness. This explanation requires the disappearance of the original oxide film during the vacuum heat-treatment at 500° C. in favour of a solution of oxygen in copper, with the internal precipitation of oxide at concentrations above the solubility limit; the continuous growth of a new oxide film at room temperature over these times of exposure to the atmosphere; and the diffusion processes above to take place at such a low rate at the reaction temperature that the new oxide film remains intact. This leads to a picture of the reaction in which the oxide film is conferring some protection on the copper, and from which it may be inferred that in the absence of such a film, as would obtain with hydrogen reduction of the specimen within the apparatus, the reaction would be very fast.

It would appear further that transport through the oxide film is largely determining the rate of reaction, superimposed on the characteristic diffusion kinetics in the growing iodide film. In view of the lack of knowledge of the thickness of the oxide films and the inherent uncertainty in this experiment of establishing precisely the time of commencement of the reaction, an analysis of these measurements has not been made. A more detailed study is, however, now in progress.

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GEOLOGY

Determination of the Age of a Basic Igneous Intrusion by the Potassium–Argon Method

Potassium–argon age determinations have been carried out mainly on minerals with a high potassium content, particularly micas and potassium feldspars, found in intermediate to acid igneous rocks, but little work has been done on minerals with a low content of potassium, such as occur in basic igneous rocks. However, Hart *et al.*[1] have shown recently that it is possible to obtain reliable ages using hornblende and pyroxene. I decided to investigate the relative argon retention properties of pyroxene, plagioclase and sanidine in two specimens from a Tasmanian dolerite body, and in so doing hoped to determine the time of intrusion of the dolerite. The presence of chilled margins to these dolerite intrusions also suggested the possibility of carrying out potassium–argon dating on a whole rock sample from such a contact.

Table 1. POTASSIUM–ARGON AGE MEASUREMENTS ON TASMANIAN DOLERITES

Sample No.	Specimen No.	Mineral	Potassium analyses (per cent)	Average potassium (per cent)	Diff. in potassium (per cent)	$^{40}Ar/^{40}K$	Air correction (per cent)	Calculated age (m.y.)
KA 811	*M*205	Sanidine	7·10, 7·05	7·075	0·7	0·008692	23·4	143·2
KA 811*R*	*M*205	Sanidine	7·10, 7·05	7·075	0·7	0·008771	14·2	144·5
KA 833	*M*207	Sanidine	6·76, 6·66	6·71	1·5	0·009569	17·2	157·0
KA 833*R*	*M*207	Sanidine	6·76, 6·66	6·71	1·5	0·009508	19·6	156·1
KA 812	*M*205	Plagioclase	0·568, 0·578	0·573	1·7	0·010181	18·1	166·7
KA 812*R*	*M*205	Plagioclase	0·568, 0·578	0·573	1·7	0·010239	25·6	167·6
KA 834	*M*207	Plagioclase	0·525, 0·519	0·522	1·1	0·009774	56·4	160·3
KA 834*R*	*M*207	Plagioclase	0·525, 0·519	0·522	1·1	0·009680	19·1	158·8
KA 813	*M*205	Pyroxene	0·245, 0·237	0·241	3·3	0·009881	22·9	162·0
KA 835	*M*207	Pyroxene	0·171, 0·169	0·170	1·2	0·01017	38·4	166·5
KA 810	*M*172	Chilled dolerite	0·747, 0·750	0·7485	0·4	0·009007	46·6	148·2

$\lambda = 5{\cdot}304 \times 10^{-10}$ yr.$^{-1}$ $\lambda_e = 0{\cdot}584 \times 10^{-10}$ yr.$^{-1}$

The Tasmanian dolerites, which are tholeiitic in composition[2], were intruded into an essentially flat-lying sequence of Permian and Triassic sediments approximately 4,000 ft. in thickness, as sheets up to 2,000 ft. thick, as large transgressive bodies and as dyke-like intrusions which exceptionally may be one mile in width. The dolerite crops out over an area exceeding 6,000 square miles, mainly in the eastern half of Tasmania. The youngest sediments which the dolerite intrudes are considered to be Middle or possibly Upper Triassic in age[3], on the basis of the plant fossils present, so that the dolerite is younger than this. Lower Tertiary fresh-water sediments occur overlying dolerite in northern Tasmania[3], placing an upper limit on the age of the dolerite. By analogy with the Karroo dolerites, which are Liassic to Lower Cretaceous in age[4,5], and which are quite similar in composition to the Tasmanian dolerites, a Jurassic age has generally been assigned to them. Analyses of the chilled dolerites from the contacts of the intrusions are all closely similar[2], suggesting that the Tasmanian dolerite magma was intruded over a relatively short period of time.

Two specimens of fayalite granophyre and a chilled contact dolerite from the large Red Hill Dyke, 20 miles south-west of Hobart in southern Tasmania, were chosen for the age determinations. The fayalite granophyre is a differentiate of the dolerite magma and consists essentially of plagioclase (anorthite$_{60}$) comprising 48 per cent by volume, pyroxene—a ferroaugite (8 per cent), fayalite (4 per cent), potassium feldspar (16 per cent), quartz (19 per cent) and iron ore (2 per cent). The potassium feldspar, which has a composition about orthoclase$_{60}$, belongs to the sanidine-anorthoclase cryptoperthite series of Tuttle[6], and by the definition of MacKenzie and Smith[7] it is a sanidine from its optical properties. The chilled dolerite is composed of microphenocrysts of plagioclase, orthopyroxene and clinopyroxene amounting to about 7 per cent of the rock, set in a microcryptocrystalline ground-mass which has the appearance of devitrified glass.

The fayalite granophyres were crushed to -60 $+100$ mesh, and plagioclase, pyroxene and sanidine were separated using bromoform. The separated minerals were split into two portions, one of which was used for duplicate potassium analyses and the other for the argon extraction. In some cases duplicate argon runs were made. The argon extraction on the chilled dolerite was run on a single uncrushed piece of the rock and the potassium on a sample crushed to -100 mesh.

The potassium analyses were made by using a Perkin–Elmer flame spectrophotometer with lithium as an internal standard. The R_2O_3 group and lime were removed by precipitation before the potassium was determined. The argon extractions were carried out in a high-vacuum line originally at a pressure of about 10^{-8} mm. mercury, and the sample was fused by induction heating. A tracer of argon-38 was added to the evolved gases, which were cleaned up by reaction in copper oxide and calcium furnaces. The isotopic composition of the purified argon was determined by means of a Reynolds-type mass spectrometer[8].

The results are set out in Table 1. The agreement between duplicate potassium analyses is in all cases better than 2 per cent of the amount present, except for pyroxene *KA* 813. The low concentration of potassium in the pyroxenes makes it difficult to attain high precision in the analysis. The rock was only crushed to -60 $+100$ mesh in order that losses of argon due to grinding would be negligible, so that some composite grains were present in the pyroxene concentrates, which could not be removed by the usual methods of purification. In both pyroxenes the impurities, which consisted mainly of plagioclase with minor sanidine, accounted for less than 5 per cent of the sample. However, because of the relatively high potassium content of the impurities, compared with the pyroxene, about half of the potassium in *KA* 813 and approximately one-third of the potassium in *KA* 835 is attributable to the impurities. The plagioclase separates were better than 98 per cent pure and those of sanidine contained approximately 5 per cent impurity of altered plagioclase.

The calculated ages range from 143 to 168 million years, but the plagioclase and pyroxene results gave ages ranging from 159 to 168 million years, a spread of less than 6 per cent. As these two minerals have quite dissimilar crystal structures this small spread of calculated ages suggests that argon retention, in both minerals, is high. Therefore it is considered that the calculated age approaches the true age closely, and that the time of intrusion of the Tasmanian dolerites was near 167 million years ago, the higher figure being preferred, as any loss of argon which may have occurred would lower the age. This corresponds to an age approximately at the boundary of the Lower and Middle Jurassic[9,10]. Although difficult to place probable errors on the physical measurements, replicate argon determinations, in all cases, agree to within 1 per cent, indicating a very high precision. The reproducibility of the potassium analyses is generally less than that in the argon measurements, showing that greater attention must be paid to refining the techniques for the determination of potassium.

The calculated ages for the sanidines are somewhat lower than those for plagioclase and pyroxene, suggesting some leakage of argon. The very low calculated age for sanidine *KA* 811 as compared with *KA* 833 is surprising, as both minerals appear to be very similar in thin section. They both appear to be homogeneous, as no unmixing has been observed, although it may be present on a submicroscopic scale. The sanidines are slightly turbid as a result of deuteric alteration, and this has probably aided leakage of argon. A satisfactory explanation for the loss of argon from potassium feldspars and, in particular, loss from the high temperature, homogeneous, monoclinic sanidines has not yet been advanced ; a thorough structural study of this group of minerals in conjunction with their observed argon retention properties is urgently required. It may be noted, however, that some sanidines, and particularly those with a high potassium content, do retain argon quantitatively (ref. 11 and Evernden, J. F., personal communication).

The single chilled dolerite result gives 148 million years, which is a surprisingly good age considering that the rock is composed mainly of devitrified glass, and hence might be expected to lose considerable argon.

In conclusion it may be stated that the Tasmanian dolerites were intruded some 167 million years ago at approximately the boundary of the Lower and Middle Jurassic, and that it has been demonstrated that minerals with a low potassium content, such as occur in basic igneous rocks, can be used for age determinations by the potassium–argon method. Chilled dolerites and possibly glassy basalts will also give some aid in the elucidation of the age of basic igneous rocks by this method.

Grateful acknowledgment is made to Dr. G. H. Curtis and Dr. J. F. Evernden for the use of their facilities and to them and Mr. J. Obradovich for their interest and help in this work. This work was carried out while I held a Commonwealth Scientific and Industrial Research Organization (Australia) post-graduate overseas studentship, for which thanks are expressed.

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Erosion Surfaces in the Caribbean and their Significance

ALTIMETRIC analysis of a series of topographic maps covering the main Cordillera of Puerto Rico indicates the existence of erosion surfaces, represented largely by concordant summit-levels, at the following average elevations : 760, 620, 470, 330 and 160 m. above present sea-level[1]. Field work at present in progress suggests that these elevations in fact represent mean elevations of groups of stepped surfaces, in some instances bearing sedimentary deposits which have been described previously as residual soils.

At the present time it is not possible to reach any firm conclusion as to the mode of origin of these surfaces ; but their distribution, at least in western Puerto Rico, suggests that they probably originated as marine cut surfaces, later modified by sub-aerial processes as they successively emerged from the sea. Whatever conclusion is eventually reached as to their origin, it seems fairly certain that they are related to changing sea-levels, the more widespread surfaces—particularly the 330 and 160 m.—presumably representing periods of relative standstill.

Examination of these surfaces was originally undertaken in the hope of determining the tectonic movements that had affected the island subsequent to middle Miocene times. However, Choubert[2] has described a series of surfaces in French Guiana with the following mean elevations : 600 (+), 500, 330, 235 and 160 m. These show a remarkable parallelism with the Puerto Rican levels, and this is borne out when the ranges of elevation of each surface are taken into account. In Puerto Rico, the 330- and 160-m. groups of surfaces are perhaps the most conspicuously developed and, from Choubert's map, this seems to be the case in French Guiana, too.

Furthermore, Martin-Kaye[3] has directed attention to the conspicuous concordance of summits in the northern Lesser Antilles at, or just above, 1,000 ft. This would seem to correlate with the 330-m. group in Puerto Rico and French Guiana. It seems scarcely likely that such correspondence of levels is a chance one, and I was reluctantly forced to the conclusion that these levels must be related to large eustatic lowering of sea-level and that the stage-by-stage emergence of Puerto Rico was not brought about by tectonic movements as had previously been taken for granted. An overall fall in sea-level since pre-glacial times has already been postulated by Wooldridge[4] and others, at least from 600 ft. above present sea-level, and even greater movements of sea-level have been hinted by Baulig[5] and Hollingworth[6].

The obvious difficulty in the way of accepting such large eustatic lowering of sea-level lies in the mechanism which would dispose of such large quantities of water. Geyl[7] has postulated a mechanism involving continental 'shift' induced by deep convection currents and isostatic readjustment of the continental areas. It seems doubtful if such readjustments, as between the continent of South America and the Caribbean islands, could be carried out without notably disturbing the relative heights of the erosion surfaces. Hence it is necessary to find a mechanism which would produce considerable eustatic lowering of the sea-level without relative vertical displacement of the land masses.

Heezen's[8] hypothesis of an expanding Earth, associated with the opening and widening of oceanic rifts, shows promise of providing such a mechanism. It is clear that, according to this hypothesis, every stage in the widening of the rift zones would add capacity to the ocean basins and thus would cause a lowering of sea-level.

A first approximation from available data indicates that a widening of the rift zones of about 150 km. would produce about 500 m. fall in sea-level. This is, of course, only a rough estimate ; but it does indicate that the order of magnitude is reasonable. As Heezen points out, the Iceland graben is opening at the rate of 3·5 m. per kilometre width per thousand years. Hence, it is not at all unreasonable to postulate widening of the oceanic rift zones by considerably more than 150 km. since middle Miocene time.

The interest in these observations is two-fold: (1) from the more general point of view, there is the further evidence of large eustatic lowering of sea-level since the middle Miocene and a possible mechanism to account for it; (2) from the regional point of view, there is the implication that the Caribbean, a tectonically active area, has suffered negligible vertical differential movements since the Miocene, and hence it would appear that any large displacements of more recent date must be confined to those in the horizontal direction.

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CHEMISTRY

Comparison of Helium and Argon in Ionization Detectors

R. BERRY[1] has recently directed attention to the use of helium in place of argon in a conventional argon detector. All the rare gases should behave in the same manner as argon, that is, collision of metastable rare gas atoms with molecules of other gases should deactivate the metastables with the possibility of forming ions from the colliding molecules in the appropriate conditions.

Owing to the high ionization potential of metastable helium atoms, it should be possible to arrange conditions so that a collision with one of these will ionize all other molecules except neon. As has been pointed out, however, cylinder helium contains far too many impurities for this effect to be noticed, and it is obvious that a very considerable purification process has to be set up to obtain helium sufficiently pure for the effect to be observed. This purification process is regularly done by the British Oxygen Company, the prime use for the gas so produced being in discharge tubes and the electronics industry.

It was decided, however, to see whether this helium was of sufficient purity for an argon-type detector to be useful. The results can now be compared with the information given by Berry. The helium used in this work was analysed by conventional means and the results are shown in Table 1.

Table 1. ANALYSIS OF PURE HELIUM

	H_2	O_2	$CO_2 + CH_4$*	H_2O
Concentration in v.p.m.	< 1	< 2	2·3	< 3

* See text.

The oxygen was determined by Hersch cell and the carbon dioxide and methane (a term used here to include all other carbonaceous material) after oxidation to carbon dioxide, by titration, the method having been checked previously against infra-red. The notable absentee in the analysis is the neon content. The helium used here was not analysed for neon. Analyses for this gas have been carried out on previous batches by mass-spectrograph, but neon was not detected. None the less, it is possible that the differences in neon content can affect the results obtained and that discrepancies between my work and that of Berry could be explained on that basis.

The apparatus used included the normal argon cell (Type *IE*.10) as manufactured by Gas Chromatography, Ltd., and the electronic equipment was one of their standard amplifiers (Type *IE*.11) modified by having an input impedance of only 71 megohms. There was no linearizing resistance in the ordinary sense, only a resistance of about 20 megohms to protect the grid of the electrometer valve.

It should be pointed out, however, that in all cases where the properties of ionization detectors are being investigated, the inclusion of both linearizing and input impedances affects the voltage across the cell once the current approaches a certain figure. As can be seen from Table 3, currents of approximately 1 μamp. are developed in the detector. This means that the resistance of the cell is about 10^9 ohms, a figure comparable with the value of the linearizing resistance used by Dr. Lovelock in his original argon detector and, so far as can be gathered, in many other versions of this instrument, and it is of such magnitude that even an input impedance of 10^8 ohms can cause the potential across the cell to be substantially less than the apparent applied potential. However, this only applies here when the maximum current is flowing and, for the purposes of most of my experiments, the total impedance of 91 megohms can be ignored compared with the resistance of the cell.

Table 2 gives standing currents and noise-levels for both helium and argon and Table 3 gives typical results for permanent gases in helium and hydrocarbons in argon.

Table 2

Voltage	Standing current $\times 10^{-8}$ amp.		Noise $\times 10^{-10}$ amp.
	Helium	Argon	
100	0·5	3·1	—
400	1·2	3·2	—
600	6·6	3·3	± 2·0*
700	28	—	—
1,000	—	4·5	± 0·6†
1,400	—	9·2	—

* Theoretical value ± 0·7.
† Theoretical value ± 0·5.

Table 3

1 Gas	2 In	3 Max. current $\times 10^{-8}$ amp.	4 Dilution at max. $\times 10^{-4}$ v/v	5 Linear from $\times 10^{-5}$ v/v	6 v.p.m. current $\times 10^{-8}$ amp.	7	8 Limit of detection v.p.m.
H_2	He	71	~10	*	~0·05	1·01	0·4
N_2	He	80	7.5	*	0·14	1·02	0·1
Ar	He	> 85	> 1·7	~ 1·3†	0·24	1·035	0·1
O_2	He	34	2·5	4	0·6	1·09	0·03
CH_4	He	> 85	⩾ 1·0	10	~1·2	1·18	0·01
CO_2	He	80	1·7	2	4·1	1·62	0·005
C_4H_{10}	Ar	19	~10	31	0·04	1·01	0·1
C_4H_{10}	Ar‡	> 85	> 4	11	0·065	1·015	0·06

Readings at 600 V.

* Non-linear positively. † Not a useful range, otherwise as N_2, H_2.
‡ at 1,000 V.

The argon results give positive non-linearity above linear point, the helium results give negative, except as stated.

In Table 3, column 4 gives the dilution of the gas at maximum current; column 5 gives the dilution of the gas when the device turns linear; column 6 gives the current at 1 v.p.m. of impurity; column 7 is the (v.p.m. current + standing current) divided by the standing current (see Berry[1]). These results were obtained by a dilution technique.

It is immediately apparent from these results that the sensitivity and linearity of different gases varies enormously in contradiction to the implication given by Berry. On the other hand, the current at 1 v.p.m., with many of the gases tested, was much higher than the figures he quotes. It seems reasonably certain that this gas is substantially purer than the helium used by Berry. The figures in Table 2, particularly the large increase in standing current with increase in voltage, indicate that there are residual impurities in the helium, but that they can easily be accounted for by the "CO_2 plus CH_4". It is thought likely that water gives a high current in helium, and this will also undoubtedly contribute to the standing current. From the cross-section of helium, compared with that of argon, and the characteristics of the cell in argon, the standing current of pure helium at voltages less than 1,000 should be about $0·5 \times 10^{-8}$ amp. If this is so, then the ultimate sensitivity of this device is likely to be at least ten times higher than the argon detector, owing to the much greater currents produced in helium.

The sensitivity at 700 V. is increased approximately in proportion to the increased standing current, but it is thought that the linearity is somewhat better at a higher voltage. Linearity may be improved by further purification.

The linearities given in Table 3 have been confirmed by injecting known quantities of gas directly on to a chromatograph. The peak heights and areas are in complete agreement with the results of Table 3.

As regards the quantity of gas that can be injected into the chromatograph, it is difficult to compare my results with those of Berry. He gives no indication of flow-rates, retention times, etc., but in my work, oxygen reaches the limit of its linear range with about 1 μl., while nitrogen has not reached saturation limit with 6 μl. My column was 4 ft. long, 4 mm. diameter, packed with Linde molecular sieve, and it was operated at a room temperature and a helium flow-rate of about 130 c.c./min. Oxygen took about 20 sec. to emerge and nitrogen about 90 sec.

There were one or two small pieces of rubber tubing in the apparatus used which, it was felt, might contribute to the standing current. A new apparatus is being devised in which these are eliminated and further tests are to be carried out. It is certain, however, that helium of sufficient purity to obtain the sensitivity which one would expect from this type of device can be prepared. There is no doubt that this device is an extremely sensitive one.

In conclusion, it should be pointed out that the process of purification of the helium may be followed by using a detector cell of this type. As the purity of the helium rises, so does the standing current. At this stage the injection of a quantity of permanent gases or other substances produces a drop in current (cf. Willis organic bleed). As the level of impurities falls, the current rises and eventually passes a maximum. After this point, injections of substances produce a *W*-shaped peak with more and more on the positive side as the purity rises further.

Finally, large positive peaks of sufficient linearity are obtained.

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IT is interesting to learn that Wiseman has obtained experimental results in general confirmation of my own. The only discrepancy concerns the sensitivity ratio for the different gases. Wiseman finds this ratio to increase through the series nitrogen, argon, oxygen, methane from 1·02 to 1·18, whereas I found the same value (1·05) for all four gases. Certainly some variation would be expected, but there is insufficient basic information available to permit this to be estimated with precision. One can say, however, on the basis of the work of Jesse and Sadauskis, that carbon dioxide would not be expected to give as high a value as 1·62, which Wiseman reports.

If no other factors interfere, the value of this ratio, for a given gas, should decrease as the impurity content of the helium increases. Since my result lies between the extremes quoted by Wiseman, deductions of the relative purity of his helium and mine cannot be made in this way. Neither does the analysis of the helium by conventional means give much help; my analysis (H_2, N_2, O_2, Ar and H_2O all < 1 v.p.m.) indicates that my helium carrier gas was of the same order of purity as Wiseman's. Since the methods are all at their limits of detection and, at these levels, contamination is very difficult to avoid, comparison is not really profitable.

It may be relevant that my method of determining the sensitivity ratio is to introduce sample volumes from 0·005 μl. (S.T.P.) upwards of the foreign gas into the helium immediately ahead of the column. Wiseman, since he injects quantities not less than 1 μl., must necessarily use a less-direct method of calibration, involving more lengthy extrapolation. It is encouraging that, despite these differences, our estimates of detection limits are of the same order of magnitude.

If helium requiring no further purification can be supplied at a price which is not prohibitive, the applicability of this method of detection will obviously be enhanced.

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Determination of Uranium as Pyrophosphate

IN the gravimetric determination of uranium as phosphate, the ignited precipitate is commonly considered[1] to be the pyrophosphate $(UO_2)_2P_2O_7$ (U = 66·67 per cent). In recent work in these laboratories uranium was precipitated at *p*H 5·5 from solutions containing ethylenediaminetetraacetic acid, nitric acid and ammonium sulphate. The results were consistently low by 2·3 per cent relative when the precipitate was ignited to constant weight at 850° C.[2] and calculations made on the basis of the formula $(UO_2)_2P_2O_7$. As no significant quantities of uranium could be detected in the filtrates to explain the low results, the ignited precipitates were examined. They were dissolved in concentrated phosphoric acid and the solutions titrated with standard potassium dichromate solutions. From the observed titrations it was calculated that the ignited precipitates contained 33·2 ± 0·3 per cent quadrivalent uranium. For precipitates ignited at 1,000° C. the corresponding result was 33·9 ± 0·1 per cent quadrivalent uranium.

From these results it is apparent that the ignited compound cannot be represented by $(UO_2)_2P_2O_7$ and the most probable formula is UO.UO$_2$.P$_2$O$_7$ (U^{4+} 34·10 per cent; total U 68·205 per cent). Supporting evidence has been obtained by thermogravimetric analysis, but attempts to use X-ray diffraction were unsuccessful because of the non-crystalline nature of the compound.

The precipitate of uranyl ammonium phosphate is crystalline, settles readily and is easy to filter and wash.

Using the formula UO.UO$_2$.P$_2$O$_7$, quantitative recovery of uranium has been obtained from solutions of known uranium content. The results obtained on a variety of impure uranic solutions have shown good agreement with those obtained using cellulose chromatography and final weighing as U$_3$O$_8$.

An account of this work will be published in detail elsewhere.

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Control of Self-heating and Gas Emission in Nitrate-containing Fertilizers

THE concentration of plant food in mixed fertilizers can be increased by the replacement of ammonium sulphate, wholly or in part, by ammonium nitrate. Granular mixtures made, *inter alia*, from ammonium nitrate and superphosphate (a mixture of monocalcium phosphate and calcium sulphate) have been found to undergo spontaneous heating when stored in large heaps, rises in temperature of 20–30 deg. C. being recorded over periods of weeks.

This spontaneous heating is accompanied by the evolution of gas, and, by measuring the rate by a simple manometric technique, we have studied factors affecting the self-heating reactions. The rate of the reaction increases with the acidity and decreases with increasing moisture content of the fertilizer. A decrease of the acidity of the mixture, sufficient to prevent self-heating, leads to a large and undesirable loss in the water-solubility of the phosphate component owing to the formation of dicalcium phosphate. An increase in the moisture content of the fertilizer is not practicable, as it increases the tendency of the mixture to cake.

Phosphate rocks contain small amounts of organic matter of unknown composition[1], and we have confirmed that this material is not destroyed during the manufacture of superphosphate. Typical amounts of organic matter in some superphosphates used in the production of fertilizers in the U.K. are shown in Table 1.

Table 1. ORGANIC MATTER IN SUPERPHOSPHATES

Source of rock for superphosphate	Organic matter content (per cent carbon)
Morocco	0·10
Nauru	0·40
Florida	0·16
Gafsa	0·50

In a mixed fertilizer made, *inter alia*, from ammonium nitrate and one of these superphosphates, it might be expected that the organic matter would be oxidized by the nitrate under acidic conditions. We have shown that the gas evolved from the fertilizer consists mainly of carbon dioxide and nitrogen (ratio approx. 1 : 5), together with small amounts of nitrogen dioxide. Acidified mixtures of some organic materials and ammonium nitrate also liberate these gases. Powdered coal reacts similarly when incorporated into a nitrate-containing fertilizer. The amount of carbon dioxide evolved from the fertilizers shows that oxidation of the organic material is far from complete, and thermo-chemical calculations on model organic systems (for example, polynuclear hydrocarbons) show that partial oxidation, to products such as phthalic acid, would liberate sufficient heat to explain the observed rises in temperature.

The self-heating of nitrate-containing mixed fertilizers may be reduced by removing, or modifying, the organic material. We have achieved this by calcining the initial rock at temperatures of 800–1,000° C. and by other means involving oxidation of the organic matter before the superphosphate is mixed with the other components[3]. Of these methods, calcination is undesirable, as it decreases the reactivity of the rock towards sulphuric acid in superphosphate manufacture.

The auto-oxidation of some organic nitro-compounds can be prevented by the addition of substituted ureas or secondary amines[2], and we have found that addition of small amounts of urea, 0·25–0·75 per cent by weight, to mixed fertilizers effectively reduces the oxidation of the organic matter and hence the self-heating of the fertilizers[3]. The effect of urea on the reactivity of laboratory-prepared samples of a mixed fertilizer (N, 10 per cent : P$_2$O$_5$, 10 per cent : K$_2$O, 18 per cent) containing 20 per cent ammonium nitrate is shown in Table 2. Each fertilizer contains 0·25 per cent urea, and the activity of the material is assessed in terms of the gas pressure developed in 48 hr. at 50° C.

Table 2. EFFECT OF UREA ON THE REACTIVITY OF A MIXED FERTILIZER

Source of phosphate rock	Percentage reduction in reactivity
Morocco	70·5
Florida	88·5
Nauru	92·0

Incorporation of urea into these fertilizers also has a marked effect on the release of nitrogen dioxide. Table 3 shows results obtained with a mixed fertilizer (N, 10 per cent : P$_2$O$_5$, 10 per cent : K$_2$O, 18 per cent), the nitrogen dioxide being evolved into a stream of air drawn through the fertilizer.

Table 3. EFFECT OF UREA ON THE EVOLUTION OF NITROGEN DIOXIDE

Source of rock for superphosphate	Urea content (per cent)	NO$_2$ evolved (ml./kgm. fertilizer) in 14 days
Nauru	0·0	120·7
Nauru	0·10	28·7
Nauru	0·50	19·7
Morocco	0·0	49·3
Morocco	0·25	9·3
Morocco	0·50	4·0

The oxidizing action of nitric acid involves the presence of trace amounts of nitrous acid, probably in the form of the nitrosonium ion[4]. It seems likely that, in the oxidation process we have studied, nitrous acid is formed autocatalytically, so that it should be possible to suppress the oxidation by removing the nitrous acid with, for example, urea. A small quantity of urea should be adequate to prevent the oxidation, and, in fact, we have observed no appreciable loss during long-term storage of fertilizers. The rate of the urea/nitrous acid reaction is undoubtedly fast enough to explain this inhibition effect[5]. It is well known that the oxidation of organic compounds during nitration can be prevented by the addition of small amounts of urea[6].

So far, approximately 1·25 million tons of mixed fertilizers containing urea as an inhibitor have been produced and have shown satisfactory storage properties, both in large heaps and in paper sacks. At the elevated temperatures attained in the absence of urea the caking tendency is increased, causing subsequent difficulty in reclaiming from the heap. Storage of the untreated fertilizer in paper sacks causes severe chemical attack on the paper (due to nitrogen dioxide) with subsequent breaking of the sacks when moved.

In conclusion, it may be noted that self-heating of fertilizer storage heaps can also occur if inter-ionic reactions (for example, between ammonium nitrate and potassium chloride) take place. Results of investigations into this problem, and a more detailed account of the urea inhibition work, will be published elsewhere.

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Solid Solutions in the System Ammonium Nitrate–Potassium Nitrate

BY phase work on the system ammonium nitrate–potassium nitrate–water, Janěcke[1,2] showed that below 23° C. two types of solid solution can exist: potassium nitrate dissolved in ammonium nitrate (form III) and ammonium nitrate in potassium nitrate (form II). At higher temperatures there is an increasing amount of another phase which is of a different crystalline form from any of the normal modifications of the two salts. Janěcke suggests that this phase is a solid solution of ammonium nitrate in a metastable form of potassium nitrate, which is only formed on cooling, in the temperature-range 128°–115° C.

By using X-ray diffraction measurements, Janěcke's suggestion has been confirmed. Further, the limits of the composition of the solid solution have been determined and also its heat of formation from the individual salts.

Aqueous solutions of ammonium and potassium nitrates were evaporated to dryness at various temperatures between 25° and 120° C. After cooling, diffraction patterns were obtained using a Geiger diffractometer, radius 24 cm.; the radiation was copper $K\alpha$, the $K\beta$ component being removed by a nickel filter. From the diffraction patterns, the relative abundance of each phase was determined.

Samples produced at temperatures of 100° C. and above, from equimolar mixtures of the two salts, consisted almost entirely of a phase having a diffraction pattern similar to that found by Kracek *et al.*[3] and by Barth[4] for the metastable form of potassium nitrate. Hereafter, this phase will be referred to as (xKNO$_3$.NH$_4$NO$_3$). If more ammonium nitrate is added to an equimolar mixture, it does not dissolve in this phase, and increasing amounts of ammonium nitrate (form III) containing dissolved potassium nitrate are found. Thus, in (xKNO$_3$.NH$_4$NO$_3$) the value of x cannot decrease below approximately 1. When the molar ratio of potassium nitrate to ammonium nitrate is greater than one, the mixture still consists essentially of a single phase having a very similar diffraction pattern. In the phase (xKNO$_3$.NH$_4$NO$_3$), x can rise to a maximum of approximately 3, this change being accompanied by a slight shift in the diffraction pattern. In Table 1 the unit cell dimensions found for solid solutions of composition (3KNO$_3$.NH$_4$NO$_3$) and (KNO$_3$.NH$_4$NO$_3$) are compared with those of the high temperature and metastable forms of potassium nitrate; Table 2 shows the corresponding diffraction patterns. All lines are listed up to a maximum Bragg-angle of 35°, the values of 'd' being accurate to 0·01 Å.

Table 1. UNIT CELL DIMENSIONS OF THE HIGH-TEMPERATURE FORMS OF POTASSIUM NITRATE, AND RELATED SOLID SOLUTIONS

Material	Space group	Cell size Hexagonal axes (3 moles/cell)	Rhombohedral axes (1 mole/cell)
KNO$_3$ (form I) at 130° C.	D^5_{3d} — $R\bar{3}m$	a = 5·44Å. c = 9·76 Å.	a = 4·54 Å. α = 73° 24′
KNO$_3$ (metastable) at 120° C.	C^5_{3v} — $R3m$	a = 5·48 Å. c = 9·27 Å.	a = 4·42 Å. α = 76° 42′
NH$_4$NO$_3$3KNO$_3$ at 20° C.	C^5_{3v} — $R3m$	a = 5·52 Å. c = 9·09 Å.	a = 4·38 Å. α = 77° 42′
NH$_4$NO$_3$.KNO$_3$ at 20° C.	C^5_{3v} — $R3m$	a = 5·56 Å. c = 9·12 Å.	a = 4·42 Å. α = 77° 54′

The chief difference between the patterns of potassium nitrate (form I) and potassium nitrate (metastable) is the absence of the (0003) reflexion in that of the former. The presence of this reflexion in all patterns from the phase (xKNO$_3$.NH$_4$NO$_3$) indicates that its structure is derived from that of the metastable form. When solutions of composition (KNO$_3$.NH$_4$NO$_3$) and (3KNO$_3$.NH$_4$NO$_3$) are heated to 130° C., the diffraction patterns show that there is an abrupt expansion of the lattice, and a total disappearance of the (0003) line, that is, the transition to potassium nitrate (form I), occurs.

When ammonium and potassium nitrates react at temperatures below 100° C. the extent of conversion to the phase (xKNO$_3$.NH$_4$NO$_3$) is considerably less, and increasing amounts of ammonium nitrate (form III), containing dissolved potassium nitrate, are found. There is also a tendency for x to have a value less than the maximum possible.

The X-ray measurements show that the solution of ammonium nitrate in the metastable form of potassium nitrate causes the lattice to expand. This is to be expected, since the ammonium ion (radius 1·42 Å.) is slightly larger than the potassium ion (radius 1·33 Å.). On cooling metastable potassium nitrate the lattice contracts until, at approximately 115° C., it collapses into a more compact form. This change is delayed by the replacement of potassium by larger ions, and in the case of ammonium ions the change does not occur on cooling to room temperature. The stability of the solid solution increases

Table 2. INDICES

Hexagonal axes	Rhombohedral axes	KNO$_3$(form I) at 130° C.		KNO$_3$(metastable) at 120° C.		NH$_4$NO$_3$.3KNO$_3$ at 20° C.		NH$_4$NO$_3$KNO$_3$ at 20° C.	
		d,Å.	$I_{rel.}$	d,Å.	$I_{rel.}$	d,Å.	$I_{rel.}$	d,Å.	$I_{rel.}$
$10\bar{1}1$	010	—	—	4·22	1	4·22	1	4·26	3
$01\bar{1}2$	110	3·38	100	3·32	100	3·30	100	3·31	100
0003	111	absent	absent	3·09	80	3·03	51	3·04	59
$11\bar{2}0$	$01\bar{1}$	2·72	22	2·74	27	2·77	28	2·78	35
$02\bar{2}1$	$11\bar{1}$	2·29	15	2·30	12	2·32	35	2·33	47
$20\bar{2}2$	020	2·12	19	2·11	13	2·11	21	2·13	33
$10\bar{1}4$	121	2·17	33	2·08	35	2·05	31	2·06	29
$11\bar{2}3$	120	2·08	17	2·05	18	2·05		2·05	33
$21\bar{3}1$	$02\bar{1}$	1·75	4	1·76	1	1·77	5	1·78	9
$01\bar{1}5$	221	1·80	2	1·72	3	1·69	8	1·70	9
$12\bar{3}2$	$12\bar{1}$	1·67	3	—	—	1·69		1·70	
$30\bar{3}0$	$11\bar{2}$	1·61	2	1·58	0·5	1·59	1	1·60	1
$21\bar{3}4$	130	1·44	3	1·42	0·5	1·41	6	1·42	11
$30\bar{3}3$	030	1·41	1	1·41	0·5	1·41		1·42	
$22\bar{4}0$	$02\bar{2}$	1·36	1	1·37	0·5	1·38	1	1·39	1
$11\bar{2}6$	231	1·40	2	—	—	—	—	—	—

as more ammonium ions are added, and it is for this reason that there is a tendency for potassium nitrate to crystallize out from the phase (3KNO$_3$.NH$_4$NO$_3$), thereby increasing its content of ammonium ions. In the presence of traces of water, the phase (xKNO$_3$.NH$_4$NO$_3$) is unstable at room temperature and changes into solid solutions of potassium nitrate in ammonium nitrate (form III) and ammonium nitrate in potassium nitrate (form II).

The heats of solution in water of the phases (KNO$_3$.NH$_4$NO$_3$) and (3KNO$_3$.NH$_4$NO$_3$) were found to be − 13·4 and − 28·5 k.cal./mole respectively. In each case the material contained approximately 95 per cent of the phase (xKNO$_3$.NH$_4$NO$_3$). Using existing values for the heats of solution of potassium and ammonium nitrates, the heats of formation of the two solid solutions from the individual salts were calculated to be − 1·4 and − 3·2 k.cal./mole respectively. These values are accurate to within ± 10 per cent, and are comparable with that for the transition from the high- to the low-temperature form of potassium nitrate, + 1·19 k.cal./mole.

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Banded Spherulites of Hippuric Acid

IT has long been known that under certain conditions many substances crystallize in the form of spherulites showing concentric extinction bands between crossed polars. The explanation of this effect, originally put forward by Michel-Levy and Munier Chalmas[1] for chalcedony, and later confirmed by other workers[2-5] for a number of substances of low molecular weight, was that the structure of the spherulite has a twist around any given radius, and that the bands correspond to those orientations in which an optic axis is parallel to the light beam. Thus, Gaubert[5] obtained spherulites of hippuric acid in which the radial twist was around the β-axis of the indicatrix, a fact which he established conoscopically, that is, by a study of the interference figures along the radii. More recently there has been a revival of interest in banded spherulites, since they are formed by many polymers (for example, polythene and terylene). These cases have been the subject of much investigation by, among others, Keller[6], and Point[7], who have shown convincingly, from the optical effects of tilting the preparations with respect to the direction of the light, that the spherulites have a radially twisted structure as for the low molecular weight substances referred to above.

In the course of a student's exercise in this Department on the crystal optics of hippuric acid we recently obtained (i) a spherulite, part of which showed a well-defined radial twist around the β-axis as observed by Gaubert (Fig. 1), and also (ii), on another preparation, a number of complete spherulites showing a radial twist around the α-axis (Fig. 2), in both cases as a result of allowing a melted film between a slide and cover slip to crystallize spontaneously on a slowly cooling metal plate. The existence of both kinds of twists, and also their sense, have been established directly from the interference figures shown by the spherulites at successive points along their radii. The figures were particularly clear in (i), in which the pitch of the twist was large (about 1 mm.). Different stages in the twist (right-handed in this case) along one radius are pointed out in Fig. 1; thus, starting with the central emergence of an optic axis A_1 (extinction band in parallel light), we pass in succession to an acute bisectrix view (Bx_a), the central emergence of the other optic axis A_2 (extinction band again), an obtuse bisectrix view (Bx_o), and, completing a rotation of 180°, axis A_1 again, and so on. (In Fig. 1 as reproduced the extinction bands are not appreciably darker than the bands marking the 'sensitive violet' tint between first order red and second order blue.) In confirmation of the evidence afforded by the interference figures, two determinations of the ratio of the path differences for adjacent Bx_o and Bx_a regions by means of a graduated quartz wedge gave values of 3·0, 3·1, while that calculated from the published refractive indices[8,9] is 2·9. Furthermore, rough measurements of the ratio of adjacent wide and narrow spacings of the extinction bands on Fig. 1 give an average value of 1·7, which agrees with that given by the published figure, 66°, for 2 V.[8,9].

In case (ii) (Fig. 2) there are no extinction bands, because the twist results in an alternation of the Bx_a (with β now normal to the twist axis) and optic normal views, and at no point does an optic axis

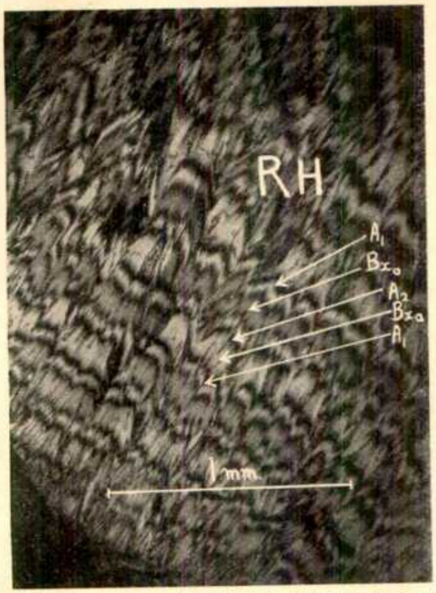

Fig. 1. Portion of spherulite showing radial twist about β-axis. Sense of twist right-handed (*RH*)

become vertical. Both right-handed and left-handed twists were found as indicated on the figure. The nature of the twist was confirmed by determinations of the path differences of adjacent optic normal and Bx_a regions, by direct identification of the polarization colours for the thicker spherulites, and by means of the graduated quartz wedge for thinner sections. Values of the ratio of these path-differences obtained were: 4·0, 3·9, 4·0; that calculated from the refractive indices is 3·9. The pitch was smaller than in (i) (for example, about 0·2 mm. for the middle region of the large central spherulite in Fig. 2) and it decreased as the borders of the spherulites were approached, that is, in the direction of falling temperature of growth. In some cases the prominent bands ceased altogether at the borders of the spherulite, and gave place to a more or less uniform structure which showed an optic normal interference figure. Examples are the two areas marked *ON* (optic normal) on the left of Fig. 2. These may have been regions in which the twisting process had ceased, or, more probably in view of the decreasing pitch in the regions which immediately preceded them, in which the pitch had become smaller than the limit of resolution of the microscope, so that the refractive index for light vibrating normally to the radii of the spherulites was an averaged value between β and γ. Whatever the explanation, it is probable that it applies also to the small unbanded spherulites seen along the left-hand margin of Fig. 2.

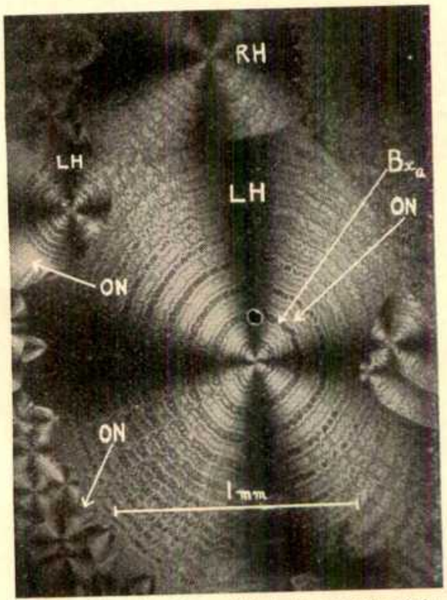

Fig. 2. Spherulites showing radial twist around α-axis. Both right-handed (*RH*) and left-handed (*LH*) spherulites present

The spherulites of both types (i) and (ii) had a markedly fibrous structure radially, as is commonly observed in spherulites, and it is of course possible that in these structures we are dealing with twisted linear aggregates of very narrow acicular crystals, which, individually, have little or no twist.

It is of interest that for both types the optical evidence suggests that it is the long axis of the molecule (presumably parallel, or nearly so, to the γ direction) which is oriented tangentially, as is the case in polymer spherulites.

Finally, it may be noted that hippuric acid belongs to class 222, one of the enantiomorphous symmetry classes[8]. Gaubert[5] was of the opinion that membership of such a class was conducive to the formation of twisted structures. It is tempting to go further, and suggest that the right- and left-handed spherulites are based respectively on the two enantiomorphous forms of the crystal.

I thank Dr. Keller for directing my attention to Gaubert's earlier work on hippuric acid.

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BIOCHEMISTRY

Nucleotides and Other Phosphorus Compounds of Cockroach Nerve

A NUMBER of phosphorus compounds, some of which have a vital role in vertebrate nerve function, have been demonstrated in the abdominal part of the ventral nerve cord of adult male *Periplaneta americana*. An estimate of their relative tissue concentrations has been made by the labelled pool technique[1]. Groups of about ten cockroaches were injected with 400 μc. of carrier-free phosphorus-32 orthophosphate per individual. They were held for seven days on normal diet to permit equilibration between injected phosphorus-32 and phosphorylated metabolites. Part of the nerve cord of each animal was exposed *in situ* by removing a strip of abdominal wall, separated from the animal by a piece of black paper and dissected free from other tissues, hæmolymph being washed off with Ringer solution. The cord was monitored using a cathode ray oscilloscope to ensure that it was capable of conducting impulses. Each electrically normal cord was raised up on two hooks 8 mm. apart, and the preparation flooded with liquid nitrogen. The frozen cord sample was transferred quickly to a homogenizer tube itself immersed in liquid nitrogen. After breaking up the tissue in 50 per cent ethanol made 1 N with respect to formic acid, the homogenizer was kept at −30° C. for 15 min. to allow thorough penetration of the medium. The tissue was warmed to 5° C., thrice extracted with 0·2 ml. extraction medium, and the extract separated by paper and elution[2] chromatography into a number of fractions. By automatic scanning of paper chromatograms[1] and Geiger counting of column effluents in solution, the size of each fraction was estimated, the results being expressed as percentages of the extracted phosphorus-32. The principal constituents of the extract were identified by co-chromatography with known substances. Where a fraction was shown to contain substantial impurity, the fact is indicated in Table 1 by a question mark and a 'less than' sign, though there may be small amounts of unidentified material in any fraction. About 60 per cent of the total phosphorus-32 of the nerve is extracted by the above procedure. The total phosphorus of cockroach abdominal nerve cord was found to be 1·41 mgm./gm. wet weight using the method of Bartlett[3]. In calculating approximate tissue concentrations of substances from these data, it has been assumed that the quantities of phosphorus-32 assayed reflect the amounts of phosphorus-31 present, both in the extract and in the insoluble residue. This assumption is not strictly valid, so the figures quoted (Table 1) are an indication of the order of magnitude of concentration of the metabolites listed.

Using an independent technique based on perchloric acid extraction and firefly luciferin assay the level of adenosine triphosphate in cockroach nerve was found to be 1,350 μgm./gm. wet weight.

In some experiments an unlabelled extract was prepared from lobster, *Homarus vulgaris*, ventral nerve cord and added to the labelled cockroach extract to reduce adsorption losses during column chromatography. This precaution proved to be unnecessary, but six compounds, coenzyme 1, cytidine monophosphate, adenosine mono-, di- and tri-phosphates, and uridine diphosphate, were tentatively identified by co-chromatography in the lobster nerve extracts.

Table 1

	Percentage of extracted phosphorus-32 (No. of observations)	Approximate tissue concentration (μgm./gm. wet weight)
Adenosine-5-phosphate	0·3 (3)	30
Adenosine-5-diphosphate	3·0 (7)	180
Adenosine-5-triphosphate	18·5 (6)	850
Cytidine-5-phosphate	0·2 (3)	20
Cytidine-5-diphosphate + ?	2·2 (6)	< 120
Cytidine-5-triphosphate	2·4 (6)	100
Guanosine-5-diphosphate	1·1 (4)	70
Guanosine-5-triphosphate	1·8 (3)	90
Uridine-5-diphosphate	1·5 (6)	80
Uridine-diphospho-glucose	1·2 (6)	90
Uridine-5-triphosphate	3·4 (3)	150
Co-enzyme 1	2·3 (7)	210
Co-enzyme 2 + ?	0·5 (6)	< 30
Orthophosphate	25·4 (6)	660
Glucose-6-phosphate	1·1 (3)	80
Arginine phosphate	7·8 (2)	540
α-Glycerophosphate + ?	3·7 (6)	< 170
Unidentified fractions	33·6	
	100·0	

Ethanolamine phosphate, riboflavin phosphate, guanosine monophosphate, uridine monophosphate and inosine monophosphate were not detected

A full account of this and related work will be published elsewhere. We are indebted to Dr. P. C. Caldwell for a gift of arginine phosphate and to Dr. N. Frontali for help in two experiments.

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Incorporation of Amino-Acids into Protein and Ribonucleic Acid of the Lens of the Rat ; Effect of Heavy Metals

THE distribution of nucleic acids and proteins within the cell varies from organ to organ. Tissues rich in ribonucleic acid (RNA) are particularly active in protein synthesis, thus indicating a close association between protein synthesis and RNA concentration[1-3]. Work from several laboratories now clearly shows that RNA is one of the essential components for protein synthesis[2,4]. Because of the relatively high concentration of RNA[5-7] and protein[8], and the absence of enzyme systems to hydrolyse adenosine triphosphate (ATP) and guanosine triphosphate (GTP) in the ocular lens[9,10], this organ may offer certain advantages for the investigation of protein biosynthesis as compared with a more complex organ such as the liver.

In order to investigate the incorporation of amino-acids labelled with carbon-14 into lens protein and RNA, lenses derived from Holtzman strain white male rats (6 weeks of age and weighing approximately 80 gm. each) were used. For the *in vitro* experiments these lenses were removed immediately after the animals were killed, weighed, and homogenized in Dounce microhomogenizers[11] at 0° C. in a medium consisting of 7 parts 0·03 M sucrose, 1 part 0·025 M potassium chloride and 1 part 0·03 M potassium bicarbonate. The capacity of such a system to catalyse the incorporation of ^{14}C-leucine, valine or histidine into lens RNA and proteins, and the experimental conditions, are summarized in Table 1.

Although the incorporation of amino-acids can occur over a broad pH range, from pH 6·0 to 10·0, all the foregoing experiments involving ^{14}C-leucine and valine were carried out at pH 8·5 in 0·1 M *tris* buffer since this was the pH optimum for the incorporation of these two amino-acids. The corresponding optimum pH for histidine was 9·0 and the experiments involving this amino-acid were all performed at this pH.

Incorporation of ^{14}C-leucine, valine or histidine into lens proteins and RNA could also be demonstrated by the injection of these amino-acids intraperitoneally into rats. The resulting *in vivo* incorporation was comparatively less than the results obtained with the *in vitro* studies, reaching a maximum 3 hr. after injection.

The results of the *in vitro* experiments carried out in the presence of cobaltous chloride are shown in Table 1. Without the addition of this metal the incorporation of carbon-14-labelled leucine, valine, or

Table 1. INCORPORATION OF ^{14}C-AMINO-ACIDS INTO LENS RNA–PROTEIN COMPLEX*

(c.p.m. per mgm. of RNA–protein complex)

	^{14}C-leucine range	^{14}C-valine range	^{14}C-histidine range
(*A*)			
Lens homogenate	3 (0–3)	—	—
,, + Mg^{++}	16 (12–19)	9 (7–14)	37 (33–46)
,, + Mg^{++} + ATP	12 (19–31)	20 (12–22)	58 (50–62)
,, + Mg^{++} + ATP + GTP	51 (44–57)	50 (38–49)	93 (90–95)
,, + ATP + GTP	16 (14–17)	16 (15–17)	21 (20–22)
(*B*)			
Lens homogenate + Co^{++}	540 (433–582)	432 (410–462)	1,252 (1,150–1,482)
,, + Co + ATP	730 (652–820)	670 (630–690)	2,058 (2,000–2,158)
,, + Co + ATP + GTP	575 (506–600)	462 (442–485)	1,320 (1,120–1,490)

* ml. of the reaction mixture contained 0·5 ml. lens homogenate (50 mgm./ml.), 20 μmoles *tris* buffer *p*H 8·5 (for leucine and valine) or *p*H 9·0 (for histidine), 5 μmoles Co^{++} (or 10 μmoles Mg^{++}), 10 μmoles ATP and 10 μmoles GTP (in the reactions indicated above), 0·56 μmole ^{14}C-valine ($8·56 \times 10^4$ c.p.m.), 0·65 μmole ^{14}C-leucine ($1·04 \times 10^5$ c.p.m.) or 1·30 μmoles ^{14}C-histidine ($9·52 \times 10^4$ c.p.m.). The reaction mixtures were incubated at 37° C. for 1 hr. and the reaction was terminated by adding 5 per cent TCA. The precipitate was washed four times with 5 per cent TCA, once with hot alcohol (50° C. for 10 min.), once with alcohol, twice with ether, and then plated and counted in a gas flow counter (Nuclear Chicago) as infinitely thick layers with a counting error of 6–8 per cent. Each result shown above represents the average value for 6–8 experiments.

histidine was low. Mg^{++} can to some extent replace Co^{++} but was much less effective; even by increasing its concentration ten-fold over the concentration of Co^{++} it was not possible to achieve an appreciable incorporation. Zn^{++}, Mn^{++}, and Ca^{++} did not have the stimulating effect of Co^{++} or the inhibitory effect of Ni^{++} on the incorporating system. Addition of 10 μmoles of Ni^{++} in the presence of 1 μmole of Co^{++} blocked 95 per cent of the incorporation of ^{14}C-leucine into lens proteins, and even in the presence of 5 μmoles of Co^{++} 90 per cent inhibition was still noted (Fig. 1).

The incorporation of labelled leucine, valine, or histidine into lens proteins and RNA was to a certain degree dependent on the addition of ATP. Furthermore, the addition of 10 μmoles of GTP instead of being stimulating actually inhibited the incorporation by 25–30 per cent. On the other hand, in experiments where Mg^{++} instead of Co^{++} was used, addition of ATP and GTP showed a stimulating effect. Activation of an amino-acid by *p*H 5·2 enzyme requires ATP[12] and for the transfer of the activated amino-acid from an AA-SRNA complex to ribonucleoprotein particles, both ATP and GTP are required[13]. The inhibitory action of GTP is not understood at the present time.

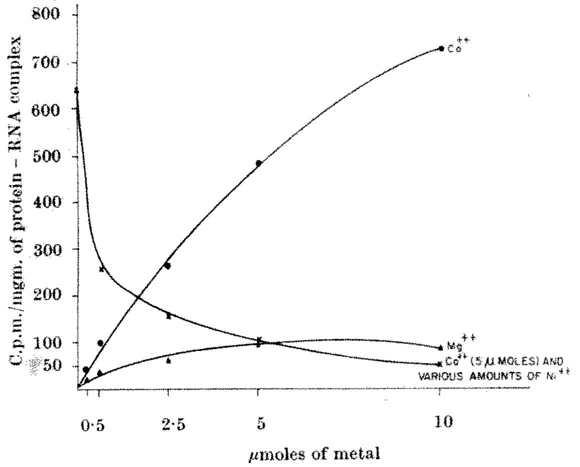

Fig. 1. Effect of Mg^{++} and Co^{++} with and without Ni^{++} on the incorporation of ^{14}C-leucine into lens protein and RNA. The assay method was the same as described above, except that varying amounts of Mg^{++} or Co^{++} were used; where the inhibitory effects of Ni^{++} were studied the latter's concentration varied as shown above. The concentration of Co^{++} was kept constant (5 μmoles). Every point represents an average of three separate experiments

In order to determine whether the effect of Co^{++} on the amino-acid incorporating systems previously described was an artefactual one (that is, due to some form of metal substrate complexing) the following experiments were performed.

(1) Two experiments were performed in which 5 per cent trichloracetic acid was added to the incubation media prior to the addition of the labelled amino-acid. After 1 hr. incubation the recovered activity was 4·6 and 6·6 c.p.m. per mgm. protein in contrast to the 540 and 432 c.p.m. obtained in the experimental reaction (cf. Table 1).

(2) There was no incorporation of these amino-acids when the incubations were performed at 0° C.

(3) Changes in the concentration of the enzyme (lens homogenate) resulted in a linear increase in the recovery of labelled amino-acids.

(4) Two experiments were performed in which the procedure was the same as originally described, but two final washings with ethyl mercaptan were added. There was no change in the recovery of ^{14}C-leucine following this procedure as compared with the values obtained when the ethyl mercaptan washings were omitted.

(5) In two experiments protamine sulphate was used instead of lens homogenate in the reaction mixture. No activity was recovered following the usual 1 hr. incubation.

(6) When Co^{++} activation was studied in rat liver microsomes and supernatant, in the presence of ATP and GTP, the count obtained was 6–8 c.p.m. and 75–80 c.p.m., respectively.

(7) The enzymic nature of the incorporating system was further indicated by the failure of this system to catalyse the incorporation of aliphatic amines such as cadaverine, putrescene and histamine labelled with carbon-14 into lens proteins. The conditions of these experiments were similar to those described by Sarkar *et al.*[14].

The results of these foregoing experiments indicate that the effects observed with Co^{++} were not artefactual. There is apparently a definitive increase in the uptake of amino-acids by lens protein and RNA which can be ascribed to Co^{++}.

The rat lens incorporating system is thus different from the rat liver incorporating system described by Zamecnik *et al.*[12]. The former does not require additional amounts of GTP, and unlike the latter requires Co^{++} instead of Mg^{++} for maximum activity. Furthermore, only 50–60 per cent of the capacity of lens protein–RNA system to incorporate amino-acids into lens proteins could be abolished by treatment with RNase prior to the addition of amino-acids. Another important property of the rat lens incorporating system is the remarkable ability of its RNA to bind amino-acids. About 80 per cent of the total amino-acid incorporated into material precipitable by trichloracetic acid is retained even after a 2 hr. incubation of the amino-acid with the rat-lens homogenate; in contrast, in the rat liver incorporating system a relatively high percentage of total amino-acid, found in TCA-precipitable material, is characteristically found in the protein fraction. Experiments are now in progress to determine whether the effect of Co^{++} can be obtained in a system in which a full complement of amino-acids are used in addition to the specific labelled ones.

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Degradation of Deoxyribonucleic Acid in *Escherichia coli* Cells treated with Mitomycin C

IT has been shown[1,2] that treatment of growing cells of *E. coli B* (strain 9637 from A.T.C.C.) with mitomycin *C* (MC) results in inhibition of deoxyribonucleic acid (DNA) formation. The synthesis of ribonucleic acid (RNA) and protein was unaffected at low levels of MC. The addition of MC in higher concentrations also blocks the synthesis of RNA and protein.

In our work on the mode of action of antibiotics upon microbiological systems[3-5]—especially on nucleic acids—we have investigated the influence of MC on DNA, RNA and protein present in cells of *E. coli B* at the end of the logarithmic phase of growth. The strain of *E. coli B* was sensitive to MC (Fig. 1). Cells grown in a salt-glucose synthetic medium for 12 hr. at 37° C. and at pH 7·0 with aeration were then treated with increasing amounts of MC. Turbidity, protein, DNA and RNA were estimated in aliquots of bacterial suspensions immediately after the addition of MC and after 4 hr. and 8 hr. of incubation at 37° C. The pH was controlled and was found to be constant during the experiment.

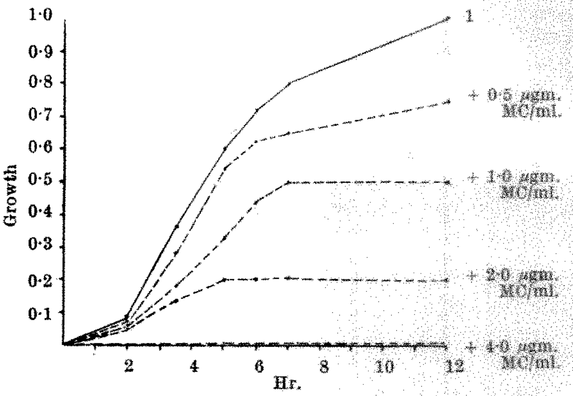

Fig. 1. Growth of *E. coli B* without (——) and with increasing amounts of mitomycin *C* added to the incubation medium (– – –). Turbidity measured at 578 mμ

Turbidity was measured photometrically at 578 mμ. Ribonucleic acid and deoxyribonucleic acid were determined after extraction (15 min. at 90° C. with 0·5 *N* perchloric acid) from acid-insoluble precipitates of bacteria with orcinol and diphenylamine respectively. The residue remaining after extraction of DNA and RNA was analysed for protein with the biuret method. The analytical values obtained in the untreated controls at the end of the experiment (8 hr.) were set at 100 and all other values were relative to them.

After the logarithmic phase there is only a slight incline in growth of the untreated control during time of incubation (Table 1). Mitomycin added to the incubation medium at the end of the logarithmic phase of growth caused a marked drop in DNA content. After 4 hr. incubation with 2·5 μgm. MC/ml. the DNA content was reduced to 60 per cent and after 8 hr. to 37 per cent.

During this time turbidity and protein content remain constant, whereas the RNA content shows only a slight decline. Furthermore, no lysis of the cells could be observed as indicated by cell count. The bacterial suspension contained 1×10^9 cells/ml., corresponding to an optical density of 1·00 at 578 mμ. The possibility that degradation of DNA was caused by induction of phage during incubation with MC—as is the case with the lysogenic *E. coli* strain K_{12} (ref. 6)—was excluded by electronmicroscopical and microbiological methods.

The remaining supernatants, after precipitation and centrifugation of DNA, RNA and protein, were checked for ultra-violet absorbancy. Aliquots taken just after the addition of MC were used for reference and the corresponding aliquots taken after 4- and 8-hr. incubation were compared with them. During incubation with MC, substances with an absorption maximum at 260 mμ appear and the ΔE values increase with increasing amounts of MC. This effect

Table 1. EFFECT OF MITOMYCIN *C* ON *E. coli B*. MITOMYCIN *C* WAS ADDED AT THE END OF LOGARITHMIC PHASE OF GROWTH (12 HR.)

Time	μgm. MC/ml.	Turbidity (per cent)	Protein (per cent)	DNA (per cent)	RNA (per cent)	ΔE_{260} mμ
After 12 hr. of growth	0	86	90	82	80	—
	0·1	89	91	85	86	—
	0·25	87	84	85	83	—
	0·50	89	82	84	89	—
	1·00	87	90	85	91	—
	2·50	89	90	84	87	—
4 hr. after addition of mitomycin *C*	0	93	100	98	100	—
	0·1	96	100	97	95	0·210
	0·25	92	98	89	95	0·230
	0·50	94	96	84	89	0·380
	1·00	90	98	79	91	0·450
	2.50	88	88	60	80	0·660
8 hr. after addition of mitomycin *C*	0	100	100	100	100	0·010
	0·1	99	100	100	95	0·440
	0·25	93	106	100	90	0·650
	0·50	90	98	72	82	0·700
	1·00	89	93	49	83	0·800
	2·50	89	105	37	76	1·000

may be caused partially by degradation products of DNA. A more detailed analysis of the acid-soluble fraction is now in progress.

These results are examples from a series of experiments under various conditions. Longer incubation of *E. coli B* cells with MC caused degradation of RNA and protein too. No degradation occurred when deoxyribonucleic acid from calf thymus and nucleic acid from herring sperm were incubated with MC *in vitro* under the same conditions as described here.

Takagi and Sekiguchi postulated that the inhibition of DNA formation in growing cells of *E. coli B* treated with MC is caused by an attack on synthesizing steps during DNA formation. However, they could not find any influence of MC on the polymerizing Kornberg enzyme. Furthermore, the block in DNA synthesis induced by MC was not overcome by the addition of thymine, thymidine or a mixture of deoxyribonucleotides to the medium.

The question arises: Is the inhibition of DNA formation in *E. coli B* treated with MC really caused by an attack in the synthesizing steps during DNA formation? The results presented here show that MC, under the experimental conditions described above, acts on the depolymerization of DNA*. If, in the presence of low levels of MC in the incubation medium of growing *E. coli B* cells, the synthesized DNA is partially degraded, the resulting picture is a block in DNA formation.

* *Note added in proof.* Tatum and co-workers obtained similar results (see ref. 7).

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Ethanol Stimulation of Oxidative Metabolism in *Euglena gracilis*

Euglena gracilis, a flagellate protozoan, utilizes acetate and ethanol as energy and carbon sources. The acetate metabolism of *Euglena* grown on ethanol differs from that grown on acetate. Ethanol-grown cells have a rate of acetate oxidation at *p*H 7·0 lower than that of acetate-grown cells[1]. However, ethanol-grown cells incubated in the presence of acetate for about 80 min. often adapt to acetate, taking on characteristics similar to those of acetate-grown cells[1,2]. Thus, it was considered of interest to determine the respiration of *Euglena* in the presence of both acetate and ethanol.

Experimental and stock cultures of a streptomycin-bleached strain, *SM–LI*, of *Euglena gracilis* var. *bacillaris*, were grown axenically on the defined Cramer–Myers medium[3] with 0·061 *M* acetate as the sole carbon source for growth. The cells were gathered during the logarithmic growth phase, centrifuged twice in basal Cramer–Myers medium (minus acetate and vitamins B_1 and B_{12}), and re-suspended in a small volume of the basal medium. Cell concentrations ranged from 1·0 × 10^6 to 3·7 × 10^6 cells per flask. Absolute ethanol, in the amounts shown in Table 1, was added to Cramer–Myers medium which was then pipetted in 1-ml. quantities into the side-arms of the respiration flasks. The acetate concentration (0·061 *M*) was held constant in all experiments. Respiration-rates were determined by the direct method of Warburg[4]. After the substrate was tipped in, readings were taken at 10-min. intervals for 60 min. Respiration-rates were measured as µl. $O_2/10^6$ cells/hr. and are reported as per cent of the acetate control. All other conditions were as reported previously[5].

Table 1. ETHANOL AND ACETATE RESPIRATION* OF INTACT, ACETATE-GROWN *Euglena*

Average Qo_2 for the acetate control (no ethanol) was 22·8 µl. $O_2/10^6$ cells/hr. Acetate concentration (0·061 *M*) was constant in all experiments. The control for each experiment was standardized at 100 per cent

Ethanol molarity	No. of experiments	Average percentage of control
0	5	100·0
6·83 × 10^{-2}	4	108·0
3·41 × 10^{-2}	3	112·0
1·71 × 10^{-2}	3	122·0
3·41 × 10^{-3}	5	129·0
6·83 × 10^{-4}	4	113·0
3·41 × 10^{-4}	4	102·0
1·71 × 10^{-4}	3	97·2
3·41 × 10^{-5}	3	97·5

* Gas phase, air; temperature, 26·5° C.

It is evident from Table 1 that the respiration of *Euglena* in the presence of acetate is stimulated by ethanol, a finding not reported before in the literature. The maximum effect of ethanol in these experiments occurs at a concentration of 3·41 × 10^{-3} *M*. Higher and lower concentrations of ethanol result in a decreasing effect on the respiration of *Euglena*.

It appears that the ethanol may be affecting the cells by being oxidized within the cells to acetate, which in turn could enter the citric acid cycle or, perhaps, stimulate the formation of lipids. Bearing on this problem is a recent observation[6] which indicates that, in *Euglena* grown in the presence of both acetate and ethanol, more acetate carbon is assimilated into protoplasm than is oxidized as compared with cells grown in the presence of acetate alone. Although it has not been determined what substances are being formed in *Euglena* cells under these conditions, further work with labelled acetate and/or ethanol should help to explain these observations.

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A Factor associated with 'Visking' Tubing capable of Antagonizing Insulin

DURING the investigation of the insulin antagonism associated with human serum albumin[1], 'Visking' tubing (Visking Corporation, Chicago, U.S.A.) was used for ultrafiltration and dialysis of protein solutions. The tubing itself has since been found to give rise to a substance which antagonizes insulin and which appears in the following circumstances:

Table 1. EFFECT OF DIALYSATES AND ULTRAFILTRATES FROM 'VISKING' TUBING ON THE GLUCOSE UPTAKE OF RAT DIAPHRAGMS, WITH AND WITHOUT THE ADDITION OF INSULIN TO THE INCUBATION MEDIUM

Mean glucose uptake above basal-level* ± *S.E.* (mgm. glucose per 100 ml. per 10 mgm. diaphragm)			
Buffer + insulin‡	Dialysate or ultrafiltrate in buffer† + insulin‡	Dialysate or ultrafiltrate in buffer† alone	Retentate in buffer† + insulin‡
16·58 ± 0·73 (10)	5·33 ± 0·86 (11)	−2·3 ± 1·5 (8)	715·90 ± 1·13 (3)

Number of experiments in parentheses.

* Amount of glucose taken up by the diaphragms when no insulin is added to the medium.

† Dialysates, ultrafiltrates and retentates were evaporated to dryness *in vacuo*, then dissolved in 9 ml. buffer for assay.

‡ Insulin added to make final concentration of 1,000μ units/ml.

(1) When distilled water is ultrafiltered through 7-ft. of heavy grade 'Visking' tubing (27/32 in. inflated diameter) supported by 'Terylene' net, under a negative pressure of 40–50 cm. mercury, over a period of three days. (2) When 7 ft. light grade 'Visking' tubing (18/32 in.) is filled with water and suspended in changes of distilled water for 2–7 days, that is, used as dialysis tubing.

The ultrafiltrate or dialysate is evaporated to dryness *in vacuo*, and the residue when dissolved in buffer[2] is found to antagonize the action of insulin in the rat diaphragm assay system[3]. Under these conditions there is no significant reduction in the normal glucose uptake (Table 1, column 3). If the wider tubing is used as a true dialysing membrane, for example the removal of trichloracetic acid and ethanol, there is no trace of this antagonist in the retentate after dialysis (Table 1, column 4).

We attempted to extract the insulin inhibitor from the 'Visking' tubing before use to avoid interference with the true insulin antagonist associated with albumin, which is dialysable. Soaking for 6 days in cold water extracted relatively little of the antagonist, for subsequent dialysates or ultrafiltrates of distilled water through the treated tubing continued to inhibit insulin action. Continuous internal and external circulation of cold water for 3 days using the wider tubing gave a similar result (Table 2). It is seen that the wash was not always collected, since more importance was attached to the effects obtained from subsequent dialysis and ultrafiltration.

Table 2. COLD AND HOT WATER WASHING OF 'VISKING' TUBING IN AN ATTEMPT TO REMOVE THE INSULIN ANTAGONIST

Experimental procedure	Mean glucose uptake above basal-level* ± *S.E.* (mgm. glucose per 100 ml. per 10 mgm. diaphragm)		
	Buffer + insulin†	Wash + insulin†	Dialysate or ultrafiltrate + insulin†
Cold water for 6 days			
18/32 in. 'Visking'	17·15 ± 1·05 (2)	3·2 (1)	11·75 ± 0·25 (2)
27/32 in. 'Visking'	22·7 ± 0·0 (2)	3·4 (1)	18·05 ± 0·75 (2)
Continuous circulation of cold water for 3 days			
27/32 in. 'Visking'	14·7 (1)	(—)	7·95 ± 0·15 (2)
Water at 80° C. for 1 hr.			
18/32 in. 'Visking'	18·40 ± 0·43 (4)	3.25 ± 1·50 (4)	16·85 ± 0·74 (4)
27/32 in. 'Visking'	20·97 ± 0·15 (3)	—	11·7 ± 1·6 (2)
Boiling in water for 30 min.			
27/32 in. 'Visking'	14·62 ± 0·77 (5)	−1·95 ± 0·50 (4)	12·58 ± 1·42 (4)

Each horizontal line represents one complete series of experiments, the figures in parentheses indicating the total number of separate determinations for each series.

* Amount of glucose taken up by the diaphragms when no insulin is added to the medium.

† Dialysates, ultrafiltrates and retentates were evaporated to dryness *in vacuo*, then dissolved in 9 ml. buffer for assay.

Soaking the 'Visking' tubing in hot water for 1 hr. also gave rise to washes inhibiting insulin action. With the lighter grade of tubing (18/32 in.) no significant insulin antagonism could be detected afterwards in the dialysate. In contrast, the ultrafiltrate of the heavier grade tubing was only rendered completely free of the antagonist if the tubing were first boiled for 30 min. (Table 2). In this case, the concentration of antagonist in the wash was probably high, and there appeared to be some inhibition of the normal glucose uptake in addition to the inhibition of insulin action. No check was made for any change in porosity of the tubing which may have resulted from the boiling.

The antagonistic substance may well be a hydrolysis product of sulphur, since 'Visking' tubing is stated to contain up to 0·1 per cent sulphur, partially extracted by a hot aqueous wash (manufacturer's specifications). However, we do not intend to investigate this further, being more concerned with the isolation and characterization of the previously reported hcrmone-dependent insulin antagonist associated with plasma albumin[4].

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Calcium and Magnesium Contents of Various Secretions entering the Digestive Tract of Sheep

THE endogenous fæcal excretion of calcium and magnesium is derived from the various digestive secretions and the desquamated epithelial cells of the alimentary tract. The relative proportions of these two fractions is unknown, but the fact that in calves[1] endogenous fæcal excretion of magnesium increases during the first few weeks of life suggests that the contribution by the digestive juices is important. There is very little information on the calcium and magnesium content of the digestive secretions of ruminants beyond the values of 1·6–3·0 and 0·6–1·0 mgm./100 ml. for calcium and magnesium respectively in mixed saliva of sheep[2]. For this reason estimates of the probable quantities of these elements secreted into the alimentary tract of sheep were made. Calcium and magnesium were estimated in solutions of the ashed secretions.

The composition of the secretions collected from the various preparations is given in Table 1 and the daily output of calcium and magnesium as a result of these secretions has been assessed in Table 2 using representative volumes per 24 hr. of the various salivary secretions[3], gastric juice[4] and bile[5,6]. The range given for bile varied from 400 to 1,140 ml. per 24 hr., which may be accounted for by the fact that in some experiments collected bile was not returned to the duodenum. A daily volume of 1 litre was considered reasonable. Estimates of the daily secretion of pancreatic juice[5,7,8] vary from 275 to 600 ml., and a conservative value of 300 ml. was used in the with

Table 1. CALCIUM AND MAGNESIUM CONTENT OF VARIOUS SECRETIONS FROM THE SHEEP'S DIGESTIVE TRACT

Secretion	No. of animals examined	No. of samples analysed	Calcium* (m.equiv./l.)	Magnesium* (m.equiv./l.)
Parotid saliva	9	12	0·74 (0·30–1·75)	0·45 (0·17–1·32)
Submaxillary saliva	8	10	1·88 (0·96–2·66)	0·50 (0·10–1·00)
Residual saliva	1	2	1·26 (1·12–1·40)	1·36 (1·06–1·66)
Gastric juice	2	45	1·68 (1·04–2·20)	0·66 (0·52–0·88)
Bile	3	30	4·56 (1·76–8·94)	1·76 (0·50–3·90)
Pancreatic juice	2	26	4·74 (3·96–5·82)	1·13 (0·66–2·38)
Brünner's glands	2	12	2·82 (2·56–3·18)	0·90 (0·68–1·22)
Cæcum	1	5	3·64 (2·24–5·66)	2·53 (1·24–3·26)
Mixed secretions:				
Bile + pancreatic juice + Brünner's glands	2	38	3·40 (1·08–6·32)	1·48 (0·20–4·32)
Bile + pancreatic juice	1	30	4·47 (3·60–5·84)	1·12 (0·72–1·44)

* Figures in parentheses denote maximum and minimum concentrations found.

given in Table 2. The volumes representing cæcal secretion and the secretions from Brünner's glands were obtained from the volumes collected from a cæcal pouch representing about half the whole organ (prepared by Dr. R. N. B. Kay) and from an isolated loop of duodenum which excluded the common opening of the bile and pancreatic ducts. The secretions of the cæcum have previously been estimated to amount to 180 ml. a day[8], a figure that is similar to the value given in Table 2. The calcium and magnesium contents of the secretions of the remainder of the small and large intestine were not estimated.

It has been calculated that a 40-kgm. sheep would contain respectively 16 and 28 m.equiv. of extracellular magnesium and calcium by assuming that the volumes of plasma and interstitial fluid represent 5 and 15 per cent of the body-weight respectively, and that the concentrations of calcium and magnesium in interstitial fluid are equivalent to the concentrations of ultrafilterable calcium and magnesium in plasma. These values are of the same order as the calculated amounts of these elements entering the digestive tract (Table 2). Calcium and magnesium secreted into the alimentary tract will be excreted in the fæces of the animal if they are not re-absorbed. It is interesting in this respect that the estimated total secretion of magnesium is of the same order as values reported in the literature for the daily endogenous fæcal magnesium in sheep, and as the contribution of magnesium by the secretion of the small intestine has been ignored, some re-absorption presumably occurs.

Table 2. TOTAL INFLUX OF CALCIUM AND MAGNESIUM IN THE SECRETIONS OF THE DIGESTIVE TRACT

Secretion	Estimated daily volume* (litres)	Daily secretion of calcium (m.equiv.)	Daily secretion of magnesium (m.equiv.)
Parotid saliva	5	3·70	2·25
Submaxillary saliva	0·5	0·94	0·25
Residual saliva	5	6·30	6·80
Gastric juice	5	8·40	3·85
Bile	1	4·56	1·76
Pancreatic juice	0·3	1·42	0·34
Brünner's glands	0·4	1·13	0·36
Cæcum	0·2	0·73	0·51
Total	17·4	27·18	16·12

* Figures arrived at from literature; for references, see text.

It would seem reasonable to assume that proportionately similar amounts of calcium and magnesium enter the bovine digestive tract. Analysis of two samples of parotid saliva revealed calcium and magnesium concentrations within the range of those found for sheep. Radioactive techniques have also demonstrated that circulating calcium is secreted into all parts of the bovine digestive tract, particularly the small intestine.

There is evidence that absorption from the digestive tract is an important factor contributing to the maintenance of magnesium concentrations in extracellular fluids of the adult ruminant, and magnesium balance studies on dairy cattle during their transfer from winter diets to diets of spring grass suggest that a reduced dietary intake of magnesium, coupled with a lower availability, is an important factor in the development of hypomagnesæmia[9]. The present investigations demonstrate that, should absorption of magnesium from the digestive tract be impeded, the continued secretion of magnesium in digestive juices would contribute to a rapid loss of extracellular magnesium.

This work was carried out at the Rowett Institute during the tenure of a scholarship from the Ministry of Agriculture. I wish to thank Dr. A. T. Phillipson for preparing the isolated loops of duodenum, Mr. R. W. Ash and Dr. R. N. B. Kay for providing samples of gastric juice and saliva respectively. Samples of bile and pancreatic juice were also kindly made available by Mr. F. A. Harrison, Institute of Animal Physiology, Babraham, Cambridge.

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Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency in West Africa

EVIDENCE is accumulating that sex-linked inherited deficiency of erythrocyte glucose-6-phosphate dehydrogenase is common in Central Africa. Frequencies of 15–30 per cent have been recorded in males living in malarious parts of the Belgian Congo[1], Nigeria[2] and East Africa[3]. In non-malarious parts of East Africa frequencies of 3 per cent or less have been found[3], and in South African Bantu the frequency is about 2 per cent[4].

The present investigation was undertaken in order to ascertain the distribution of this trait in West Africa. Males only were examined. Blood specimens were obtained from northern Nigerians through the courtesy of Dr. H. M. Archibald and Dr. C. G. Berry, Kano. The majority of subjects were Hausa, but small numbers of Fulani and other tribes were included. The samples from southern Ghana were obtained from two schools near Accra; 64 were Akim and the rest Ga.

Table 1. FREQUENCY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN WEST AFRICANS

Locality	No. tested	No. of enzyme deficient	Percentage of enzyme deficient
Northern Nigeria	136	28	20·6
Southern Ghana	96	23	24·0
Gambia	103	16	15·5

The Gambian sample was taken from adult Mandinka living at Keneba in the West Kiang District of the Gambian Protectorate. Blood specimens were collected into acid-citrate-dextrose and sent in thermos flasks to London for testing. Glucose-6-phosphate dehydrogenase activity in erythrocytes was assayed by the technique of Motulsky. The results are shown in Table 1. All three groups showed enzyme deficiency, the frequency being highest in the sample from southern Ghana. These findings suggest that the enzyme-deficiency gene has a widespread distribution in West Africa. Tests on subjects such as the southern Liberians, who show low frequencies of hæmoglobins *S* and *C*, would be of interest.

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A New Technique of Chromatography and Ionophoresis on Ion-exchange Paper. Application to Separation of Compounds of Toxicological Interest : Separation of a Mixture of Promazine, Quinine, Sulphacetamide and Acetophenetidin

IN two previous communications, we[1,2] have shown that a mixture of phenobarbitone, acetophenetidin, salicylate and *p*-acetylaminophenol can be separated in 60–75 min. by a combined technique of chromatography and ionophoresis on Whatman ion-exchange cellulose papers. We have now been able to effect the resolution of a mixture of basic and neutral drugs, namely, promazine, quinine, sulphacetamide and acetophenetidin.

This separation was achieved by applying solutions of these drugs to a sheet of diethylaminoethylcellulose paper and carrying out chromatography in a 0·1 *M* solution of the disodium salt of ethylenediaminetetraacetic acid for 20 min. At the end of this period the wet sheet was subjected to ionophoresis at a constant current of 10 m.amp. for 30 min. Both the chromatography and the ionophoresis were carried out in the same tank as described previously[1,2].

Fig. 1 shows the results of this experiment, from which it is seen that chromatography effects a separation into three parts, namely, promazine, quinine and a mixture of sulphacetamide and acetophenetidin. The sulphacetamide and acetophenetidin are quite clearly separated by ionophoresis. Examination of the wet paper in 254 mμ light shows promazine as a blue fluorescent spot, sulphacetamide as a light-blue absorbing spot and acetophenetidin as a dark-purple absorbing spot. Quinine is not visible on the wet paper. After drying the paper, which takes 3–4 min. with a hair-dryer, the promazine shows up as a brilliant yellow fluorescent spot. Quinine can be revealed as a bright blue fluorescent area by holding the paper over the mouth of a bottle of concentrated hydrochloric acid. The other two compounds show the same absorption colour as on wet paper.

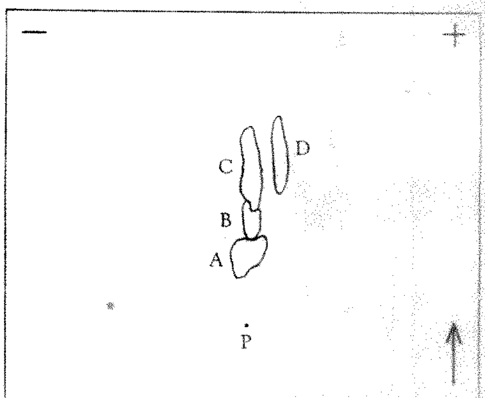

Fig. 1. Chromatography in 0·1 *M* ethylenediaminetetraacetic acid (20 min.) followed by ionophoresis in 0·1 *M* ethylenediaminetetraacetic acid (30 min., 10 m.amp.) of a mixture of promazine (*A*), quinine (*B*), acetophenetidin (*C*) and sulphacetamide (*D*). Mixture applied at point marked *P*. Arrow indicates direction of ascending solvent flow during chromatography

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Detection of Small Quantities of 3,4-Benzpyrene directly from Chromatographic Paper

W. Davies and J. R. Wilmshurst recently published a report[1] on the detection of 3,4-benzpyrene, under the most favourable conditions, in a concentration of 10^{-9} gm./ml., using fluorescent spectrography (Hilger medium spectrograph).

We detected 3,4-benzpyrene directly from chromatographic paper in a quantity of 10^{-9} gm. on the chromatographic spot 60–90 mm.[2] in size. This was done by means of fluorescent spectrography (Hilger medium spectrograph, quartz optics). Actually we applied this procedure for detecting carcinogenic substances from mixtures of organic compounds as follows: an extract of coal soot, previously chromatographed on an alumina and silica column, was transferred to chromatographic acetylated paper[3] (Whatman No. 1). The spots developed on the chromatogram and which showed fluorescence were set directly facing the spectrograph slit and were illuminated with Wood's light (Quarzlampen, Hanau S., 300 *PL* 12; 220 V.; 2·5 amp., with Wood's glass filter). From one of the spectrographed spots we obtained the characteristic band spectrum of 3,4-benzpyrene.

This method, a combination of paper chromatography and fluorescent spectrography, facilitates greatly the detection of minute quantities of 3,4-benzpyrene and other fluorescent substances with a characteristic band spectrum, as in this way it is possible to avoid elution and subjecting the solution to spectrographic analysis.

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PHYSIOLOGY

Effect of Feeding Two Levels of Dietary Calcium on the Growth of Broiler Chickens

The dietary requirements of calcium for chickens 0–18 weeks of age has been stated by the U.S. National Research Council[1] to be 1·0 per cent of the diet. In the United Kingdom commercial broiler rations contain at least 1·0 per cent but may contain up to 1·4 per cent. Davis[2] has presented evidence taken mainly from laboratory animal experiments indicating that the utilization of dietary protein, fat minerals, and vitamins may be reduced appreciably when the dietary calcium-level exceeds 1·0 per cent. When there is a limited intake of these nutrients, the inclusion of a high level of calcium in the diet may result in a clinical deficiency of them.

During the course of a large-scale experiment conducted by us it was possible to compare the effect of a diet containing approximately 0·8 per cent calcium with that of a diet containing approximately 1·3 per cent calcium on the rate and efficiency of gain of broiler chickens from one day to ten weeks of age. The results of this experiment provide further evidence that the efficiency of utilization of nutrients may be affected by the level of dietary calcium.

The experiment was conducted during March–May 1960 in a commercial-type broiler house designed to accommodate thirty pens, each housing one hundred and fifteen chickens from day old to ten weeks of age. Fifteen pens were allocated at random to a commercial broiler ration containing 1·35 per cent calcium, and the other fifteen pens of chickens were fed the same commercial ration with 0·83 per cent calcium. The latter ration was prepared by excluding calcium carbonate which normally was included to bring the level of calcium up to 1·0 per cent and above. The average levels of protein, calcium and phosphorus determined from five samples of each of the two rations are given in Table 1.

The average gain in live-weight, the average food consumption, and the average food conversion efficiency observed for the first six weeks and for the total ten weeks of the experiment are presented in Table 2. The birds fed the 0·83 per cent calcium diet grew 3·4 per cent faster up to six weeks and also up to ten weeks of age than the birds fed the 1·35 per cent calcium diet. These increases in gain were highly significant ($P<0·001$). Over the first six weeks there was no significant difference in food consumption, but over the total ten-week period the birds on the 0·83 per cent calcium diet consumed 2·7 per cent less food than the birds fed the 1·3 per cent calcium diet. This result was highly significant ($P<0·001$).

Table 1. Levels of Crude Protein, Calcium and Phosphorus in Rations

Ration	Crude protein (per cent)	Calcium (per cent)	Phosphorus (per cent)
0·8 per cent calcium	24·38 ± 0·49*	0·83 ± 0·08	0·52 ± 0·02
1·3 per cent calcium	23·63 ± 0·55	1·35 ± 0·11	0·53 ± 0·01

* *S.D.*

Table 2. Average Live-weight Gain, Food Consumption and Food Conversion Efficiency to Six Weeks and Ten Weeks of Age

Ration	Average gain (lb.)	Average food consumed (lb.)	Average food consumed/lb. live-weight gain (lb.)
	0–6 weeks		
0·8 per cent calcium	1·784*	3·606	2·022*
1·3 per cent calcium	1·725	3·601	2·087
S.E. of difference	± 0·014	± 0·047	± 0·017
	0–10 weeks		
0·8 per cent calcium	3·702*	9·334*	2·522*
1·3 per cent calcium	3.580	9·593	2·680
S.E. of difference	± 0·026	± 0·070	± 0·012

* $P < 0·001$

Over the six-week and ten-week stages, respectively, the birds fed the 0·83 per cent calcium diet converted their food 3·1 and 5·9 per cent more efficiently than the birds fed the 1·3 per cent calcium diet. Again these results were highly significant ($P<0·001$).

The birds were slaughtered at ten weeks of age, and the resulting carcases were graded according to normal commercial standards. The resulting data are presented in Table 3. There was no significant difference in the percentage of grade *A* or grade *B* carcases from the two experimental groups. However, the average carcase weight of birds fed the 0·83 per cent calcium diet was 3·9 per cent higher than that of the birds fed the 1·35 per cent calcium diet. This highly significant difference ($P<0·001$) resulted wholly from the increased weight of grade *A* carcases, since the weight of grade *B* carcases was not significantly affected.

Table 3. Carcase Quality Data

	0·8 per cent calcium diet	1·3 per cent calcium diet	*S.E.* of difference
Percentage of 'Grade *A*' carcases	78·0	82·4	± 2·29
Percentage of 'Grade *B*' carcases	22·0	17·6	± 2·29
Average weight of all carcases (lb.)	3·23*	3·11	± 0·02
Average weight of 'Grade *A*' carcases (lb.)	3·18*	3·06	± 0·02
Average weight of 'Grade *B*' carcases (lb.)	3·33	3·38	± 0·07

* $P < 0·001$.

It should be noted that although the 0·83 per cent calcium diet averaged 0·75 per cent more protein than the 1·35 per cent calcium diet, it is unlikely that this extra protein would have produced the effects which we have attributed to the difference in level of calcium.

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Replacement of Adrenaline and Noradrenaline in the Adrenal Gland of the Rat, following Depletion and Redepletion with Reserpine

In a previous communication[1], we showed that reserpine causes a similar percentage loss of adrenaline and noradrenaline from the adrenal glands of the rat. Recovery of the catechol amines was slow, the amine content returning to normal within 21 days. The adrenaline content gradually returned to the control value, whereas the noradrenaline content first increased rapidly to a level well above normal at 7 days before falling to the control value.

To determine whether or not the recently replaced amines are bound in the medullary cells in a different manner from the amines that are not released during depletion, it was decided to redeplete the glands at 7 and 21 days. Redepletion at 7 days would detect any difference in the sensitivity to reserpine of the recently replaced amines and redepletion at 21 days would reveal whether or not the glands had, at that time, returned to normal with respect to their sensitivity to further reserpine treatment.

In this series of experiments groups of 5 male albino rats weighing 150–190 gm. were used. The treatment they received and the schedule of killing are shown in Table 1. Four 7-day and two 21-day redepletion experiments were performed. In all 88 groups of 5 rats were used. Each test group had its own control group also of 5 rats. In every instance except the 10-day point in the 7-day redepletion experiments, the control groups consisted of glands from rats given the reserpine solvent only. The control group for the 10-day point was obtained from the glands of rats given reserpine on days 0, 1 and 2 and killed on day 10 (Table 1). This represents the amine content that the redepleted group would have attained if it had not been further treated with reserpine. Subsequent to this 10-day point the controls were the normal solvent-injected rats, because it was found that the relative amounts of adrenaline and noradrenaline following redepletion were very similar to the amounts following the initial depletion. The glands were extracted with *N*/10 hydrochloric acid and assayed biologically as described previously[1].

As can be seen from Fig. 1, redepletion at 7 days caused a preferential loss of noradrenaline, the newly replaced amine thus appearing more susceptible to the action of reserpine than that remaining after the initial depletion. The subsequent recovery of the noradrenaline content was similar to the initial recovery, with a peak of noradrenaline occurring 7 days after the beginning of redepletion. The noradrenaline content was still slightly raised above the control-level at 21 days. The replacement of adrenaline was similar to that which occurred in the initial depletion experiments.

Table 1

Group	Day						
	Reserpine (1 mgm./kgm. subcutaneously) administered						Killed
Depletion	0	1	2				3
	0	1	2				7
	0	1	2				14
	0	1	2				21
7-day redepletion	0	1	2	7	8	9	10
	0	1	2	7	8	9	14
	0	1	2	7	8	9	21
	0	1	2	7	8	9	28
21-day redepletion	0	1	2	21	22	23	24
	0	1	2	21	22	23	28
	0	1	2	21	22	23	35
	0	1	2	21	22	23	42

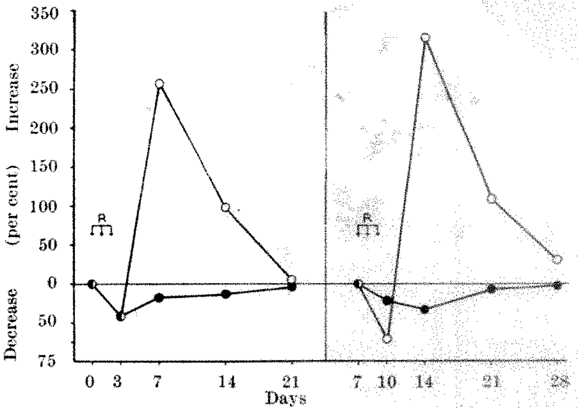

Fig. 1. Effect of redepletion with reserpine, at 7 days, on the adrenaline (●) and noradrenaline (○) content of rat adrenal glands. Left, depletion; right, redepletion

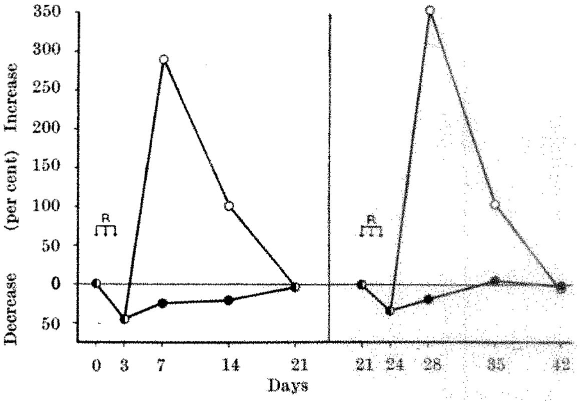

Fig. 2. Effect of redepletion with reserpine, at 21 days, on the adrenaline (●) and the noradrenaline (○) content of rat adrenal glands. Left, depletion; right, redepletion

Redepletion at 21 days (Fig. 2) caused losses of both adrenaline and noradrenaline similar to those which had occurred in the initial depletion experiments. The recovery of both amines was similar to that obtained initially with a peak of noradrenaline occurring at 28 days, that is, at 7 days following the beginning of redepletion.

Thus it appears that at 21 days the rat adrenal gland has recovered its normal catechol amine content following a 50 per cent loss produced by reserpine. However, the preferential loss of noradrenaline occurring when redepletion occurred at 7 days resulted in the noradrenaline content of the glands being depleted to the same absolute amount as that following the initial depletion. Thus it seems possible that, under the experimental conditions described here, it is the newly synthesized noradrenaline that is released before there is time for it to be converted to adrenaline.

We wish to thank Ciba Laboratories, Ltd., for generous gifts of reserpine.

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Choline Acetylase in the Human Placenta at Different Stages of Development

It has long been known that there is acetylcholine in the human placenta[1] and Comline's[2] experiments established that choline acetylase, the enzyme which synthesizes acetylcholine, is in the placenta as well. The rate of acetylcholine synthesis, which he observed in extracts of acetone powders, made from mature placentæ, was between 0·6 and 0·75 mgm./gm. acetone-dried tissue/hr. Later in this laboratory, when a coupled enzyme system was used to provide an independent supply of the substrate acetyl-co-enzyme *A*, rates of 5–6 mgm./gm./hr. were obtained[3].

More recently we have re-measured the choline acetylase activity in mature and in some early placentæ (at 15, 24, 25 and 34 weeks of development) and have found that the choline acetylase activity of the early placentæ is higher than of those which reach term. But the activity even in the fully developed placentæ may be much greater than that reported earlier. Expressed (in terms of the chloride) as the amount of acetylcholine produced per gm. acetone powder, per hr., rates as high as 34 mgm. have been observed. Neither the fœtal blood nor the other fœtal membranes (amnion, umbilical cord) have any significant activity and the placental tissue washed free of blood is found to synthesize acetylcholine at the same or little higher rate than samples that contain blood. In this respect the enzyme differs from placental cholinesterase, since it appears from Ord and Thompson[4] that cholinesterase of the placental tissue and of the blood which it contains is about equal.

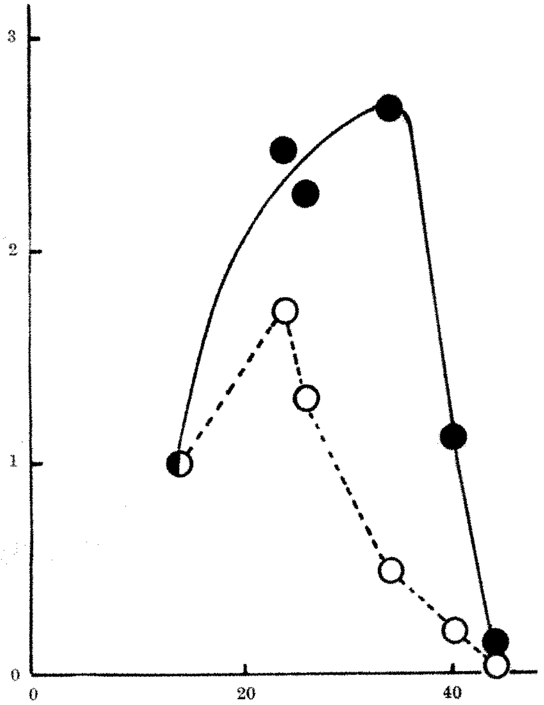

Fig. 1. Choline acetylase activity of the human placenta at different stages of development. The activity is determined by measuring the amount of acetylcholine produced by incubating an extract of a known quantity of tissue (equivalent to 1 mgm. or more) for 1 hr. at 38·5° C. in a system containing coenzyme *A*, acetylphosphate, phosphotransacetylase, choline, *l*-cysteine, potassium chloride, magnesium chloride, eserine sulphate and sodium phosphate buffer (*p*H 6·9). The ordinate is the amount of acetylcholine expressed as gm. acetylcholine-chloride and the abscissa is the developmental age of the placenta in weeks. ○, activity per 100 gm. of tissue; ●, activity per total weight of organ

By comparison with the mature placentæ those in which development had been interrupted at an early stage had extraordinarily high concentrations of choline acetylase. The highest activity was exhibited by a placenta thought to be 24 weeks old. Acetone powders made from this specimen had an average activity of 130 mgm. acetylcholine-chloride/gm./hr., equivalent to 17·1 mgm./gm. fresh tissue and a total activity for the whole organ of about 2·0 gm./hr. The specific activity of the extracts, or the amount of acetylcholine formed per mgm. of protein was 7·6 μM. So far as we are aware, this is the highest activity yet reported for any vertebrate tissue.

Fig. 1 shows how both concentration and total activity vary during development of the placenta. Although, as already mentioned, the concentration was highest at 24 weeks, the total amount was higher in the 34-week placenta. This suggests that the tissue containing the enzyme continues to grow for the first 24–34 weeks; but that some time before 34 weeks its growth is overtaken by the growth of other tissue elements with the consequence that its concentration falls. At the end of pregnancy, however, there is a decrease both in concentration and total amount of choline acetylase. This might be due either to involution and degeneration of the tissue containing the enzyme, or its denaturation. We have some evidence to suggest that this might occur by oxidation of its sulphydryl groups.

The results are of interest in that they strengthen the view that acetylcholine is of importance in placental function during pregnancy. Present indications are that the enzyme is located in the chorionic villi, in the cell layers separating maternal from fœtal blood. We are tempted to suggest therefore that its function is in the active transport of materials across this barrier.

We are grateful to Dr. J. Dickie at the County Maternity Hospital, Cambridge, for enabling us to obtain the placentæ and providing the clinical history of each.

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Effect of Dimethyl Sulphoxide on the Cryo-tolerance of Mitochondria

The integrated structural and functional complexes of many biological entities have been conserved by freezing and storage at low temperatures. The cryo-tolerance of enzymes, viruses, bacteria, cells or tissues has been increased by the inclusion of 'protective substances' in the suspending medium[1,2]. We have reported recently the role of glycerol in protecting oxidative phosphorylation by suspensions of mitochondria during freezing or freezing and storage at low temperatures[3]. Dr. Audrey Smith, of the National Institute for Medical Research, London, suggested to us that dimethyl sulphoxide could replace glycerol. Our preliminary work has shown dimethyl sulphoxide to be equal to glycerol as a protective substance and to be superior in certain instances.

Suspensions of mitochondria were prepared from the livers of Sprague-Dawley rats. Two procedures were used: (1) mitochondria were prepared in isotonic sucrose containing dimethyl sulphoxide; (2) the total isolation of mitochondria was carried out in isotonic sucrose and dimethyl sulphoxide was added to the final suspension. Both preparations contained $3{\cdot}5 \times 10^{-5}$ M versene. All preparative steps were at 0–2°. Oxidative phosphorylation, using α-ketoglutarate as substrate, was measured by a modification[4] of the method of Lardy and Wellman[5]. Aliquots of suspensions of mitochondria, 0·8 ml., were placed in tubes and frozen slowly by immersion into −76° baths, 'dry ice' and acetone, with continuous swirling to distribute the mitochondria evenly on the sides of the tubes. Frozen suspensions were thawed by placing the tubes at 30° until melting was initiated; the samples were then kept in an ice-bath until used.

Because of the intimate contact between mitochondrial elements and the substance to be tested, the several concentrations of dimethyl sulphoxide were tested first by procedure 1. The concentrations of dimethyl sulphoxide of those preparations showing the best overall retention of oxidative phosphorylation after freezing or freezing and storage at low temperatures were tested further by procedure 2. The results of these investigations are shown in Table 1. Differences of 3 μmoles in the esterification of inorganic phosphate or 2 μatoms in the uptake of oxygen were statistically significant[3].

In procedure 1, oxidative phosphorylation was not affected in suspensions of mitochondria prepared in isotonic sucrose containing 5 or 10 per cent dimethyl sulphoxide. Phosphorylation was significantly lower in suspensions prepared in 20 per cent dimethyl sulphoxide; oxidation was not altered. Concentrations of 30 per cent were toxic. A significant decrease in oxidative phosphorylation was observed in suspensions containing 5 per cent dimethyl sulphoxide and frozen slowly at −76°; the decrease in phosphorylation was greater than that of the control frozen similarly. Freezing did not alter further oxidative phosphorylation by suspensions containing 10 and 20 per cent dimethyl sulphoxide. Phosphorylation was significantly less in all groups after storage at −65° for 18 days, averaging 11·7 per cent in relation to the values of frozen suspensions; oxidation was unchanged in groups exposed to 10 or 20 per cent dimethyl sulphoxide and significantly lower in the 5 per cent group.

In procedure 2, the values for oxidative phosphorylation by non-frozen, frozen or frozen and stored suspensions of mitochondria isolated in isotonic sucrose prior to the addition of dimethyl sulphoxide, in amount to give a 10 per cent solution, were not significantly different from that of the non-frozen control. Oxidative phosphorylation by non-frozen suspensions of mitochondria containing 20 per cent dimethyl sulphoxide was not different from the non-frozen control. The oxidative and phosphorylative capacities of the suspensions above following freezing or freezing and storage were not different from those suspensions containing 20 per cent dimethyl sulphoxide and prepared by the first procedure.

Neither 5 nor 10 per cent dimethyl sulphoxide nor glycerol affected adversely oxidative phosphorylation by suspensions of mitochondria (all references to glycerol are based on data in ref. 3). The losses in phosphorylation by suspensions of mitochondria prepared with 20 per cent dimethyl sulphoxide (procedure 1), were approximately one-half those observed when 20 per cent glycerol was used, being 16 and 32 per cent respectively; oxidation was affected similarly. No losses were found in oxidative phosphorylation by suspensions containing 20 per cent dimethyl sulphoxide. Suspensions prepared by procedure 2, and containing 20 per cent glycerol, on the other hand, lost approximately 25 per cent of their ability to carry out oxidative phosphorylation. Mitochondria exposed to 30 per cent glycerol (procedure 1), were inactivated; suspensions exposed to 30 per cent dimethyl sulphoxide retained their capacity for oxidative phosphorylation, but at a greatly reduced level. These results indicate that dimethyl sulphoxide is less toxic to mitochondria than glycerol.

Both 10 per cent dimethyl sulphoxide and glycerol protected fully oxidative phosphorylation by suspensions of mitochondria frozen slowly at −76°. In the case of the former, both procedures were equally effective; the latter, on the other hand, was effective fully only with procedure 1. At a diminished concentration (5 per cent), procedure 1, greater protection was observed with dimethyl sulphoxide. Thus, with comparable concentrations and techniques of application, dimethyl sulphoxide was equal or superior to

Table 1. EFFECTS OF SEVERAL CONCENTRATIONS OF DIMETHYL SULPHOXIDE ON OXIDATIVE PHOSPHORYLATION BY SUSPENSIONS OF MITOCHONDRIA FROZEN SLOWLY AT −76° OR FROZEN SLOWLY AT −76° AND STORED AT −65° FOR 18 DAYS

Dimethyl sulphoxide (per cent)	Treatment	Procedure 1*			Procedure 2†		
		P (μmoles)	O (μatoms)	P : O	P (μmoles)	O (μatoms)	P : O
0 (control)	Non-frozen	35·48	11·61	3·06			
	Frozen	29·95	10·77	2·78			
	Stored	26·24	10·17	2·58			
5	Non-frozen	33·60	10·44	3·22			
	Frozen	25·79	8·13	3·17			
	Stored	22·63	6·47	3·65			
10	Non-frozen	35·59	10·40	3·42	36·83	11·24	3·28
	Frozen	36·86	10·64	3·46	35·59	11·00	3·24
	Stored	31·67	9·84	3·22	36·23	11·71	3·09
20	Non-frozen	29·54	10·49	2·82	34·18	11·15	3·07
	Frozen	30·78	10·56	2·91	30·26	10·16	2·98
	Stored	27·68	10·53	2·63	29·78	10·18	2·93
30	Non-frozen	10·40	4·83	2·15			
	Frozen	0·00	0·00	—			

* Mitochondria prepared in isotonic sucrose containing dimethyl sulphoxide.
† Dimethyl sulphoxide added to isolated mitochondria suspended in isotonic sucrose.

glycerol for protecting the oxidative and phosphorylative capacities of suspensions of mitochondria following freezing.

No losses in oxidative phosphorylation were found when 10 per cent glycerol (procedure 1), or 10 per cent dimethyl sulphoxide (procedure 2) were used to protect mitochondria during storage at −65° for 15 and 18 days respectively, the only comparison possible at present. However, procedure 2 is allied more closely than procedure 1 to the methods of preserving biological materials, that is, putting cells and tissues in solutions containing the protective substance prior to freezing and storage. Therefore, it is likely that dimethyl sulphoxide, by protecting mitochondrial elements with greater efficiency than glycerol, will be found to protect cells more effectively than glycerol from freezing and storage at low temperatures.

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Preservation of Mouse Bone Marrow at −79° C. with Dimethyl Sulphoxide

DIMETHYL sulphoxide was used by Lovelock and Bishop[1] to protect bovine erythrocytes against the damaging effects of freezing to, and thawing from, low temperatures. The bovine erythrocyte is impermeable to glycerol, which does not protect it from the damage caused by freezing. Dimethyl sulphoxide has recently been shown to protect mouse bone marrow from the damaging effects of freezing to −79° C. (ref. 2). After thawing, the protected cells retained between 50 and 60 per cent of their ability to synthesize lipids and proteins. Fluorescence microscopy with acridine orange revealed the presence of many intact erythroid and myeloid cells. It has now been demonstrated that mouse bone marrow stored for one month at −79° C. in the presence of dimethyl sulphoxide will prevent the death of lethally irradiated mice.

Bone marrow cells were obtained from the femora of 12-week old female mice of an inbred *CBA* strain. The cells were suspended in Parker '199' tissue culture fluid containing 33 per cent rat serum and 15 per cent (v/v) dimethyl sulphoxide. Ampoules containing 50×10^6 nucleated cells in 1 ml. were cooled slowly to −79° C. in the apparatus described by Polge and Lovelock[3]. Marrow cells were also frozen in the absence of dimethyl sulphoxide. After one month at −79° C., ampoules were thawed rapidly at +37° C. and the bone marrow cells injected intravenously into lethally irradiated mice.

Table 1. SURVIVAL OF LETHALLY IRRADIATED MICE INJECTED WITH ISOLOGOUS MARROW PRESERVED AT −79° C. FOR ONE MONTH WITH DIMETHYL SULPHOXIDE

Treatment (No. of nucleated cells)	No. of mice	No. living at 30 days	Survival (per cent)
Controls, injected with Parker/serum/dimethyl sulphoxide medium (0·5 ml.)	19	0	0
Fresh marrow; 5×10^6 in 0·5 ml. of Parker/serum/sulphoxide	18	15	83
Marrow frozen without dimethyl sulphoxide; 5×10^6 in 0·5 ml. of Parker/serum	26	2	7·7
Marrow frozen in the presence of 15 per cent dimethyl sulphoxide; 5×10^6 in 0·5 ml. of Parker/serum/sulphoxide	27	19	70

Female *CBA* mice of the same strain and age as the donor animals were irradiated with 1,000 r. of X-rays (LD_{99} at 30 days). The X-ray apparatus used was a Newton Victor *G* × 10 operated with the following factors: 120 kV.; 5·5 m.amp.: 1 mm. aluminium filter; dose-rate of 48 r. per min. Immediately after irradiation, the mice were allocated at random to four groups. One group received 5×10^6 fresh marrow cells; a second group received 5×10^6 cells which had been preserved at −79° C. for one month with dimethyl sulphoxide; the third group was injected with the same number of cells frozen for one month without dimethyl sulphoxide, and the last group was injected with Parker-serum-sulphoxide medium only. The number of mice alive 30 days post-radiation in the four treatment groups is shown in Table 1. These results indicate that marrow preserved by dimethyl sulphoxide at −79° C. is only slightly inferior to fresh marrow in preventing radiation death.

A second experiment was designed to study the hæmatological response of lethally irradiated mice to fresh marrow and preserved marrow. Mice were killed at intervals following the injection of different numbers of fresh cells and a standard dose of frozen cells preserved in dimethyl sulphoxide. Re-population of the marrow cavity of the femur was evident on the eighth day after irradiation in the group of mice which received the marrow frozen in the presence of sulphoxide (see Fig. 1). By the twelfth day the cellularity of the marrow was almost normal, and cells of all types, including megakaryocytes, were present. Mice which had been injected with the same number (5×10^6) of fresh marrow cells showed evidence of re-population of the femur between the sixth and seventh day. The femora of mice which had received marrow frozen in the absence of dimethyl sulphoxide and of those which had been injected with cell-free medium showed no signs of re-population and most of these animals were dead by the tenth day.

Leucocyte counts on the peripheral blood from these animals and of others which had been injected with smaller doses of fresh bone marrow are shown in Fig. 2. The leucocyte response of mice which had received frozen marrow preserved with dimethyl sulphoxide was slightly better than the response obtained with 1×10^6 fresh cells. This implies that more than 20 per cent of the myeloid stem cells in the frozen marrow sample were viable.

Blood leucocyte-levels were normal (greater than 9,000 per c.mm.) within 14 days in those mice treated with 5×10^6 fresh cells and within 17–18 days in mice treated with the same number of cells frozen in the presence of sulphoxide. In both cases, however, the

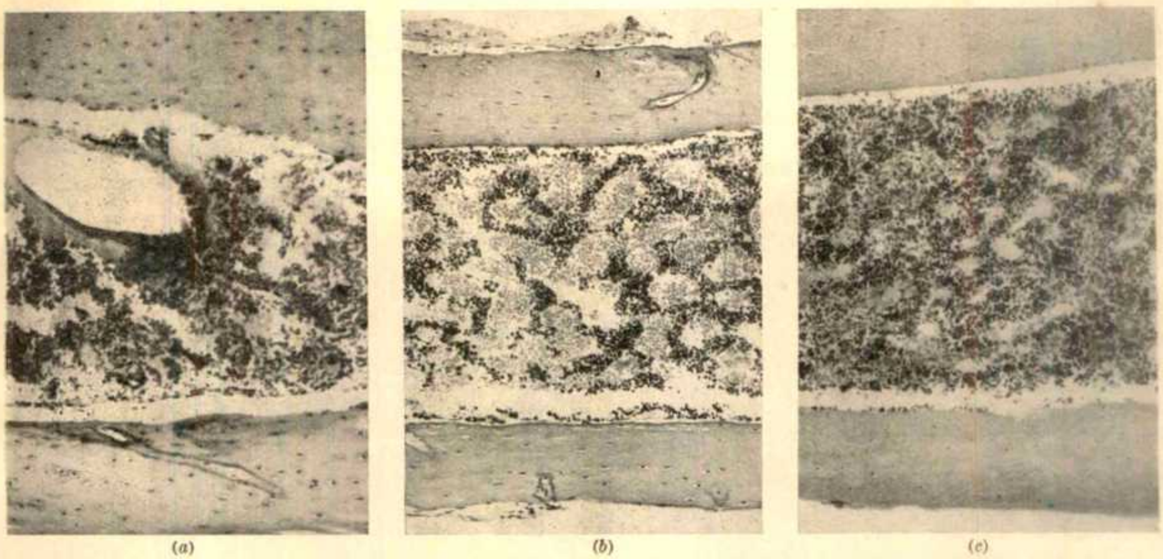
(a) (b) (c)

Fig. 1. Femora of X-irradiated mice (hæmatoxylin and eosin). (a) Untreated control 8 days after X-irradiation. (× 85); (b) treated with bone marrow stored at − 79° C.; 8 days after X-irradiation (× 67); (c) treated with bone marrow stored at − 79° C.; 12 days after X-irradiation (× 75)

differential counts were abnormal. Nearly 75 per cent of the leucocytes were polymorphs and only 25 per cent were lymphocytes and monocytes. This is almost a quantitative reversal of the normal differential blood picture.

Work is in progress to compare the hæmatological response of lethally irradiated mice with marrow preserved with glycerol and dimethyl sulphoxide.

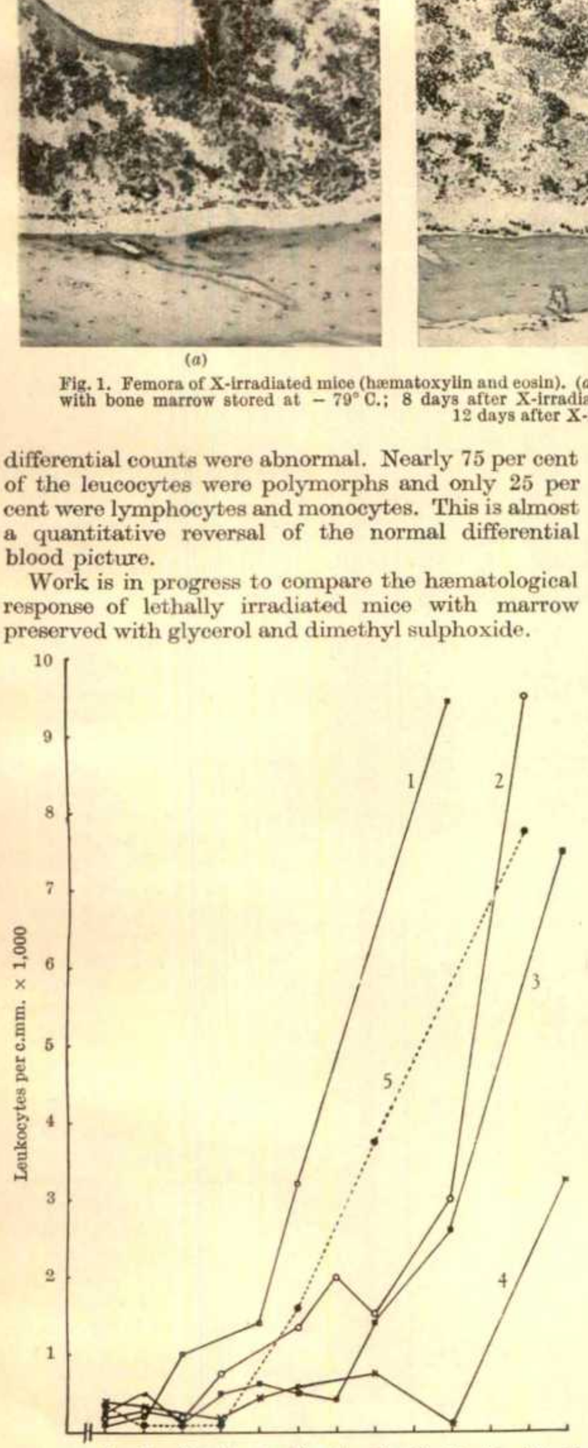

Fig. 2. Effect of fresh marrow and marrow preserved at − 79° C. with dimethyl sulphoxide on the recovery of blood leucocytes in lethally irradiated mice. (1) 5 × 10^6 cells; (2) 1 × 10^6 cells; (3) 0·5 × 10^6 cells; (4) 0·1 × 10^6 cells; (5) 5 × 10^6 frozen cells

I wish to thank Dr. A. U. Smith for useful suggestions.

Note added in proof. Since this work was completed, comparisons with marrow preserved with dimethyl sulphoxide and with 15 per cent glycerol have indicated the superiority of dimethyl sulphoxide as a low-temperature preservative agent. The number of animals which survived lethal X-irradiation when treated with either marrow preserved with glycerol or sulphoxide was about the same. The onset of reticulocytosis and the start of the leucocyte response, however, occurred about two days later in mice injected with 5 × 10^6 cells which had been kept for one month at −79° C. in the presence of 15 per cent glycerol.

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Role of the Mucous Membrane in the Peristaltic Reflex in the Isolated Ileum of the Guinea Pig

Bülbring, Lin and Schofield[1] have presented evidence which suggested that receptors of the sensory neurones subserving the peristaltic reflex in the isolated small intestine of the guinea pig, rabbit and cat are present in the mucous membrane. Ginzel[2], on the other hand, showed that necrosis of the mucous membrane brought about by 30 per cent silver nitrate or 20 per cent tannic acid did not abolish the peristaltic reflex. He concluded that mucosal nerve fibres were not essential for this reflex.

When the peristaltic reflex is investigated by the technique of Trendelenburg[3], the fluid which distends the lumen and is then ejected by the peristaltic movement is not renewed during the experiment; thus any material which may be shed by the mucous membrane will accumulate in this fluid. If the preparation is left in the organ bath for some hours and the peristaltic reflex elicited at intervals, it may be noted that the fluid ejected from the lumen becomes increasingly turbid, and at the same time the intestinal wall appears more and more transparent. This suggested to us that a part of the mucous membrane is lost while the ileum is in the organ bath.

This possibility was tested in a series of experiments in which pieces of ileum were left in the organ bath for varying periods, during which the peristaltic reflex was elicited every 30 min. At the end of the experimental period the gut was fixed for histological examination.

Changes in the villi occurred as early as 30 min. after setting up the preparation and consisted of a swelling of the stroma of the villi, particularly at their tips (Fig. 1, *B*); at this stage most of the epithelial cells were still in place although they showed degenerative changes. Half an hour later (Fig. 1, *C*), the swollen distal ends of the villi had been shed into the lumen, where chains of epithelial cells were found. In the course of the next few hours more and more of the mucous membrane was lost until, after 18 hr., very little live mucosal tissue was left (Fig. 1, *D*–*F*). No obvious changes were observed in the muscle fibres.

The segments of the ileum from which the sections shown in Fig. 1, *B*–*E* were obtained, that is up to 8 hr. after setting up the preparation, still exhibited a normal peristaltic reflex. In the ileum which, after 18 hr. in the organ bath, had the histological appearance of Fig. 1, *F*, distension of the lumen elicited a reflex as late as 16 hr. after setting up the preparation. From these experiments it may be concluded that, if stretch receptors subserving the peristaltic reflex are present in the mucous membrane, they are situated in its basal layers.

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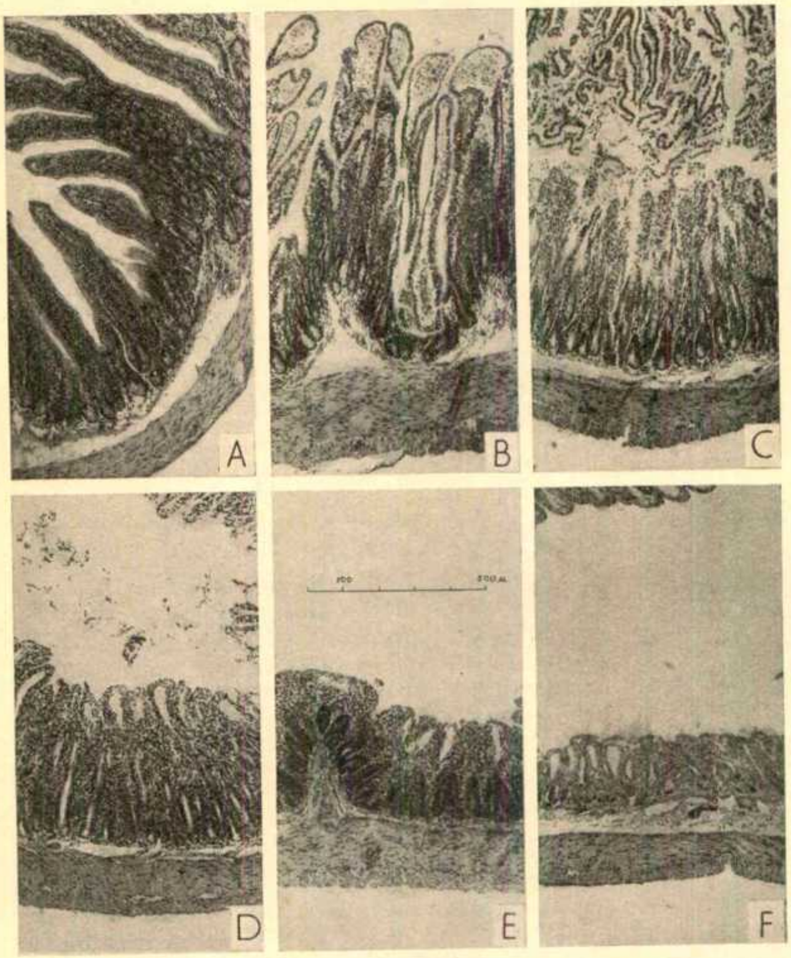

Fig. 1. The histological appearance of the isolated ileum of the guinea pig at intervals after setting up in an organ bath. *A*, Control, immediately after excision; *B*, after 30 min.; *C*, after 1 hr.; *D*, after 4 hr.; *E*, after 8 hr.; *F*, after 18 hr. Tyrode solution oxygenated with oxygen was used in *B*, *C*, *D* and *F*, while Krebs's solution oxygenated with 95 per cent oxygen and 5 per cent carbon dioxide was used in *E*. Temperature of bath, 35° C.

HISTOCHEMISTRY

Histochemical Demonstration of Aldehydes by *p*-Phenylenediamine

A HIGHLY specific and sensitive reaction has already been proposed[1] for the microscopic detection of aldehydes in the tissues. Improvements have now been made which meet the needs of practical histochemistry.

The oxidation velocity of *p*-phenylenediamine with hydrogen peroxide is greatly increased by aldehydes[2]. Only nitriles, aldehyde ammonia and aldehyde bisulphite compounds behave similarly to aldehydes[3]. The black precipitate formed on the tissues as a consequence of this peroxidase activity of aldehydes in neutral solution was found suitable for microscopic observation after stabilization with gold chloride.

The following reagents are used: (1) 1 per cent *p*-phenylenediamine stock solution (to reduce storage oxidation, previously boiled water must be used and the solution kept in a tightly stoppered dark flask); (2) 1·5 per cent hydrogen peroxide; (3) 1 per cent gold chloride neutral solution.

The procedure used was as follows. Fix and section by any standard technique. Take sections down to distilled water in the usual manner and treat them for release of aldehydic groups (periodic acid was used in the present experiments). Wash thoroughly with distilled water. Before use, mix equal volumes of reagents (1) and (2) and immerse sections for 15 min. (operate in dark dishes, because light accelerates oxidation of *p*-phenylenediamine). Wash thoroughly with several changes of distilled water. Immerse in reagent (3) for 5 min. Wash, dehydrate and mount in the usual way

(Canada balsam). Structures containing aldehyde are stained bluish-violet.

The results obtained with *p*-phenylenediamine using sections treated in this way are comparable with those obtained with Schiff's reagent, though greater sensitivity and selectivity is obtained.

As a control on the selectivity of the method, the following reagents for blocking aldehydic groups were used before treatment with *p*-phenylenediamine: (1) 0·2 per cent 4-nitrophenylhydrazine solution in 50 per cent v/v ethanol containing 1 per cent sodium acetate, incubation for 2 hr. at 60° C.; (2) saturated hydroxylamine hydrochloride, ethanolic solution, 2 hr. at 60° C.; (3) 1 per cent aniline solution in 75 per cent v/v ethanol, 2 hr. at 60° C. All these reagents completely prevented the peroxidase oxidation of *p*-phenylenediamine.

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PATHOLOGY

Growth of Tumours in Rats fed with 4-Dimethylaminoazobenzene injected with Homogenized Tumour mixed with Freund Adjuvant

THE enhancing (or *XYZ*) effect on the growth of tumour homografts in animals pre-treated with non-living donor tissue first described by Flexner and Jobling[1] led to many fruitful immunogenetic investigations in oncology. In order to explain the phenomenon, several working hypotheses have been evolved: the 'blockage' hypothesis was advocated by Medawar and co-workers[2] and Snell[3], 'immunoselection' by Hauschka and co-workers[4] and the hypothesis of some 'physiological' alteration in the tumour cells was argued by Kaliss[5]. According to experiments of Casey and co-workers[6], it is possible to enhance the tumour growth in the strain from which the tumour originated. It is difficult to explain these results on the hypotheses mentioned above.

I have tried to enhance the growth of indigenous tumours. Albino rats of mixed breed were subjected to a carcinogenic diet supplied with 4-dimethylaminoazobenzene. The daily dose of 3·5 mgm. of the drug per rat given during a period of 203 days induced liver carcinoma after a latent period of nearly 120 days after stopping the drug. The incidence of hepatoma was 68 per cent.

In the experiment to be described, 120 rats fed 4-dimethylaminoazobenzene for 203 days were divided at random into five groups and injected with normal liver tissue or liver carcinoma of the same origin, both mixed with Freund's adjuvant. The antigen mixture was heated on a water-bath at 60° C. for 45 min. No palpable liver tumour was present in animals at the time of injection, so it was assumed that the animals were in a pre-cancerous stage. The intracutaneous injections, 8 × 0·2 ml. of antigen mixture, were given along both flanks of the rats simultaneously on the fourth day after stopping the carcinogenic diet. The three intraperitoneal injections (1 ml. each of antigen mixture) were given at intervals of 1 week, the first being given on the same day as intracutaneous injections. On the twenty-second day after stopping the carcinogenic diet, the animals were killed, and diagnosis was by gross inspection and histological examinations of the liver.

Table 1. INCIDENCE OF CARCINOMA HEPATITIS IN PRE-CANCEROUS RATS TREATED WITH TUMOUR TISSUE AND NORMAL LIVER TISSUE MIXED WITH ADJUVANT

	Treatment				
	Intraperitoneal		Intracutaneous		
Animals	Tumour tissue	Liver tissue	Tumour tissue	Liver tissue	None
Pre-cancerous	12/24	0/24	0/24	0/24	0/24
Normal	0/20		0/18		

The results presented in Table 1 show that intraperitoneal application of the antigen mixture containing cancer tissue was in this instance only suitable for inducing growth promotion of the cancer. It is worth mentioning that inflammatory reaction in the abdominal cavity which occurs after intraperitoneal injection of adjuvant alone or mixed with some tissue was negligible in tumour-bearing rats in comparison with other animals injected intraperitoneally.

As already stressed[7], the enhancement of tumour growth and tumour graft may show a specific difference between cancerous and normal tissues. The effect of enhanced tumour growth produced by the stimulating effect of antibodies might be explained simply by assuming that antibodies are complementary bodies, which act on normal tissue by destroying it and on cancerous tissue on the contrary by enhancing its growth. Cachexia and enhanced tumour growth in cancer-bearing animals could thus be ascribed to a manifestation of the same process in tissues of different physiological and functional state. Further experiments are in progress to investigate the problem.

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Interaction of Enzymes with Normal and Tumour Cells

WHEAT germ lipase (Worthington) has been shown[1] to reduce the adhesion of washed mouse ascites cells to glass. This was considered to be due to the adsorption of the enzyme on to the cell surface. The same lipase preparation has also been found[2] to inhibit the growth of hamster kidney tumour cells in tissue culture at certain concentrations (0·01–0·1 per cent), without inhibiting growth *in vitro* of hamster normal kidney epithelial cells from which the tumour was derived.

An investigation has been made into the cytological effects of lipase on these normal and tumour cells growing in tissue culture, using 'Anoptral' contrast microscopy. At an enzyme concentration of 0·1 per cent, the tumour cells showed extensive vacuolation

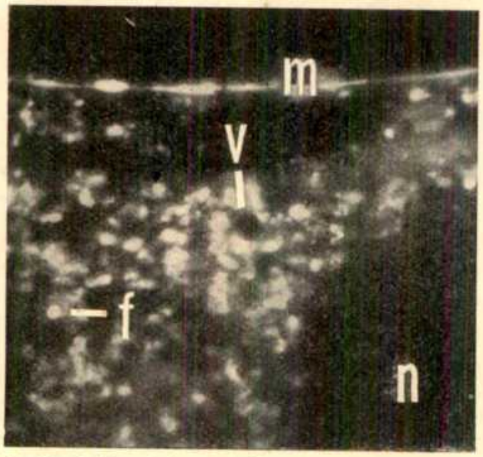

Fig. 1. Marginal part of a normal hamster kidney epithelial cell in a monolayer culture, 5 hr. after adding fluorescein-labelled lipase (0·1 per cent). Ultra-violet illumination (× 1,600). *f*, Fluorescent vacuole; *m*, cell membrane; *n*, nucleus; *v*, non-fluorescent vacuole

of the cytoplasm by the third day after addition. Such vacuolation was not found in treated normal cells.

The site of action of lipase has also been followed, by labelling it directly with the fluorescent dyes fluorescein and lissamine rhodamine *RB* 200 (ref. 3). The enzyme was assayed against triacetin before and after labelling, to check whether the conjugation procedure had destroyed its activity. It was found that the fluorescent conjugate showed half the aliesterase activity of the untreated enzyme. Schiller *et al.*[4] provide evidence that directly labelled protein conjugates are reasonably stable for at least 24 hr. under *in vivo* conditions.

Cultures were incubated in growth medium (0·25 per cent lactalbumin hydrolysate, 10 per cent calf serum, 0·001 per cent sodium bicarbonate) containing 0·1 per cent lipase conjugate. They were mounted in this medium without fixation and observed under a Reichert fluorescence microscope. In the normal cultures, many large and small droplets were taken in within the first few hours, and an intense staining of the cell membrane in some cells was observed 5 hr. after addition. Membrane staining was not observed before this period had elapsed, and was not seen later than 8 hr. after addition. Non-viable cells did not show staining of the cell membrane. As a control, lissamine rhodamine *RB* 200 was applied to growing normal cultures at the same concentration as in the conjugate-treated cultures. The dye was taken in as droplets, but did not stain the membrane.

Tumour cultures also took up many large and small droplets, but there was no obvious staining of the cell membrane, even in cells washed prior to treatment.

Lipase conjugate has also been applied to Landschütz ascites tumour cells at the same concentration, under both *in vivo* and *in vitro* conditions. About 5 per cent of the ascites cells took up pinocytosis droplets *in vivo* during the first day after intraperitoneal injection of the conjugate, but there was no staining of the membrane. Washed ascites cells after 2-hr. treatment with lipase conjugate *in vitro* did, however, show surface staining, confirming that the enzyme is adsorbed on to the surface of washed ascites cells.

Observations using fluorescent-labelled lipase indicate that the conjugate is readily taken up by pinocytosis within a few hours by normal hamster kidney and hamster kidney tumour cells *in vitro*, and by mouse ascites cells *in vivo*. The transient staining of the normal cell membrane, which has not been observed in the tumour cells, suggests that there is some accumulation of the enzyme preparation at the surface. Such an accumulation has not been detected with other fluorescent-labelled enzyme conjugates. The difference in vital staining between the normal and the homologous tumour cells provides further evidence for the altered properties of the tumour cell surface.

We wish to express our gratitude to Dr. R. D. Andrews and his staff of Glaxo Laboratories for preparing the cultures.

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HÆMATOLOGY

Acetylcholinesterase and the Life-span of the Erythrocyte

The function of the relatively large amount of the enzyme acetylcholinesterase which is present in erythrocytes is unknown. The enzyme was thought to be essential for the maintenance of normal permeability of the erythrocyte[1], but experiments on cells deficient in acetylcholinesterase[2] have failed to demonstrate this function. Interest in red-cell acetylcholinesterase has been stimulated by the recent finding that in some patients with paroxysmal nocturnal hæmoglobinuria (P.N.H.) the enzyme is deficient[3]. The enzyme activity in patients with P.N.H. varies from 6–134 per cent of average normal activity[4], and there is a correlation between the degree of enzyme deficiency and some other features of P.N.H.[4]. It is thus possible that the shortened erythrocyte life-span in P.N.H. may be related to the enzyme deficiency, and that normal amounts of acetylcholinesterase are necessary for normal survival of erythrocytes.

To determine whether normal levels of acetylcholinesterase are necessary for normal erythrocyte life-span, the enzyme was inhibited *in vivo* in five hæmatologically normal subjects by the administration of octamethylpyrophosphoramide (OMPA) 5 mgm. daily, and erythrocyte survival measured with chromium-51. The administration of OMPA results in irreversible inhibition of erythrocyte acetylcholinesterase[5]. Erythrocyte acetylcholinesterase was measured according to the method of Michel[6]. Erythrocytes were labelled as described by Mollison and Veall[7] and the results expressed as the time taken for circulating radioactivity to reach 50 per cent ($T\frac{1}{2}$ ^{51}Cr). Mean cell-life was calculated after correcting for elution ($T\frac{1}{2}$ elution 64 days).

In three subjects, erythrocytes were labelled (on day 0) and OMPA administered from day 6 to day 13–18, during which time the enzyme activity was reduced to less than 50 per cent of the initial level. In spite of deficient acetylcholinesterase during the period of investigation (50 days), erythrocyte life-span was within normal limits in all subjects (Table 1). In two further subjects OMPA was administered for a period of 21 days before labelling the erythrocytes. The life-span of the erythrocytes was within normal limits in both subjects (Table 1).

Table 1

Case	Duration of administration of OMPA (days)	Erythrocyte acetylcholinesterase (Δ *p*H/hr.) Before OMPA	After OMPA	$T\frac{1}{2}$ ^{51}Cr (days)	Mean cell life (days)
1	7	0·66	0·32	28	110
2	7	0·79	0·36	33	111
3	12	0·98	0·32	32	113
4	21	0·72	0·25	36	120
5	21	0·68	0·35	29	114

Thus in five subjects where erythrocyte acetylcholinesterase was inhibited to levels comparable to those found in patients with P.N.H., shortening of erythrocyte life-span could not be demonstrated. These results suggest that normal amounts of the enzyme are not essential for the maintenance of red-cell life-span, and that the low levels of enzyme in P.N.H. do not directly contribute to the diminished erythrocyte life-span in the disease.

We wish to thank the Director, South African Institute for Medical Research, for facilities to carry out this work.

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Fructose as a Component of the Fœtal Blood in Several Mammalian Species

In the examination of fœtal bloods from several mammalian species, Goodwin[1] suggested that fructose is a characteristic component of the fœtal blood of the Ungulata and Cetacea. We wish to support this generalization by reporting the presence of fructose in the fœtal blood and fœtal fluids of several further species within the order Ungulata, and the essential absence in a member of the Edentata.

The specimens from the dromedary were made available through Dr. W. R. M. Morton, of the Queen's University, Belfast, the sloth material obtained while one of us (A. St. G. H.) was in Brazil, through the kindness of Prof. Nelson Chaves, of the University of Recife. The remainder of the specimens were obtained by Mr. Tong and his staff at Whipsnade Zoological Park. Samples were obtained from the placenta at a variable interval of time after extrusion. The material was placed in bottles containing 100 mgm. powdered sodium fluoride and potassium oxalate in the ratio 1 : 3 and refrigerated pending transfer to the laboratory. After deproteinization, the filtrates were estimated for glucose and fructose by the glucose oxidase and resorcinol methods respectively. The concentrations obtained (Table 1) must be considered as minimal values, since there is evidence of a preferential utilization of glucose in the placenta after it is shed.

It is interesting perhaps to note that in the spectrum of mammalian species which have been examined by us and by others[2-4], in which fructose is present in substantial concentrations, all belong to a group of animals having either the epitheliochorial or syndesmochorial type of placenta. The latter type, typified by the sheep, is known from direct experimentation to produce fructose[5].

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Table 1. Glucose and Fructose Concentrations in Samples obtained from Species within the Orders Ungulata and Edentata (mgm./100 ml.)

Species	Maternal blood Glucose	Fructose	Fœtal blood Glucose	Fructose	Fœtal fluids* Glucose	Fructose
Père David's deer (*Elaphurus davidianus*)	—	—	364	61	12	209
Père David's deer (*Elaphurus davidianus*)	—	—	18	57	22	36
Huanaco (*Lama huanacus*)	—	—	32	148	14	218
Llama (domestic breed)	—	—	15	43	10	223
Giraffe (*Giraffa camalopardalis*)	—	—	8	8	—	—
Rhinoceros (*Rhinoceros unicornis*)	—	—	15	31	—	—
Hippopotamus (*Hippopotamus amphibius*)	—	—	9	60	—	—
Bactrian camel (*Camelus bactrianus*)	—	—	—	84	—	—
Bactrian camel (*Camelus bactrianus*)	—	—	9	63	13	376
Dromedary camel (*Camelus dromedarius*)	97	0	UA† 42 UV† 43	40 41	6	352
Three-toed sloth (*Bradypus tridactylus*)	124	1·5	28	1·5	13	1·5

* Amniotic or allantoic or a mixture of both fluids.
† UA and UV refer to umbilical artery and vein samples respectively.

Transferrin-groups of Fœtal Calf-Serum

Intensive work on the occurrence of blood-group factors in embryos of cattle[1] led to the result that the pattern of blood-group factors, as special individual molecule structures, are genetically dependent and, consequently, are already fixed in the mother cells of the erythropoietic system. The substances of the blood groups undergo a maturing process before birth during embryonic and fœtal growth. With the help of strong immunosera, the blood-group factors already can be identified in the early embryonic stage in the immuno-hæmolytic and in the inhibition test. The

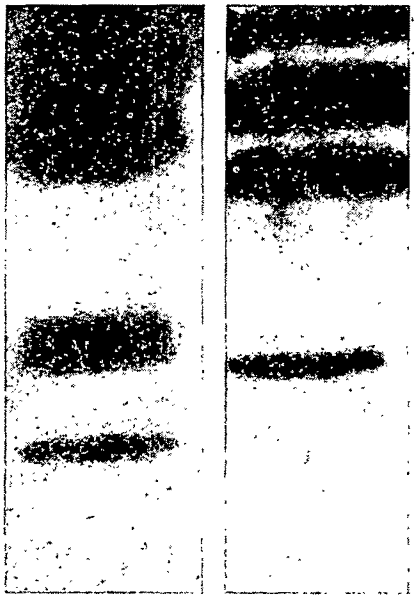

Fig. 1. Left, starch-gel electrophoresis pattern of fœtal calf serum (transferrin bands); right, starch-gel electrophoresis of fœtal calf serum after 90 per cent enrichment of fetuin (fetuin bands)

youngest cattle embryo, the blood-group factors of which we have already identified, had a head to tail length of 17 mm. This corresponds to an age of 28–35 days.

The study of fœtal sera was performed with the help of starch-gel electrophoresis[2]. As buffer solution, we used a mixture of *tris*(hydroxymethyl)aminomethane, disodium salt of ethylenedinitrolotetraacetic acid and boric acid. The migration time of the proteins was 3·5 hr. in an electric field of 7–8 V./cm. Here they showed a clear difference in the albumin/globulin ratio between fœtal serum and serum of adult cattle. The serum of embryos and fœtuses showed a minor content of beta-globulins and a relatively higher content of alpha-globulins. Between the region of the alpha-globulin complex and the post-albumin region in the serum of adult animals, in fœtal sera an additional separation appeared in wide bands (Fig. 1).

By isolating fetuin, which as mucoprotein is a regular part of calf serum, using the precipitation method of Deutsch[3] with a 90 per cent enrichment and a joining starch-gel electrophoresis, we came to the conclusion that the observed additional bands must be attributed to the fetuin fraction.

By the separation of the beta-globulins in the fœtal sera, all transferrin-groups until now observed in the serum of adult cattle of highland breeds (Fleckvieh, Brown Swiss) could be shown. Until now, neither with the adult cattle nor with cattle fœtuses has the serum-transferrin group *E*/*E* been discovered.

The smallest embryo from which we could successfully get enough serum for starch-gel electrophoresis had a head-to-tail length of 42 mm., corresponding to an age of 7 weeks.

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RADIOBIOLOGY

Changes in X-ray Sensitivity of HeLa Cells during the Division Cycle

MEASUREMENT by Puck and Marcus of the reproductive survival of randomly dividing cultures of HeLa *S*3 cells after X-irradiation revealed an exponential response to dose following a shoulder[1]. However, the possibility was not excluded that these populations are in fact heterogeneous, the cells undergoing small, or large but brief, fluctuations in sensitivity during the division cycle. We have examined this possibility by irradiating synchronously growing populations at various times after mitosis.

Synchrony was achieved by selectively harvesting mitotic cells from cultures of HeLa *S*3–9*IV* (ref. 2) growing as monolayers in medium *N*16*HHF* (ref. 3). Dividing cells, being less firmly attached to the plastic culture vessels, were removed by repeated rinsing with growth medium. About 1 per cent of the cells were so gathered, and 80–90 per cent of them were in metaphase to late telophase. Their plating efficiency was 84–90 per cent; 90–100 per cent of those which attached to new plastic culture dishes completed division within 3 hr. after collection. The next division cycle did not commence for an additional 17–21 hr. Fig. 1 shows typical growth-curves for synchronous and random cultures, as determined by repeated microscopic observations of specified fields[4]. The rise in mitotic frequency in the synchronous culture at about the time of division is also shown in Fig. 1. Details of this technique will be reported more fully elsewhere.

Fig. 2 shows typical survival curves obtained for cells irradiated at 37° with 220 keV. X-rays (constant potential; 15 m.amp.; 1 mm. aluminium + 0·5 mm. copper added filtration; about 30 rads per min.; half-value layer 1·9 mm. copper) at selected times after collection. The zero-hour curve represents cells in mitosis, and if the time-course of the synthesis of deoxy-

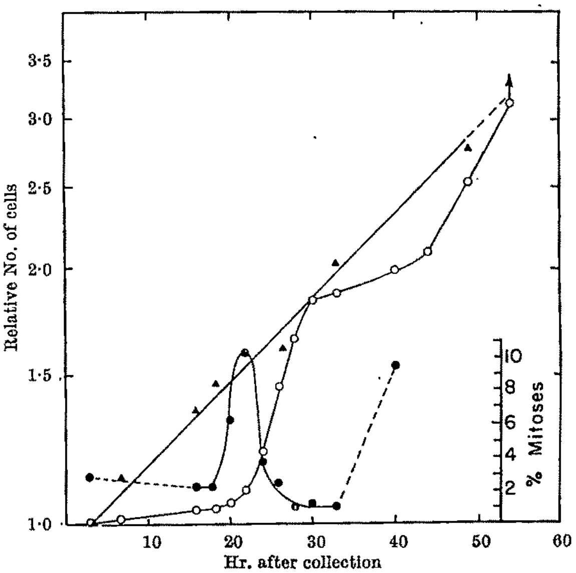

Fig. 1. Synchronous and random division of HeLa *S*3-9*IV* cells. Relative cell numbers, as determined by repeated microscopic observation of specified fields, and mitotic activity, as determined from stained preparations, are plotted as functions of time after harvesting mitotic cells. The cell counts at 3 hr. were 103 for the synchronous culture, and 94 for the random culture. The arrow at 54 hr. indicates that the value plotted was a minimum one. ▲, Random population; ○, synchronized population; ●, mitotic frequency

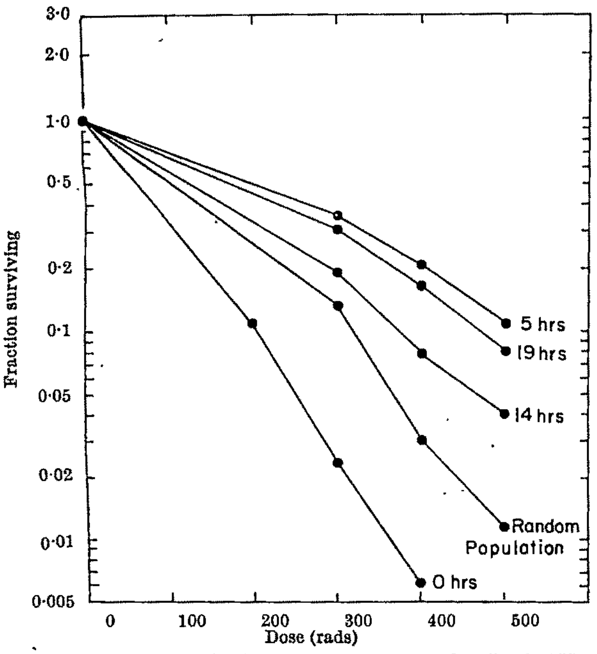

Fig. 2. Survival of colony-forming capacity of HeLa *S*3-9*I V* cells after irradiation with 220 keV. X-rays at the times in the division cycle indicated. Random population was irradiated immediately after trypsinization, while cells were still in suspension; 3 hr. later such cells showed a lower sensitivity

ribonucleic acid (DNA) as determined by Painter and Drew[5] for HeLa *S*3 cells applies to our system, then 5 hr. after cell collection lies in the pre-synthetic (G_1) phase, 14 hr. in the synthetic (S) phase, and 19 hr. in the pre-mitotic (G_2) phase.

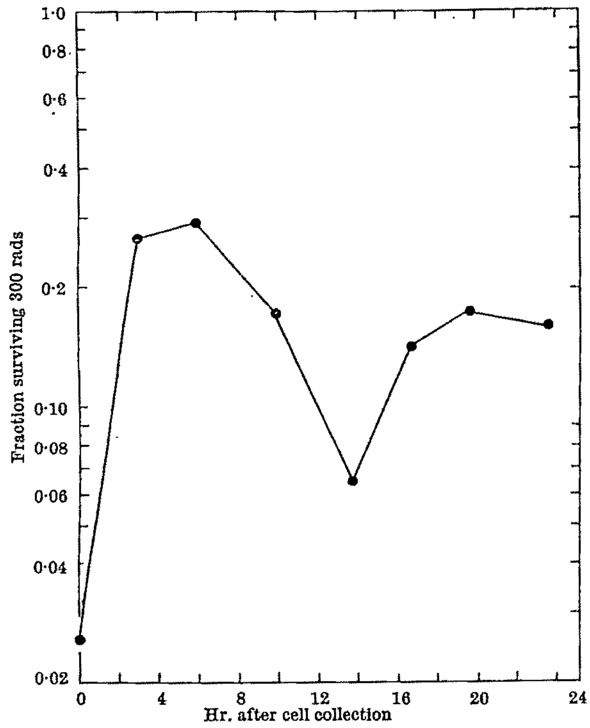

Fig. 3. Fractional survival of HeLa *S*3-9*I V* cells after exposure to 220 keV. X-rays at various times during the division cycle. Data obtained from single doses of 300 rads are plotted on semi-logarithmic co-ordinates, as this representation corresponds to changes in mean lethal dose, if the extrapolation number is constant

It is clear that mitotic cells are more sensitive (mean lethal dose, $D_0 = 59$–82 rads) than are random populations ($D_0 = 100$–120 rads). Such heightened sensitivity is probably correlated with the condensed state of mitotic chromosomes[6]; increased chromosome damage has previously been observed in cells during mitosis[7]. We are unable, with this technique, to determine changes during the various phases of mitosis.

Fluctuations in sensitivity also occur during interphase. As shown in the data of Figs. 2 and 3 (the latter showing cell survival following administration of 300 rads at different times in the division cycle), and of several other experiments, cells are relatively resistant 3–7 hr. after collecting, become progressively more sensitive during the period 7–14 hr., and then more resistant again from 14–18 hr. The data do not permit detection of small changes in extrapolation number during the cycle, but no gross changes are apparent. Accordingly, most or all the fluctuation shown in Figs. 2 and 3 is attributable to changes in D_0. Making this assumption, in four experiments D_0 decreased 16–60 per cent between 3–7 and 14 hr.

The causes of these intermitotic fluctuations in sensitivity are not apparent. The occurrence of a peak in sensitivity at about 14 hr. after mitosis suggests that no simple model will account for the fluctuations, which may result from alterations in damage, or in repair processes, or both. Further indications of the complexity of the system have been found in other experiments (Terasima, T., and Tolmach, L. J., unpublished work) which show that X-ray-induced divisional lag fluctuates in parallel with sensitivity from zero to 14 hr. after mitosis, but not over the remainder of the cycle.

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Trace-labelling of Insulin with Iodine

A RECENT technique for the immunological assay of human plasma insulin requires a radioactive bovine insulin of very high specific activity[1].

Crystalline bovine insulin is labelled with iodine-131 using the only method reported to produce a suitable activity, to the order of 75–300 mc. iodine-131/mgm. insulin[2]. We encountered some difficulties with this method and have made certain modifications. The following procedure has proved more reliable, consistently yielding a specific activity of 300–600 mc. iodine-131/mgm. insulin. Also there is less radiation hazard in that chloroform extraction of up to 80 mc. of iodine-131, part of the original method, is avoided, and so the number of transfers of solutions of high radioactivity into different containers is reduced.

20 μl. of iodine monochloride and 10–20 mc. of thiosulphate-free, carrier-free iodine-131 in minimal $N/50$ sodium hydroxide are added virtually simultaneously to a fresh solution of 20 μgm. of crystalline bovine insulin in borate buffer, *p*H 7·8, in a perfectly clean glass tube[3]. The tube, sealed by a secure glass stopper, is shaken vigorously for 4 min., and a third of the insulin is immediately dialysed. Several changes of distilled water, 2 l. at a time, rid the protein solution of unbound iodine-131.

Some insulin is inevitably damaged by irradiation. The amount damaged is roughly proportional to the specific activity. The damaged fraction is separated from the undamaged fraction on a cellulose column[2]. Damaged insulin labelled with iodine-131 is biologically and immunologically inactive and runs just behind albumin on chromato-electrophoresis on Whatman 3 *MM* paper strips, while the undamaged 'free' insulin is adsorbed at the origin (Fig. 1). After purification almost all the damaged fraction is removed (Fig. 2). The undamaged insulin is now available for investigation requiring truly tracer quantities of radioactive hormone.

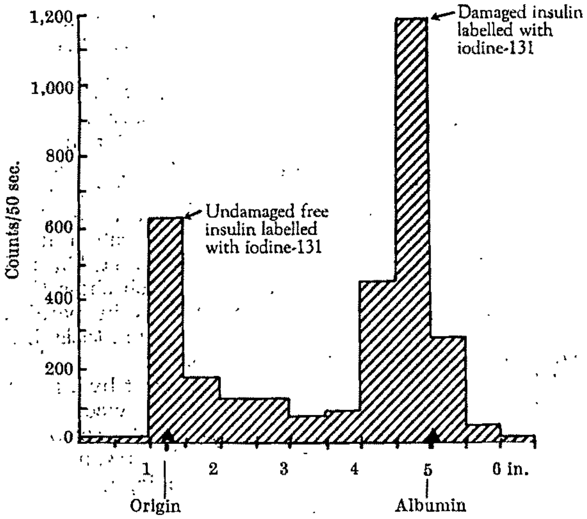

Fig. 1. Damaged insulin labelled with iodine-131 predominates on scanning the radioactivity of a paper strip after chromato-electrophoresis of the first fraction collected from a cellulose column, during purification of crystalline bovine insulin labelled with iodine-131 (specific activity 550 mc. iodine-131/mgm. insulin). Human plasma was present in this mixture

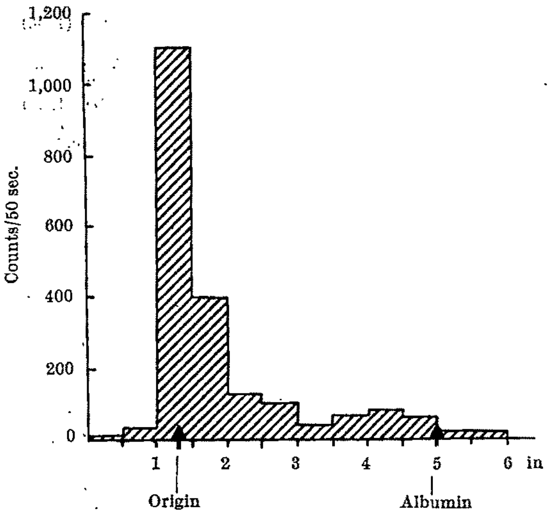

Fig. 2. Radio-chromato-electrophoretograms of a later eluate from the cellulose column, showing purified, 'free' insulin labelled with iodine-131 adsorbed at the origin

It has been suggested that damaged insulin binds indiscriminately to serum proteins and hence migrates away from the origin. We have found, however, that a solution containing damaged insulin, free of serum and albumin, shows identical migration of damaged insulin, as in Fig. 1. We have also separated the two fractions on cellulose acetate strips pre-soaked in non-radioactive insulin, confirming that damaged insulin has an electrophoretic mobility of its own.

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Radiation Effect on Yeasts of the Genus *Candida* Berkhout

So far investigations of radiation damage on yeasts were solely done on ascosporogenous yeasts of the genus *Saccharomyces* (Meyen) Reess, mostly on the species *cerevisiae* Hansen. Major radiation damage to this genus is known[1-4]. We irradiated an anascosporogenous yeast *Candida tropicalis* (Cast.) Berkh. with X-rays (1·54 Å.) and ultra-violet light. This genus is characterized by the ability to form pseudomycelium, that is, filaments similar to the hyphæ of moulds, but consisting of elongated cells which have originated by budding. Formation of pseudomycelium takes place when growth goes on and cell division (budding) is inhibited. By irradiation of *C. tropicalis* we found all the types of radiation damage discussed in refs. 1–4 and a new radiation effect, the formation of pseudomycelium. The equations of the regression-lines for this effect are: X-rays, $y = 3{\cdot}18 + 2{\cdot}78x$; $PPD_{50} = 3{\cdot}6$–$4{\cdot}47$–$5{\cdot}13$ kr. Ultra-violet light: $y = -3{\cdot}66 + 4{\cdot}7x$; $PPD_{50} = 64{\cdot}5$–$69{\cdot}7$–$74{\cdot}1$ sec.

PPD_{50} is the dose necessary to induce in 50 per cent of the irradiated cells the formation of pseudomycelium. It seems interesting to note that simultaneously with the formation of pseudomycelium, the cells lose their Gram-positivity to become Gram-negative instead. Gram-positivity is a function of ribonucleoproteins; by destroying or extracting them, the cells become Gram-negative. So, as a result of irradiation by ultra-violet light or X-rays (1·54 Å.), the metabolism of ribonucleic acid (RNA) is disturbed. In the case of X-rays, the formation of pseudomycelium results much earlier than the induction of lethal mutations, $PPD_{50} = 4{\cdot}47$ kr., whereas $LD_{50} = 10$ kr. So the first attack of X-rays on yeasts of the genus *Candida* is inhibition of cell division, expressing itself in formation of pseudomycelium coupled with damaging of the metabolism of RNA.

Coloration of the irradiated cells with Unna-Pappenheim-stain (after Brachet) confirms the extensive damaging of the metabolism of RNA.

A possible explanation that the first attack is to be found in the inhibition of budding, combined with the damaging of the synthesis of RNA, could be found in the fact that in both cases sulphydryl enzymes are involved.

W. J. Nickerson and G. Falcone[5] described evidence supporting the conclusion that the process of cellular division in yeasts is the result of a chain of events, beginning with transfer of hydrogen by protein-disulphide-reductase to —S—S— linkages in a polysaccharide-protein complex of the yeast cell-wall. Thus rupture of covalent bonds in this major component of the cell wall becomes possible. Following this hypothesis[5], the free radicals produced by irradiation would oxidize the SH-groups and so make budding impossible since pseudomycelium is formed. That this is the case is supported by the fact that by feeding certain sulphydryl-compounds to *Candida* for irradiation (*M*/300 thiourea in glucose-peptone-solution for 18 hr. under continuous stirring at 27°) all the radiation damage is greatly reduced.

This work forms part of a research work contract from the International Atomic Energy Commission, Vienna.

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BIOLOGY

A Rare Earless Monitor Lizard from Borneo

Lanthonotus borneensis Steindachner—the Borneo earless monitor lizard (which forms, with two American lizards, the family Helodermatidae) is known from less than ten specimens, all from Sarawak (three in Kuching, one in Singapore, the rest in American and British museums), none of which has been collected in recent times or with satisfactory field data. Thus, although the importance of this reptile's purely taxonomic characters in lizard-snake evolution has been examined in detail[1], no supporting information on behaviour has been available.

For some years this Museum has been associated with the Chicago Natural History Museum in a reptile-amphibian collecting project[2], under the general guidance of its curator, Dr. R. Inger. Particular attention has been devoted to a search for *Lanthonotus* throughout West Borneo. Not until January 1961 was any positive report obtained. Then, a live specimen measuring 13 in. (about average size to date) was obtained only a mile from our own archæological base camp at the Great Caves of Niah, centre of intensive reptile collecting since 1954.

After an initial period in which the lizard refused any sort of food and spent most of the time in a state resembling a coma (superficially 'dead') it then responded to the raw eggs of the green turtle, *Chelonia mydas*, the distribution of which in Sarawak happens to be controlled by this Museum[3]. It sucked raw turtle yolk every night for two months. Then circumstances made us bring it back to Niah, where fresh turtle eggs are not available. Chicken eggs were accepted as substitutes ; but the animal died on April 11, 1961.

In most of its behaviour it resembled a nocturnal snake. Though taken from a hole in the ground, the front legs are so weak that it is difficult to conceive of its burrowing with these. The strong snout and head were used to enlarge any ground weakness, however. A very good swimmer, it could relax on the bottom of a tank for up to half an hour. This capacity to relax was also a striking characteristic on land ; when resting it could literally collapse along the whole length of the body. Then, with very low, slow respiration it was difficult to decide whether it was alive. Evidently, in a natural state, it is able to remain dormant for long periods underground without food and very little air. This, plus nocturnalism and a capacity to carry about earth adhering to the nodular skin, make it most difficult to notice.

When handled, however, it would quickly flatten the body into semi-rigidity. It soon showed evidence of enjoying handling and became 'tame', in a way already known for the related 'Gila monster' *Heloderma suspectum*[4]. It showed no inclination to bite either the handler or anything else (including food). It seems unlikely, therefore, that it is poisonous as has often been suggested[5].

Detailed day and night observations and film records have been made[6]. Further search is continuing at Niah ; the results so far are negative.

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Growth Substances separated from the Root of Water Hyacinth by Paper Chromatography

EVIDENCE for the presence of growth substances in the root extract of water hyacinth (*Eichhornia speciosa* Kunth), presumably related to the rapid multiplication of the weed, has been reported previously from this laboratory[1]. A comparison of the effects of the extract with those of indole acetic acid or gibberellic acid indicated a different substance or substances present in the extract.

Paper chromatographic separation has now been attempted to reveal the nature of the active factors. 30 gm. of fresh root were extracted with chilled absolute alcohol at 0° C. for 18 hr. The extract was filtered and alcohol distilled off under reduced pressure. The aqueous residue was acidified with sulphuric acid to *p*H 3 and extracted with peroxide-free ether for 1½ hr. The ether-soluble factors were then partitioned into acidic and neutral fractions following Kefford[2]. The ether-insoluble substances were neutralized with barium hydroxide, filtered and evaporated to dryness. The dry mass was then dissolved in 0·5 ml. of distilled water and treated overnight with 0·5 ml. of absolute alcohol for chromatography. Acid and neutral ether fractions were evaporated separately and the dry mass dissolved in

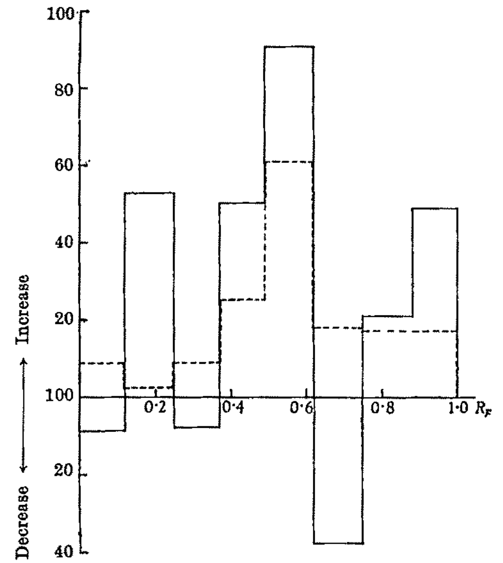

Fig. 1. Root growth (——) and shoot growth (- - - -) of rice seedlings treated with the ether-insoluble water fraction. Solvent: *iso*propanol/ammonia/water

1 ml. of dry peroxide-free ether or absolute alcohol. Three fractions were then chromatographed in Whatman paper No. 1 for 6–8 hr. with *iso*propanol/ammonia/water (16 : 3 : 1). After running the solvent for 20 cm., the air-dried filter paper was cut into 8 pieces and eluted overnight with distilled water in the cold. Root and shoot growth of the intact sprouted rice seed var. Chinsurah Boro in the eluates was used for the bioassay of growth substances. Chemical identification was sought from the R_F of chromatograms with different solvents: *iso*propanol/ammonia/water (16 : 3 : 1); benzene/acetic acid/water (4 : 1 : 2); alcohol/water (7 : 3) and *n*-butanol/acetic acid/water (4 : 1 : 1).

In the neutral ether fraction, no growth-promoting substance was detected either by bioassay or spot colour reaction. The acid-ether fraction did not show significant growth promotion in bioassay, but the colour reactions of the chromatograms run with *iso*propanol/ammonia/water were noticed at R_F 0·73–0·75, 0·76 and 0·91–0·99. The growth substance at R_F 0·73–0·75 was indole-3-acetic acid as confirmed by synthetic indole-3-acetic acid run with the extract in different solvents. The colour reaction at R_F 0·76 was obtained by potassium permanganate, but not with Salkowski or Ehrlich reagent, and was also inactive in the rice-seedling bioassay. The growth factor at R_F 0·91–0·99 appeared to be an indole compound as it gave a spot colour reaction with Salkowski and Ehrlich reagents. The ether-insoluble or aqueous fraction showed marked promotion of growth at three zones: R_F 0·12–0·25, 0·37–0·62 and 0·75–1·0 (Fig. 1). Inhibition of root growth was also observed at R_F 0·62–0·75. Positive Salkowski and Ehrlich reactions were given by the fractions with R_F 0·71–0·75 and 0·97–0·99, suggesting the presence of indole compounds. It is interesting to note that the substance present in the fraction with R_F 0·49–0·60 has marked growth-promoting activities both in the root and shoot of the test material. It gives a colour reaction with potassium permanganate like gibberellic acid but at widely different R_F. On the basis of these results it is concluded that the root extract of water hyacinth contains growth substances in addition to indole compounds or gibberellins.

Further work on the chemical nature and propertie of the substances is in progress in this laboratory.

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Life-span of the Fronds of the Giant Kelp, *Macrocystis pyrifera*

THE large brown attached alga, *Macrocysti pyrifera*, member of a widespread genus, is c considerable importance as the direct and indirec source of food for a large number of near-shor animals; it is also gathered by man, to be used a fertilizer, food additives, and it is processed fo certain of its chemical constituents. The frond develop continuously from growing points located o the perennial hold-fast at the base of the plant Little information exists about the life-span of th fronds, although this is a factor of considerabl importance in management of the resource and i computing the productivity of the kelp grove Authors have estimated the life-span as severa months[1,2]. Further information is now available a a by-product of work on rate of growth in severa kelp-beds spread over a geographical range of abou 150 km.

Individual fronds were tagged with numbered smal white plastic squares, by divers using self-containe underwater breathing apparatus (SCUBA)[3]. Tw tags were attached to each frond by a rubber ban loosely encircling the stipe; thus if one marke disappeared, replacement could be effected withou loss of the identity of the frond (in practice, howeve few tags were lost). Two or more fronds per plan were always tagged. It has always been possible t relocate the plant on subsequent visits, except whe the entire organism was removed by a storm or b concentrated grazing. Elimination of the paren plant was disregarded in the statistical calculation of the loss of fronds.

Results for eleven different time periods and thre locations are presented in Table 1. For work o rate of growth, fronds were tagged when quite smal 30–100 cm. long; measurements of length wer made at intervals as they developed. Experiment 1, 2, 4 and 5 show consecutive measurements o groups of fronds tagged at the beginning of eac series. For experiments 2 and 3 many of these tim intervals are lumped in Table 1 and only a few c the visits are represented. In preparing Table 1, frond was considered as being defunct when it ha lost the distal growing tip from which all its blade originate. Measurements have shown that althoug elongation can occur after loss of this apical blad overall growth-rates quickly slow down and no mo blades are produced (as was also observed in *Macro cystis integrifolia*[1]).

The first experiment in Table 1 summarizes long-term investigation that began with a larg number of young stipes. Experiment 4 in Table was started at about the same time, at Pt. Loma about 16 km. away. Both beds showed similar rate of mortality, but the Pt. Loma site was abandone after the final observations shown in experiment 4

Table 1. MORTALITY OF *Macrocystis pyrifera* FRONDS AT DIFFERENT SEASONS AND LOCATIONS

Experiment	Dates of observations		No. of intact tagged fronds		Elapsed time (days)	Loss (per cent)	Half-life (days)	Depth (m.)
	Initial	Subsequent	Initial	Subsequent				
(1) (La Jolla)*								
a	6/12/59	7/1/60	66	25	32	62	23	20
b	7/1/60	16/5/60	25	0†	131	100	—	20
(2) (La Jolla)								
a	9/5/60	20/7/60	9	8	72	11	400	20
b	20/7/60	22/8/60	8	3‡	33	63	24	20
(3) (La Jolla)	23/7/60	27/8/60	14	13	35	7	340	20
(4) (Pt. Loma)								
a	28/12/60	22/1/60	106	74	25	30	47	15
b	22/1/60	18/2/60	34	23	27	32	48	15
(5) (Punta Banda)								
a	28/6/60	26/7/60	49	24	28	51	27	2–16
b	26/7/60	11/8/60	24	18	16	25	38	2–16
c	11/8/60	2/9/60	14	7	22	50	22	2–10
d	2/9/60	23/9/60	7	2	21	71	12	2–10

* A composite sample of plants along a straight transect line 125 m. long; experiments 2 and 3 were conducted on 2 groups of plants along this same line, separated by about 100 m.

† 3 incomplete fronds were found remaining.

‡ These 3 fronds, although complete, seemed unhealthy, fragile, and brittle, and were heavily grazed and encrusted.

because of the encroachment of a horde of sea-urchins, *Strongylocentrotus franciscanus*, which destroyed all the plants at their bases. At La Jolla, in experiment 1, only 3 incomplete fronds remained after 162 days, and these bore evidences of heavy grazing and deterioration. Their lengths were 28, 32 and 35 m.; the stipes were so fragile and chewed that they broke in several places during recovery and measurement, although extreme care was used in handling. Had the fronds been complete, their lengths would probably have been increased by perhaps as much as 5–10 m. Growing conditions during the period of this experiment were favourable with respect to temperature and water clarity, throughout the water column, but a series of storms presumably took a considerable toll of both the Pt. Loma and the La Jolla fronds.

Experiments 2 and 3 describe late spring and summer observations on other groups of fronds when the ocean was comparatively calm. After 72 days, only 1 of the original 9 fronds in experiment 2 had broken, indicating a much slower rate of mortality than had been observed in the winter and spring in experiments 1 and 4. Subsequent to the final observation of this group, the fronds grew up into the warm water above the thermocline and rates of growth quickly decreased practically to nil. All the fronds took on an unhealthy appearance, involving loss of pigment, and became brittle. The mortality-rate increased markedly. A younger set of 14 fronds nearby, below the thermocline, constituting experiment 3, exhibited a very low rate of mortality and normal growth-rates during the period when the older fronds of experiment 2 were suffering heavy losses.

Experiments 5*a*–*d*, which were run consecutively in a very small bed in a cold upwelling area at Punta Banda, Baja California, illustrate a continuous attrition process, as was likewise demonstrated by experiments 1 and 4. The loss per unit of time was somewhat variable in this bed. The half-life expectation of the fronds ranged from 12 to 38 days (Table 1). These values compare with a winter half-life of 23 days at La Jolla (experiment 1) and of 47 to 48 days at Pt. Loma (experiment 4). The chief damaging factor in the Punta Banda bed was apparently an excessively thick and continuing encrustation of the bryozoan *Membranipora serrilanella*, which restricted growth, deformed the tissues, and induced brittleness and breakage by water movements.

It is apparent from the examples just cited that considerations of the life-span of kelp fronds can be quite complex. It is important to define accurately the terms used. In some circumstances the average age of a group may be considerably less than the maximum age. The age of our experimental fronds at the time of tagging may be safely estimated from our growth data as 1–2 months, hence the observations indicate that the maximum age of a frond in 20 m. of water under favourable growing conditions is about 6 months, confirming the values of Scagel[1] and Brandt[2]. The average age, however, may often be only 2–3 months.

If unfavourable conditions, such as the temperature destruction of the fronds in experiment 2, develop suddenly, the maximum age of a population of fronds may almost coincide with the average age, and may be substantially less than 6 months.

Support of this work by the California Department of Fish and Game and the State Water Pollution Control Board is gratefully acknowledged.

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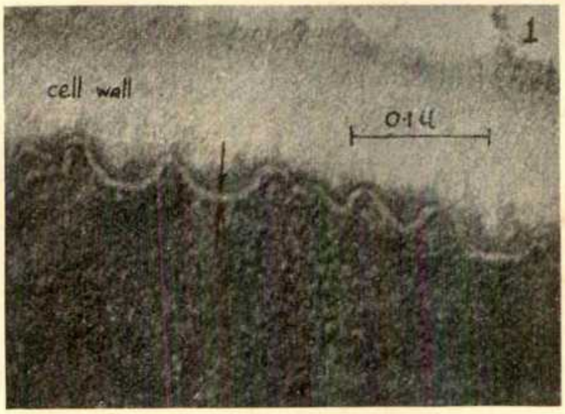
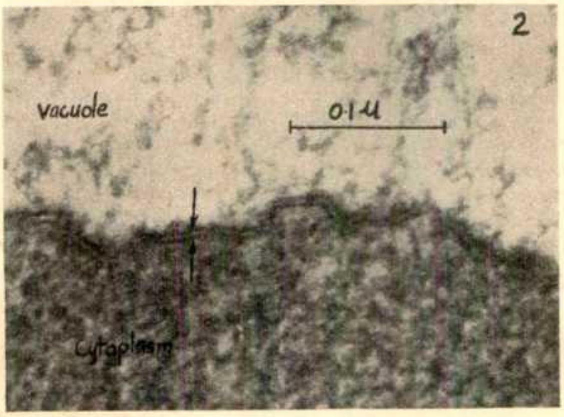
Method for revealing the Membrane Systems in Micro-organisms

THE use of potassium permanganate as a biological fixative for revealing the membrane systems of cells is well known to electron microscopists[1]. This fixative has been successful in preserving the fine morphology of bacteria and yeast; but, up to date, workers have failed to show the true nature of the membranes in these organisms[2,3]. This communication describes a method for stabilizing and increasing the electron density of membranes in micro-organisms by post-fixing them with a uranium salt.

The organisms are fixed in suspension with a 2 per cent solution of potassium permanganate buffered with veronal acetate. This is followed by washing them in several changes with a 2 per cent solution of uranyl nitrate and allowing them to remain in the





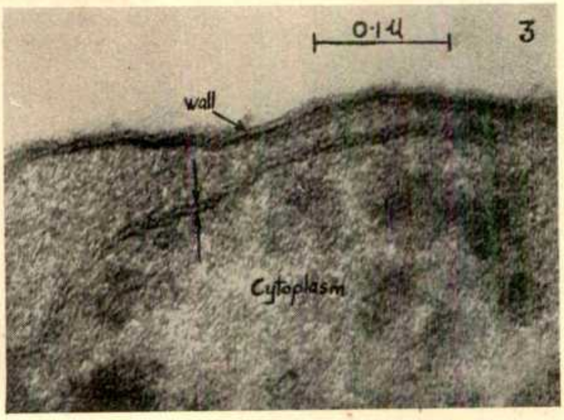


Fig. 1. Yeast cell wall and the cytoplasmic unit membrane
Fig. 2. Yeast vacuolar membrane
Fig. 3. Wall of *Rhizobium* and the limiting cytoplasmic membrane

final change for 1 hr. Washing, dehydration, embedding and sectioning are then carried out in the conventional manner.

Robertson[4,5] regards the unit membrane as two protein layers each about 20 Å. thick separated by a lipid layer approximately 35 Å. thick. The use of post-fixation has enabled me to resolve these layers in the nuclear membrane, cytoplasmic membrane, mitochondrial membranes and vacuolar membrane of yeast and has also enabled me to show a limiting cytoplasmic unit membrane in the bacterium *Rhizobium trifolii*.

In the three electron micrographs (Figs. 1–3) the components of the unit membranes are resolved. The two electron-dense lines represent the protein layers and the clear space dividing them the lipid layer.

I prefer to call the uranyl nitrate treatment a post-fixation since, fixing with permanganate followed by the staining of sections with uranium on the grid in the usual fashion fails to show the membranes satisfactorily. It appears that post-fixation retains something in addition to that retained by the permanganate alone.

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Function of the Caudal Appendage of *Priapulus caudatus*

THE caudal appendage of *Priapulus* consists of central hollow stalk from which numerous finger-like tubes project. The lumen of the appendage communicates with the voluminous cœlomic cavity by an opening surrounded by circular muscles[1]. Severa authors ascribe a respiratory function to the appendage[2,3], but other theories have also been proposed[4] The following observations may throw light on th function of the appendage.

Ten specimens of *Priapulus caudatus* were store overnight in 100 ml. sea-water. The next day th animals were motionless and probably asphyxiated but when new sea-water was added the animal started lively movements. At intervals the cauda appendage and its processes contracted vigorously (Fig. 1), injecting cœlomic fluid into the body cavity Shortly afterwards the appendage relaxed an cœlomic fluid streamed back into it. The streamin of cœlomic corpuscles could be observed through th transparent walls of the appendage. In othe experiments specimens of *Priapulus* were asphyxiate by bubbling nitrogen through the water. Also i these cases rhythmical contractions and relaxation of the caudal appendage followed the addition o aerated sea-water.

From the observations, it seems probable that th caudal appendage has a respiratory function. It

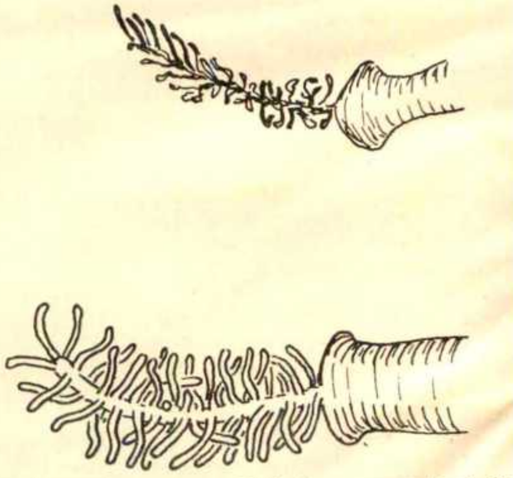

Fig. 1. *Priapulus caudatus*. Respiratory movements of the caudal appendage, contracted stage (above), relaxed stage (below)

thin walls allow a rapid uptake of oxygen, and the rhythmic muscular contractions bring oxygenated cœlomic fluid into the body cavity. The presence of a respiratory pigment in the cœlomocytes[5,6] makes the cœlomic fluid well suited for transport of oxygen. That the pigment is a hæmerythrin, as stated previously[5,6], was confirmed by a spectrophotometric examination of a hæmolysate with distilled water. The appearance of an absorption maximum at 4900 Å. agrees well with observations on *Sipunculus*[7,8].

In spite of its content of a respiratory pigment the oxygen capacity of the cœlomic fluid is rather small. By measurements with a van Slyke apparatus the value of 0·014 ml. oxygen per ml. fluid was obtained. For sea-water the value 0·006–0·007 was found. The oxygen dissociation properties of cœlomic fluid was studied by a crude colorimetric method. Half saturation was obtained at an oxygen tension of 60 mm. mercury, which indicates a slowly sloping dissociation curve. However, the measurements were made at 19° C., which is above the temperature of the natural environment of *Priapulus caudatus*.

It is an unsolved problem how respiratory movements of the caudal appendage can be triggered by an increase of oxygen tension in the surrounding sea-water. Probably, the movements are initiated from the nervous system, because the muscles of the appendage are innervated from the abdominal nerve trunk[1].

We are grateful to Dr. Knut Schmidt-Nielsen of Duke University, Durham, North Carolina, who made the van Slyke analyses.

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ANATOMY

Properties of Stressed Bone

PROF. DREYER's communication[1] raises a matter of considerable interest and importance. Engineers know that all materials react similarly on broad principles. That is, they obey Hooke's law, within wide ranges, of course, and conform to other physical changes under stress such as Poisson's ratio.

There is no reason to doubt that bone material differs in principle from other created material. As such it should be, and probably is, elastic up to a yield point. Experiments on living bone may be difficult, but if Young's modulus for bone tissue, or the range of elastic limit, could be determined it would be most helpful. Moreover, its subsequent behaviour beyond that point up to fracture should be established, and would then provide an answer to the problem under investigation by Prof. Dreyer.

Moreover, the amount of compression and extension under stress, as well as the degree of bending under torque, or eccentric loading, are clearly forms of bone characteristics that ought to be known. Naturally the reactions on colour referred to by Prof. Dreyer are a measure of the reaction set up to applied stresses, and are familiar to every engineer. 'Perspex' and celluloid, in the form of full-size models, indicate changes in colour under stress, and show the pattern and distribution of stresses set up under applied loads. They can also be used to reveal the effects and redistribution of stress under fatigue due to repetitive loading. A breakdown then occurs of normal strength leading to failure at a much lower stress. This, of course, is of considerable importance with a lowering of the elastic limit by age.

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THE problem that needs elucidation is to determine the nature and origin of the osteoblastic and osteoclastic stimuli which, when associated with a normal metabolism, will influence the deposition or removal of bone.

These stimuli may originate as a result of function or non-function of a bone. It is therefore tempting to suggest that stress produces some change in the inorganic or organic portion of the bone. The stress is then converted to a stimulus which affects the neighbouring osteogenic tissue.

The crystalline structure of bone had led us to believe that these stimuli may be electrical. The piezoelectric properties of bone have been investigated, but so far my results have not been encouraging. Thus when I found the alteration in staining properties and birefringence of bone as a result of stress I felt that these changes may well be related to the origin of locally produced osteoblastic and osteoclastic stimuli in bone.

I am not sure that the phenomenon that I have described is quite comparable with the changes seen in colour in stressed 'Perspex', celluloid, transparent 'Balelite' and 'Araldite' (Ciba).

When these materials are stressed with mild forces the changes in colour occur almost immediately and conversely disappear as soon as the force is released. My *in vitro* experiments show that the birefringent portion of the bone does not, even after 24 hr., revert to its unstressed state when the stress is removed; yet *in vivo* this must be a reversible process. Of course, the picture with massive forces and the influence of temperature is an entirely different matter.

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Influence of Large Doses of Œstrogens on the Structure of the Bones of Some Reptiles

IT was first shown by Kyes and Potter[1] that, during reproductive activity, the marrow cavities of the bones of female pigeons become filled with endosteal bone. It is thought that this medullary bone is formed under the synergistic action of the androgens and œstrogens[2] which are produced in the female bird during the breeding season. Similarly, injections of œstrogens induce the formation of medullary bone in male birds. Medullary bone differs from cortical bone histologically[3,4], and it appears to act as a labile calcium store which can be mobilized rapidly during the formation of the eggshell.

Although medullary bone has been found in all species of birds which have been investigated it has only been possible to demonstrate it in one species of mammal, the mouse[5], although six other species have been investigated. Medullary bone never occurs naturally in mice and there is in fact evidence to suggest that even when it is induced experimentally it differs from that which occurs normally in birds[6].

The question arises therefore whether the early reptiles possessed medullary bone, and the ability to form it has been lost in mammals with the loss of the calcified eggshell; or whether the ability to store calcium in this way has been evolved only in the birds.

Some answer to this question is provided in the recent work of Edgren[7], who found that there was a fall in the density of the bones of female turtles during the reproductive season. This indicates that calcium is withdrawn from the long bones of the limbs to provide calcium for the eggshell. If calcium were stored before the formation of the eggshell, this would have been detected as an increase in the density of the bones, but this was not found. This would appear to indicate that there is no formation of medullary bone which could act as a special store of calcium, but that the cortical bone is resorbed during the formation of the eggshell.

The work reported here concerns two observations which are pertinent to the scheme implied by Edgren's work. A male eastern painted turtle, *Chrysemys picta picta*, was caught, in Georgia, United States, during August and given an initial injection of 1 mgm. of œstrone (10,000 International Units) in 1 ml. of peanut oil. The animal was then given daily intramuscular injections, into alternate hind limbs, of 5,000 I.U. of this hormone for 12 days and it was then killed. At death it weighed 200 gm., of which the shell comprised 63·5 gm. The femurs were cleaned and fixed in 5 per cent formalin. Pieces of bone were decalcified and sectioned.

A similar experiment was performed on a female gopher tortoise, *Gopher polyphemus*, which at death weighed 350 gm., of which the shell comprised 169·5 gm. Although the ovary contained ova of up to 1 cm. in diameter, there were no eggs in the oviduct. The femurs were again examined histologically.

The bones from these animals contained many trabeculæ, possibly related to their curved shape. There was no medullary bone present. Methylene blue extinction values were taken at various *p*H-levels using barbitone buffers[8]. In both cases the bone had values of about *p*H 4, whereas avian medullary bone has a value below *p*H 3 (ref. 4).

The experiments described must be regarded as preliminary ones, but they provide evidence from an entirely independent source to confirm the system suggested by the work of Edgren. Thus it appears that these Chelonia do not form medullary bone under the influence of the sex hormones and do not store calcium before egg-laying. There is therefore a definite difference in the calcium metabolism of these reptiles and the birds.

It is a pleasure to acknowledge the assistance of Hugo Greiner and Miss E. M. H. Wilson in these experiments. The work reported was performed in the Anatomy Department, Emory University, Georgia, United States, through the hospitality of Prof. G. H. Bourne and during the tenure of a Lalor Foundation research award for the summer of 1960 and a Wellcome research travel grant.

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IMMUNOLOGY

Partial Inhibition of Antibody Formation by means of Anti-Ribonucleoprotein Serum

NUCLEOPROTEINS seem to be effective antigens[1], and anti-nucleoprotein sera produced in rabbits exhibit a definite cytotoxic activity on the cells of different lines grown in tissue culture[2]. On the other hand, it is generally believed that ribonucleic acid and ribonucleoprotein are instrumental in protein synthesis, and that these cell constituents provide also the patterns for *de novo* production of highly specific proteins, such as immune antibodies. It may be deduced, therefore, that the capacity of antibody-protein formation can be affected by the action of an anti-nucleoprotein serum. The present experiment was done in order to investigate the immunological events which are associated with the activity of anti-ribonucleoprotein and anti-deoxyribonucleoprotein sera.

Ribonucleoprotein-rich fraction[3], 11·0–15·0 mgm./ml. of protein, 45–70 γ/ml. of deoxyribonucleic acid (DNA), and 1,300–1,600 γ/ml. of ribonucleic acid (RNA) and deoxyribonucleoprotein-rich fraction[4] (9·2–11·6 mgm./ml. of protein, 975–1,250 γ/ml. of DNA, and 300–420 γ/ml. of RNA) were isolated from Rhode Island Red chicken organs (spleen, liver and lung). Immune sera were prepared by injecting chicken ribonucleoprotein and deoxyribonucleoprotein into rabbits. Two three-injection series were performed, with an interval of 10 days between them (3 ml. of nucleoprotein per injection subcutaneously). Ten days after the last injection the rabbits were bled and γ-globulin fraction (96·25 mgm./ml. of protein) of anti-ribonucleoprotein serum, and γ-globulin fraction (76·37 mgm./ml. of protein) of anti-deoxyribonucleoprotein serum were prepared as described by Bassett *et al.*[5]. The γ-globulin fraction (89·31 mgm./ml. of protein) of normal rabbit serum was also used in the experiments. Rabbit antisera were investigated for the presence of specific antibodies by diffusion-gel technique and complement fixation[6]. The supernatants obtained by centrifuging at 3,000 r.p.m. for 30 min. chicken nucleoprotein fractions served as antigens. The supernatant of ribonucleoprotein contained 1,915 γ/ml. of protein, 25 γ/ml. of DNA and 450 γ/ml. of RNA, and that one of deoxyribonucleoprotein 2,065 γ/ml. of protein, 900 γ/ml. of DNA and 160 γ/ml. of RNA. Chicken ribonucleic (RNA) and deoxyribonucleic (DNA) acids were also used in complement fixation. In the agar-diffusion reaction a maximum of five zones was observed with

protein and corresponding anti-
well-defined precipitin lines with
onucleoprotein and anti-deoxyribo-
serum. Normal rabbit serum did not
gar medium with chicken nucleoproteins.
anti-ribonucleoprotein serum in comple-
on were 0–1/2 with RNA and 1/64–1/128
ucleoprotein, and those of anti-deoxyribo-
otein serum were 1/8–1/32 with DNA and
28 with deoxyribonucleoprotein.

ode Island Red chickens were divided into three
oups: the first group received γ-globulin of anti-
ucleoprotein serum, the second group γ-globulin
nti-deoxyribonucleoprotein serum, and the third
up γ-globulin of normal rabbit serum (control). A
rst injection (0·3 ml. of corresponding γ-globulin)
was given to 6-day-old embryos. The mortality-rates of embryos were 60·7 per cent for the first, 66·7 per cent for the second and 57·2 per cent for the control group. The other four injections were performed on chickens at the age of 32, 35, 38 and 41 days (1 ml. of corresponding γ-globulin per injection intravenously). After a rest of one day, all three groups were injected intravenously with 1 ml. of a 10 per cent suspension of human *O* red cells. Three days later the first group of chickens received the last 1 ml. of anti-ribonucleoprotein, the second group 1 ml. of anti-deoxyribonucleoprotein and the third group 1 ml. of normal rabbit γ-globulin. Seven days after the final injection, the chickens were bled for the first time, and further bleedings were performed every 7 days. A semi-quantitative agglutination reaction was used to test the presence of agglutinins for human *O* red cells in chicken sera.

Table 1. EFFECT OF RABBIT ANTI-NUCLEOPROTEIN SERA ON HÆMAGGLUTININ PRODUCTION IN CHICKENS RECEIVING ONE INJECTION OF HUMAN *O* RED CELLS AT THE AGE OF 42 DAYS

Days after red cell injection	Chickens treated with γ-globulin from anti-deoxyribonucleoprotein serum	anti-ribonucleoprotein serum	Normal rabbit serum (control)
7	7·52 (10)	6·47 (13)	7·72 (15)
14	6·65 (9)	5·86 (13)	7·05 (15)
21	6·32 (8)	5·23 (12)	6·53 (14)
28	5·69 (8)	4·65 (10)	6·09 (10)
35	4·82 (8)	4·43 (10)	5·43 (10)

The values represent mean peak agglutinin titres ($\log_2$). In parenthesis is the number of chickens tested.

The results are presented in Table 1. The rates of appearance of hæmagglutinins in two experimental groups of chickens are similar to the control one. Peak agglutinin titres in the sera of chickens treated with anti-deoxyribonucleoprotein serum were lower than those obtained in control group, but not so low that the difference observed can be considered significant. However, partial suppression of agglutinin production occurred among the chickens injected with anti-ribonucleoprotein serum.

Although the mechanism by which anti-ribonucleoprotein serum causes a decrease in antibody-forming capacity is not clear, it may be postulated that its action is rather at the level of nucleoproteins and cellular membrane (Colter *et al.*[2]). Since the metabolic activity of antibody-forming cells is accompanied by an increased amount of ribonucleic acid[7], it may also be supposed that the anti-ribonucleoprotein serum exhibits an indirect effect on ribonucleic acid, by acting on nucleoproteins which are bound with this nucleic acid. This view seems to be supported by the observation made in the present work, namely, ribonucleic acid scarcely showed antigenic properties, while its protein component reacted rather well with anti-ribonucleoprotein serum. It must be pointed out that the promising results obtained in the present experiments are preliminary ones, and that further investigations are planned in which more specific techniques will be used, including the tissue culture of antibody-producing cells.

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MICROBIOLOGY

Formamidase in Mycobacteria and its Use in differentiating Saprophytic Mycobacteria from other Mycobacteria

RECENTLY, biochemical methods have been proposed to differentiate various types of mycobacteria without resorting to their growth characteristics or animal pathogenicity. Thus human tubercle bacilli can be distinguished from all other types of mycobacteria by determining their nicotinic acid production (Konno's niacin test)[1]. Bovine and avian tubercle bacilli would be differentiated from other mycobacteria by determining the activity of nicotinamidase[2] and urease[3], respectively. As for the other types of mycobacteria, namely, unclassified mycobacteria and saprophytic mycobacteria, however, such a reliable method has hitherto not been reported. Bönicke[4] and Kitamura[14] attempted to sub-divide unclassified mycobacteria by the amidase activity of bacterial suspension.

In this communication, we report the occurrence in mycobacteria of an enzyme which catalyses the formation of ammonia from formamide. This enzyme activity was found, among various mycobacteria tested, only in saprophytic mycobacteria. Amidase activity towards formamide has not so far been demonstrated with various preparations, such as rabbit liver, yeast, and *Torula utilis*, and assumed to be non-existent[5]. Halpern and Grossowicz suggested its occurrence in some mycobacteria[6]; but no other report has appeared at present on this enzyme. The natural production of formamide was reported in 1955 as the final product of histidine metabolism in *Aerobacter aerogenes*[7] and *Clostridium tetanomorphum*[8].

Twenty-three strains of various mycobacteria were grown on the surface of Sauton's synthetic liquid medium for 3 weeks (one week for saprophytic mycobacteria). The pellicles were filtered off, and washed four to five times with water. The cells (about 3 gm.

wet weight) were ground in a mortar by hand with equal amount of sea-sand for 30 min. Cell-free extracts were prepared by extracting the paste with five volumes of water for 10 min. and centrifuging at 10,000*g* for 40 min.

The reaction mixture contained 0·5 ml. of the cell-free extracts (approx. 2 mgm. protein), 10 µmoles of formamide, 1·0 ml. of 0·05 *M* phosphate buffer (*p*H 7·0) in a total volume of 2·0 ml. Solutions of enzyme without substrate and substrate alone were used as controls, which were essential. The mixtures were incubated for 4 hr. at 37°. The ammonia formed was determined colorimetrically by Imai's modified procedure[9] of Russel's phenol-hypochlorite method[10].

Among 23 strains tested, the activity of this enzyme was observed only with extracts from saprophytic mycobacteria, as shown in Table 1. With other types of mycobacteria, no activity was detected. Thus, it might be possible to distinguish saprophytic mycobacteria from all other mycobacteria by determining this enzyme activity.

Table 1. FORMAMIDASE ACTIVITY OF EXTRACTS FROM VARIOUS MYCOBACTERIA

Type	Strain	Activity (NH_3 formed, µmoles*)
Human tubercle bacilli	*H37Rv*	0
	H37Rv–INH–R	0
	H37Ra	0·2
	Aoyama B	0
Bovine tubercle bacilli	Bovine D_4	0
	Bovine Miwa	0
	BCG (Japan)	0
	BCG–INH–R	0
Avian bacilli	11755	0
	4110	0
Unclassified mycobacteria	*P*–1 (Photochromogens)	0
	P–8 (Photochromogens)	0
	P–6 (Scotochromogens)	0
	100616 (non-photochromogens)	0
	Kofu (non-photochromogens)	0
	M. fortuitum	0
Saprophytic mycobacteria	*M. phlei*	5·7
	M. smegmatis	7·8
	M. 607	10·0
	Asami	9·5
	Guinea pig I	10·1
	Soil III	7·3
	M. takeo	8·6

Substrate, formamide 10 µmoles; *p*H 7·0; incubation at 37° for 4 hr.

* Values are corrected for controls: substrate only and enzyme blank.

Some properties of the enzyme were examined with the preparation from saprophytic *Mycobacterium* 607. This preparation split 79·3 per cent of substrate within 2 hr. under the above assay condition. The rate of the reaction remained constant for more than 2 hr. At a concentration of 5 µmoles formamide per ml. the rate was almost maximum. Dialysis against distilled water had no effect on the activity.

The enzyme solution showed a considerable activity, besides formamide, towards nicotinamide, pyrazinamide, benzamide, *iso*-nicotinamide, L-asparagine, and urea; only weak activity towards L-glutamine, glycinamide, and acetoamide.

The enzyme activity towards formamide was observed over a wide *p*H range (6·0–9·0), with an optimum at *p*H 7·0–8·0. The *p*H activity curve was, though similar to that of nicotinamidase activity, slightly but definitely different from that of benzamidase activity, and markedly different from that of urease activity. This fact seems to suggest that the enzyme might be different from urease, and possibly from benzamidase.

Heating the enzyme solution [illegible] 10 min. abolished about 80 per ce[illegible] activity towards nicotinamide, pyra[illegible] amide, and *iso*-nicotinamide to the sam[illegible] none of the activities towards formamid[illegible] and urea was impaired. Heating at 60° [illegible] complete loss of the aromatic amidase acti[illegible] as asparaginase without affecting the activit[illegible] formamide and urea. (By this heat-treatm[illegible] *p*H curve for formamide was not affected.[illegible] enzyme activity was destroyed by heating at [illegible] These results render it probable that the enzyme responsible for formamide is not identical with any of these known amidases.

Evidence for this hypothesis was provided by t[illegible] mixed-substrate method[11]. Further addition o[illegible] nicotinamide, benzamide, asparagine, or urea to an enzyme system already saturated with formamide increased the rate of ammonia production additively. This indicates that these added substrates are hydrolysed by different enzymes from that concerned with formamide.

Our results seem to indicate that the activity observed towards formamide is due to a new, distinct enzyme which we propose to call 'formamidase'. We are aware that kynurenine formamidase (formylase) is called sometimes by this rather misleading name[11]; but as for this enzyme we think it is more appropriate to call it either 'kynurenine formamidase' as used at present[12] or simply 'formylase' as originally proposed[13]. The name 'formamidase' should be reserved strictly for the enzyme specific for formamide.

This work was aided by a grant from the Committee on Medical Research of the American Thoracic Society, medical section of the National Tuberculosis Association.

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ENTOMOLOGY

Resting Habits of *Anopheles gambiae* and *Anopheles pharoensis* in Salt Bush and in Crevices in the Ground

THE failure to eradicate *Anopheles gambiae*, the most important vector of malaria in tropical Africa, by spraying the inside of houses with insecticides, had directed attention to the importance of alternative resting places. Box shelters[1] around cattle enclosures were used to assess the numbers of *A. gambiae* biting cattle during an experiment to break malaria transmission in the Pare-Taveta area of East Africa. Catches in the boxes, in the village of Bumba, showed peaks that corresponded to the dry months June–October, and were thought to be due to the artificial shelters competing favourably against the nearby vegetation as resting places. This is supported by complementary catches in natural resting places outdoors at Bumba between September and June.

Searches, for a total of 333 man/hours, were made in grass, foliage of trees, cacti (*Opuntia* sp.) earth banks, crevices in the ground and salt bush (*Suaeda* sp.) within a radius of three hundred yards of the village, and the mosquitoes collected by sucking tubes. The results are summarized in Table 1. Salt bush and crevices in the ground were the chief sources of *A. gambiae* and were studied in detail; for *Anopheles pharoensis* grass was also an important resting place. The salt bush covered an area of approximately 1,500 sq. yd., stretching along two sides of the village near the huts and cattle enclosures and then beyond for 300 yd. Where the villagers had taken branches for firewood and for enclosure walls, parts of the bush were thin and sparse, but other parts had been left to grow thick to give cover for places used as latrines. The terrain between the village and rice swamps comprised grassland in which deeply creviced patches of bare ground appeared during the dry season, but which were usually absent during the rainy season, November–May. The catches included *A. gambiae* and *A. pharoensis* in all stages of ovarian development, but recently-fed mosquitoes predominated. Precipitin tests on both species, carried out by Mr. B. Weitz, of the Lister Institute, London, showed that specimens from salt bush and crevices in the ground had fed almost entirely on cattle.

Table 1. AVERAGE NUMBER OF *A. gambiae* AND *A. pharoensis* CAUGHT PER MAN/HR. IN DIFFERENT RESTING PLACES

Resting place	Wet season (November–May)		Dry season (June–October)	
	A. gambiae	*A. pharoensis*	*A. gambiae*	*A. pharoensis*
Thick salt bush	3·6	0·1	0·4	0
Thin salt bush	0·3	2·1	—	—
Crevices near village	0·03	0	36·6	0·5

A. gambiae was found in greatest numbers in salt bush in which the foliage was dense. The characteristic resting place was in the large salt bushes, on the main branches inside the cavity produced by the arching of the branches as they inclined to the ground. Thin salt bush, either young bushes or new growths from old bushes that had been cut down, was little used as a resting place. In the dry season, dense salt bush lost much of its luxuriant growth and became ess suitable as a resting place. Thus the shortage of natural outdoor resting places in the vicinity of the box shelters during the dry season accounted for the seasonal increase in the catches of *A. gambiae* in the boxes. The shortage of outdoor resting places in the dry season was reflected again in the high concentra-ion of *A. gambiae* in a relatively small area of reviced ground about 300 yd. from the boxes. This inding also shows that there was a seasonal change of esting place depending on the availability of dense alt bush in the wet season and crevices in the dry eason.

A. pharoensis was quite different in its resting habits and was found in greatest numbers in thin salt bush during the wet season, where it rested on the outer leaves of the veegtation. Small numbers of *A. pharoensis* were found in crevices as well as in grass during the dry season, and it was noted that they rested higher up the crevices than *A. gambiae*.

After establishing that dense salt bush was the principal resting place of *A. gambiae* in the wet season, the bush was cut down at the end of June. Subsequent catches in the box shelters showed that the peak in them was transposed to the wet months[2], thus confirming that, in the absence of nearby natural resting places outdoors, *A. gambiae* entered artificial shelters in large numbers in areas where many cattle were penned outdoors at night.

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Members of the *Culex pipiens* Series in New Caledonia and the New Hebrides

IN 1952–54, during studies of South Pacific mosquitoes and the ecology of their larval habitats, *Culex* (*Culex*) *pipiens fatigans* Wiedemann was recorded from numerous localities[1]. At that time, *C. pipiens australicus* Dobrotworsky and Drummond[2] was known only from Australia. However, this sub-species has since been reported from New Caledonia[3] and the New Hebrides[4], although the latter record is queried in a recent review[5]. Furthermore, another member of Edward's *Culex pipiens* series, *C. iyengari* Mattingly and Rageau, has now been described from the Loyalties and New Caledonia[6].

Accordingly, all specimens identified as *C.p. fatigans* in the account of my investigations[1] have been re-examined. The material still proves referable to this sub-species, with three exceptions, the collection numbers quoted being those of the account referred to:

Collection 2 (in part), ex whaling try pot, Anelgauhat, Aneityum Id., New Hebrides, August 3, 1952.
Collection 49, ex pot hole in rock, Uala, Art Is., Belep Is., New Caledonia, November 28, 1952.
Collection 56 (in part), ex 44 gallon drum, Poum, northern New Caledonia, December 3, 1952.

All fourth instar larvæ of collection 2 have four pairs of subventral tufts on the siphon, which in most cases tapers most pronouncedly in its distal half, its index being 4·2–4·4. Such larvæ are regarded as undoubted *C.p. fatigans*, although their antennæ are unusually long—0·45–0·52 mm., as compared with a New Caledonian figure of 0·41–0·44 mm. (ref. 3) and 0·41–0·43 mm. in examples from Polynesia (Samoa and Aitutaki) in my own collection. Seven others have an evenly tapered, very slightly curved siphon, its index ranging from 5·8 to 6·2, and their antennæ are 0·55–0·63 mm. long. In all respects other than siphonal curvature, which is characteristic of the Australian type strain of *C.p. australicus*[2], they agree with the New Caledonian larvæ of this sub-species examined by Marks and Rageau[3].

Iyengar's[5] doubt as to the occurrence of *C.p. australicus* in the New Hebrides presumably stemmed from the low siphonal index (4–4·5) given by Rageau and Vervent[4] for their material from Espiritu Santo. The presence of the sub-species in the southern part of the Group, at least, may be due to human agency, for Anelgauhat was formerly a watering point for ships[7].

Collection 56 includes both *C.p.fatigans* and larvæ in which the siphon has a distinct sinusoidal flexure, an index of 5·0–6·1, and only three pairs of sub-ventral tufts. These features, in combination with the length of the antennæ (0·48–0·52 mm.), identify them as *C. iyengari*[5,6]. All specimens of collection 49 (originally assigned to *C.p. fatigans* with some reluctance[8]) are also referable to *C. iyengari*. This species was previously known from an outlier of New Caledonia, the Isle of Pines, as well as from the mainland and the Loyalty Group. Its range is now extended to the Belep Islands, Art lying as far to the north-west of New Caledonia (approximately 30 miles) as the Isle of Pines does to the south-east.

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VIROLOGY

Negative Evidence against Passage of Whole Plant Virus Particles through Gut Wall in Aphid Vectors

IN transmission of so-called persistent aphid-borne plant viruses, an incubation period in the insect is generally observed. It is understood that this time is required for the virus circulation in the insect body. Virus taken up with the plant juice is supposed to penetrate the intestinal wall and later the wall of the salivary glands, transport of the virus from the former to the latter being brought about by the blood. This theory is supported strongly by the fact that blood from infective aphids injected into non-infective individuals has been shown to make the latter able to transmit the virus in question. This has been demonstrated for potato leaf roll virus[1–3] and (Ossiannilsson, F., unpublished), beet yellow-net virus[3], and pea enation mosaic virus[4].

Nothing is known, however, about the form in which the virus is thus moving in the body of the vector. From work found in the literature on these matters one is apt to get the impression that the virus is supposed to pass through the insect tissues in the shape of complete molecules, that is, particles like those observed by electron microscope examination of infective plant sap or purified virus preparations. The size of these particles is, however, extremely large, as we know. The molecules of normal protein are very much smaller, but nevertheless the protein has to be broken down by the digestive enzymes before absorption by the mid-gut cells can take place. Since phagocytosis of food particles by the mid-gut cells is unknown in insects[5], a penetration of the intestine wall by intact plant virus particles would be possible only if there existed special pores or ducts through this wall. Results of recent experimental work[6] indicate the presence of capillary leaks of at least 120 Å. radius in the blood-lymph barrier in *Canis*. Analogous conditions might possibly be present in some part of the alimentary canal of aphids. This point does not seem to have been investigated so far, in spite of its significance in the present connexion.

In order to find out whether any such ducts do exist, I have examined the ultrastructure of the wall of the alimentary canal of *Myzus persicae* (Sulzer). These investigations were pursued for two years (1958–60) and concerned four parts of the intestine, namely, the œsophagus, the stomach, the posterior slender part of the mid-gut, and the hind-gut. The aphids were dissected under water, and the parts to be investigated were fixed in osmic acid and embedded in 'Methacrylate'. Thin sections were made by aid of a Philips ultra-microtome and examined in the electron microscope. The results of this work yield a negative answer to the question in point. No ducts whatever through the intestinal wall have been observed, with the exception of tracheoles. It is very difficult to see how the latter could possibly take any part in transport of virus, of course. The intestinal wall is throughout very compact, and a passage of bodies with the dimensions of, for example, 32 × 300 mμ (particles of beet yellow-net virus[7]) through this wall appears to be out of the question. The base membrane of the posterior part of the mid-gut is 0·1–0·3μ in thickness, and no pores could be observed. Moreover, the fore-gut as well as the hind-gut are lined in the interior by a cuticular intima. This is certainly very thin (in the œsophagus <0·02μ) but devoid of pores visible at a magnification of ×27,000. After treating the hind-gut with 10 per cent potassium hydroxide, examination in the electron microscope revealed a reticulate structure of the intima, recalling conditions in the rectal cuticle of *Calliphora*[8], but in this region, too, no open holes or pores could be established.

In work[9] on the ultra-structure of the salivary glands of *Myzus persicae*, Wohlfarth-Bottermann and Moericke established that the basement membrane enclosing these glands, as well as the salivary ducts, has a very dense microstructure, apparently making penetration by virus particles from the hæmocœle into the salivary glands impossible. The conclusion of these authors is that the virus must be assumed to enter the glands in the shape of smaller units. The same conclusion seems to be applicable also for the transport of virus through the wall of the gut, according to the results summarized here.

This work was supported financially by the Swedish Natural Science Research Council.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, June 26

University College (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Dr. Severo Ochoa (New York University School of Medicine): "Propionic Acid Metabolism in Animal Tissues".*

Monday, June 26—Wednesday, June 28

National Physical Laboratory; National Engineering Laboratory; and the Building Research Station (at the National Physical Laboratory, Teddington, Middlesex)—Conference on "The Control of Noise".

Wednesday, June 28

Bone and Tooth Society (at 234 Great Portland Street, London, W.1), at 4.15 p.m.—Annual General Meeting, followed by a programme of Casual Communications.

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. N. L. Flacon: "Major Earth Flexuring in the Zagros Mountains of South-West Iran"; Dr. M. H. P. Bott: "Geological Interpretation of the Magnetic Anomalies over the Askrigg Block".

Medical Research Council (at the National Institute for Medical Research, Mill Hill, London, N.W.7), at 5 p.m.—Dr. Severo Ochoa (New York University School of Medicine): "The Biosynthesis of Ribonucleic Acid".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Research Assistant in the Department of Geology, for work on hydrated silicate melts and glasses—Prof. F. W. Shotton, University of Birmingham, Edgbaston, Birmingham 15 (June 28).

Assistant in the Departments of Logic and Metaphysics and Moral Philosophy in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (June 30).

Assistant Lecturer in Psychology—The Registrar, The University, Manchester 13 (June 30).

Assistant Lecturer (with a good honours degree or equivalent together with industrial experience, and preferably an interest in research) **in Mechanical Engineering**—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11 (June 30).

Lecturer (with experience in electrical measurement or measurement and control) in Electrical Engineering—The Secretary, Queen's University, Belfast (June 30).

Lecturer (with experience in the use of digital computers, preferably in applications to crystallography or business calculations) **in the Computing Laboratory**—The Secretary of University Court, The University, Glasgow (June 30).

Organic Chemist (recent graduate, or undergraduate now taking final examinations) **in the Nutrition Department**, for work on the synthesis of substances of biological interest—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (June 30).

Research Assistants (2) (with a first- or second-class honours degree in mathematics) **in the Mathematics Department**, to work in one of the following fields: hydrodynamics, elasticity, numerical methods and computing—The Clerk to the Governors, Woolwich Polytechnic, Woolwich, London, S.E.18 (June 30).

Scientific Assistant, Abstractor (with a degree in agriculture or natural science, and able to read Russian)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire (June 30).

Assistant Lecturer in the Department of Biochemistry—The Dean, St. Thomas's Hospital Medical School, London, S.E.1 (July 1).

Junior Research Fellow in Pure Mathematics—The Registrar, The University, Sheffield (July 1).

Research Assistant (Biologist or Chemist), to assist in research on the tank culture of bivalve shellfish at Poole, Dorset, under the supervision of the Professor of Zoology—The Secretary and Registrar, The University, Southampton (July 1).

Junior Lecturer in Organic Chemistry—The Registrar, Trinity College, Dublin (July 3).

Assistant (with a first- or second-class honours degree in astronomy, mathematics or physics, and preferably an interest in radio astronomy) **in Astronomy**—The Secretary of University Court, The University, Glasgow (July 7).

Junior Lecturer in Mathematics—Prof. E. C. Titchmarsh, Mathematical Institute, The University, 10 Parks Road, Oxford (July 7).

Research Fellow (preferably organic chemist with a Ph.D.) in the College of Science and Technology (Department of Chemistry), for work in connexion with the development of organic dye filters which will be used for nebular and cometary night-sky photography for astronomical purposes—The Registrar, Manchester College of Science and Technology, Manchester 1 (July 8).

Chair of Microbiology in the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, July 15).

Lecturer or Assistant Lecturer (with qualifications in mathematical analysis and/or statistics) **in Mathematics** at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 17).

Assistant in Research (with an honours degree in the natural sciences with chemistry, geology or mineralogy as principal subjects) **in Soil Science**—The Secretary, School of Agriculture, The University, Cambridge (July 19).

Lecturer or Assistant Lecturer (with a recognized honours degree or equivalent qualification and experience in teaching and research) **in the Department of Chemistry**, University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Kuala Lumpur and London, July 27).

Chair of Education in Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 31).

Chair of Geography in Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 31).

Lecturer (applied mathematician with experience of numerical analysis and the use of digital computers for the solution of engineering problems) **for the Nuclear Power Group**—Dr. P. J. Grant, Department of Mechanical Engineering, Imperial College of Science and Technology, London, S.W.7 (July 31).

Lecturer in the Department of Mineralogy and Geology, University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (July 31).

Reader; a Senior Fellow; and Research Fellows in the Department of Nuclear Physics, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 31).

Reader in Bacteriology at St. Bartholomew's Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 31).

Research Assistant (graduate with research experience, an interest in numerical analysis or in some field of the application of numerical computers) **in the Computing Laboratory**—The Director, University Computing Laboratory, 1 Kensington Terrace, Newcastle upon Tyne 2 (July 31).

Senior Technician in the Department of Zoology, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 31).

Foundation Chair of Pure Mathematics in the School of Mathematics, University of New South Wales, Kensington, New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2 (Australia and London, August 14).

Senior Lecturer (with an engineering degree and knowledge and experience of industrial or production engineering, or a science or arts degree with qualifications in statistical methods and operations research) **in Industrial Engineering** at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 31).

Assistant, Grade B (honours graduate, preferably with teaching or research experience), **in Mathematics**; and an **Assistant**, Grade B (honours graduate, preferably with teaching or research experience), **in Geology**—The Principal, Kingston Technical College, Fassett Road, Kingston-upon-Thames.

Chemist or Biochemist (with a first- or second-class honours degree) **in the Radiobiochemistry Department**, to join a small team working on the metabolism of fat in the ruminant, and in particular involves a study of the biochemical forms of radioactivity in blood, milk and various organs after the administration of labelled lipids——The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. No. 61/20.

Graduate Research Assistant in the Department of Biology and Geology, to work in a team engaged on a survey of marine plankton—The Principal, Norwood Technical College, Knight's Hill, London, S.E.27, quoting Ref. FE.3/N/1553/6.

Lecturer or Assistant Lecturer (with qualifications in mathematical analysis and/or statistics) **in Mathematics** at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Lecturer (with teaching experience and preferably qualified to undertake some teaching of biology) **in Physiology**—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

Principal Scientific Officer (with training in physiology and/or biochemistry, and preferably also in physics and experience in work on blood) (Ref. 61/18); and an Assistant Experimental Officer (preferably with a pass degree or equivalent and experience in biological work) (Ref. 61/19), for work on the flow and coagulation of blood—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting appropriate Ref. No.

Research Students (with, or anticipating to have, a good honours degree in chemistry, Grad.R.I.C., or Dip.Tech. in chemistry) in the Department of Pure and Applied Chemistry; research topics will be selected from the following fields: the chemistry and origin of coal tar constituents; low temperature carbonization studies; physico-chemical studies, including electro-chemistry, surface chemistry, and states of aggregation in solution—Head, Chemistry Department, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

Senior Assistant (with physics and/or mathematics to degree standard and some secretarial experience), for work which will include preparation of material for publication, correspondence in connexion with publications, a small amount of committee work and responsibility for day to day running of the office; and an Editorial Assistant (with physics and/or mathematics to degree standard, and preferably able to type), to assist in the preparation of material for publication—The Editor and Deputy Secretary, Institute of Physics and the Physical Society, 1 Lowther Gardens, London, S.W.7.

Senior Lecturers and Lecturers in Applied Psychology (Psychometrics and Experimental Psychology); Botany; Mathematics; and Physics in the Faculty of Science, University of New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2, and The Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia, quoting Ref. SA1.

TECHNICIAN IN THE RESEARCH LABORATORY, to assist in the preparation of material for human chromosome studies—The Administrative Office, Mount Vernon Hospital, Northwood, Middlesex.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

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